The process of thicket encroachment in semi-arid savanna: community patterns and biotic interactions

by

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Submitted in fulfilment of the academic requirements of

Master of Science

Department of Botany

15 February 2022

RHODES UNIVERSITY
Where leaders learn

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PREFACE

The research contained in '**The process of thicket encroachment in semi-arid savannas: community patterns and biotic interactions**' was completed by the candidate while based in the Discipline of Botany, Department of Botany, Rhodes University, Makhanda, South Africa. The research was financially supported by the NRF-ACCESS (ACyS) Grant ACFP171012265469.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

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PLAGIARISM DECLARATION

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ABSTRACT

Bush encroachment in savannas is widespread in South Africa and is concerning, from both socio-economic and conservation viewpoints, as it affects ecosystem services, functioning and productivity. This phenomenon depends on multiple factors such as history, vegetation, management and environmental conditions, and their interplay. Encroachment into savannas has been relatively well-documented, however understanding of the different roles of tree-tree interactions between species that occur during this process is still limited. This includes the interactions causing spatial patterning, or how interactions and outcomes change over time in terms of encroachment succession from open savanna to closed-canopy thicket. The main objectives of this research are to document thicket establishment in a savanna ecosystem and consider the ecological roles of the key woody species and the abiotic properties of their micro-sites. Determining interactive effects of species co-occurrence is critical to understanding or predicting patterns and changes in biodiversity, nutrient distribution and available water resources. It is also imperative in determining correct and effective land management practices, particularly for reducing bush encroachment and its negative effect on rangelands. All data were collected on Endwell farm, located in the Smaldeel region of the Eastern Cape, South Africa. Endwell farm is a semi-arid savanna with a mean annual rainfall of 730 mm.

First, I examine and describe the thicket encroachment process by exploring the associations between species and their size classes in the field. This was done by using plot-based belt transects and looking at changes in species size-class compositions from early to late successional stages. Association rules (market basket) analysis was used to identify the most common species size-class association patterns. The association between the savanna tree *Vachellia karroo* and the thicket pioneer *Scutia myrtina* was the most prevalent at all stages, with *V. karroo* being central to all associations in the first stage of encroachment; during later stages of encroachment, associations shift to incorporating other thicket pioneer species. The demography and clump formation of *S. myrtina* was strongly linked to associations with *V. karroo* to initiate bush clump formation. Results suggest that mature *V. karroo* facilitate the establishment and growth of *S. myrtina*. These two species were the focus of more detailed investigations to explore the nature and magnitude of their interspecific interactions.

I then examined the effects of pairwise tree interactions between *V. karroo* and *S. myrtina* on soil and leaf nutrient content. I measured differences between inter-canopy and sub-canopy

soil nutrient content, and the effect of associations on plant leaf nutrients, between pair-size combinations and individual controls. Results confirmed that pair-size tree interactions affected both soil nutrient and leaf nutrient content. All individuals increased soil K, N and organic C in the sub-canopy, while association with *V. karroo* increased *S. myrtina* foliar N, P and K. In contrast, association with *S. myrtina* lowered *V. karroo* foliar N, P and K. Small *S. myrtina* individuals were found to benefit most from establishing and growing next to a large *V. karroo* individual, through mechanisms affecting soil and foliar nutrients. *Scutia myrtina* individuals establishing in association with smaller size classes of *V. karroo* showed no significant effects.

I tested for positive and negative effects of pairwise tree interactions between *Vachellia karroo* and *Scutia myrtina* on available soil water and plant water potential (Ψ). This was done by looking at differences between inter-canopy and sub-canopy soil moisture and bulk density and associations on plant water stress (pre-dawn and mid-day leaf Ψ), between pair-size combinations and individual controls. I also selectively removed large *V. karroo* individuals from pairs to confirm the effects of competition and facilitation. Similar to other studies, results confirmed positive and negative effects of pairwise tree interactions. Small *S. myrtina* individuals were most facilitated by establishing and growing up next to a large *V. karroo* individual, through mechanisms affecting soil water content, bulk density and leaf Ψ. *Scutia myrtina* establishing in association with other size classes of *V. karroo* were much less facilitated, showing no significant effects. In contrast, large *S. myrtina* showed competitive interactions with *V. karroo*.

ACKNOWLEDGEMENTS

I would like to make special acknowledgements to my supervisors Susanne Vetter and Tiffany Pillay: their continued guidance, encouragement, engagement, input, availability for discussions and problem-solving skills throughout the duration of this master's thesis have been invaluable.

A number of other people also deserve specific mention:

Brad Ripley, for access and input to knowledge and guidance on plant ecophysiology, theory, methodologies, equipment and technical support.

Craig Peter, for help in acquiring and use of equipment, and problem solving of technical issues around tree removal in the field.

Riaan Strauss and Barry Hartley, for technical knowledge, guidance, support, acquisition and use of the laboratory, equipment and materials.

Marina Khoza, for help in application and calculation of field site cover change using digital aerial imagery, as well as help in data collection and species identification in the field.

Nozuko Ngqiyaza, Keith Steunenberg, Rachel Putzier and Nompumelelo Baso for aid in transportation and data collection.

Tony Painter, for access to his farm to collect data, accommodation, equipment and who went out his way to help with tree removal.

Busi Goba, for carrying the administrative burden, crucial knowledge and information, acquiring forms, funding materials and assistance in many aspects.

Jan Nell and Kim Nell, for continuous support, and Caileigh Ramsay for both support and help in data collection and sample processing.

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CHAPTER 1: Introduction

1.1. Bush encroachment in savannas

Savannas are one of the world's major biomes and are important from both socio-economic and conservation viewpoints (Sankaran *et al.*, 2004, 2005). Bush encroachment can have effects on ecosystem services, functioning and productivity, an increasing dynamic that is of global interest. Bush encroachment is a concern in South African rangelands (O'Connor *et al.*, 2014) and is likely to have negative impacts on agriculture and conservation (O'Connor *et al.*, 2014; Scholes & Archer, 1997).

The causes of this phenomenon depend on multiple factors and the interplay of history, vegetation, management and environment (O'Connor *et al.*, 2014). In South Africa, using data from 23 studies, it has been estimated that the rate of woody cover change has ranged between -0.131 and 1.275% y^{-1}, with changes being fastest on small protected areas and slowest in large natural environments with megaherbivores present (O'Connor *et al.*, 2014). It has also been estimated that bush encroachment has affected the agricultural productivity and biodiversity of about 10 to 20 million hectares of South Africa in 2005 (Ward, 2005).

Bush encroachment is defined as the expansion and increase in density of an indigenous woody species in its original domain at the expense of C_4 grass cover (Hudak & Wessman, 2001; Richter & Meyer, 2001). Bush encroachment can cause changes in woody cover due to an increase in density of trees. This could be due to one of two similar yet distinct woody thickening processes; depending on which species are responsible, whether or not the C_4 grass layer remains intact and if the system is able to easily revert to its original state or not (Parr *et al.*, 2012). The first processes is called 'savanna thickening' and occurs when savanna tree and shrub species increase in density but do not fully suppress the C_4 grass understory. Savanna thickening can be a cyclical process in which thickening and thinning may occur as encroached savanna is ecologically similar to that of open savanna and is able to easily revert from closed to open savanna (Parr *et al.*, 2012). The second processes is called 'thicket expansion', which occurs when savanna and broad-leaved thicket species increase in density and suppress the C_4 grass understory (Parr *et al.*, 2012). Thicket expansion is not as much a cyclical process and is not readily reversible over short timespans. Once thicket expands into savanna it causes a biome switch, because species traits alter the disturbance regimes as densities of relatively fire tolerant, more shade-tolerant, broad-leaved thicket species increase (Parr *et al*., 2012). The woody thickening process of thicket expansion alters the ecosystem functioning and ecological processes much more significantly than savanna thickening (Parr *et al*., 2012).

‘Thicket’ in South Africa was first classified on the basis of dominant life-form combinations of plants and climatic features (Rutherford & Westfall, 1986). Typical thicket, referring to a structural type of vegetation, is defined as a tangle or dense growth of shrubs or trees (Rutherford & Westfall, 1986). Savanna and Thicket are structurally identified as separate biomes based on differences in tree species assemblages and are also different in functional traits related to fire, grazing and competition for light (Calitz *et al*., 2015; Charles-Dominique *et al*., 2015, 2018). To distinguish between the thicket biome that occurs naturally (in areas climatically unsuitable for savannas) and thickets formed through the encroachment of savannas, one needs to examine what species are present and their abundances. This distinction is important because not all thicket species are involved in the encroachment process. Thicket pioneer species are a subset of thicket species more generally that encroach; see Volk *et al*. (2003) for delimitation and characterisation of thicket vegetation in south-eastern South Africa.

Jamison-Daniels *et al*. (2021) provided a detailed examination on the biology of early vs. late clump-forming species. Key findings included a similar species composition between young individuals in early clumps and older individuals in large clumps, rather than between individuals in small and large clumps (Jamison-Daniels *et al*., 2021). Traits of forest specialization were also found to increase as clump area got larger, indicating late successional bush clumps have more species with forest-type traits (Jamison-Daniels *et al*., 2021). Their results also showed that light intensity, temperature, relative humidity and soil moisture are all related to changes in species composition throughout the encroachment gradient (Jamison-Daniels *et al*., 2021).

Encroachment of some semi-arid savannas through the process of thicket expansion has been identified with different encroaching species, causing changes in vegetation structure and spatial ecology of patterning in encroaching species (e.g., Mureva & Ward, 2016). Encroachment via thicket expansion involves a vegetation dynamic known as ‘thicket clump formation’. This occurs over time as thicket species recruit, increase in size and eventually form bush clumps around species that initially encroach (Archer *et al*., 2017; O'Connor & Crow, 1999). A bush clump is defined as the association of two or more woody thicket species with continuous canopies that are separated from other woody species by a grass layer (O'Connor & Chamane, 2012). These bush clumps may eventually amalgamate to form a closed canopy thicket (O'Connor & Chamane, 2012).

One of the most prolific and widespread encroaching species that is increasing in density and expanding its distribution in the Eastern Cape is *Vachellia karroo* (Balfour & Midgley, 2008). *Vachellia karroo* has been recorded in the encroachment process in the Eastern Cape as far back as the 1950s (Acocks, 1953, O'Connor *et al*., 2014). *Vachellia karroo* is also a major species responsible for encroaching Eastern Cape Thicket vegetation, Bisho Thornveld and Amathole Montane Grassland vegetation types in South Africa (Acocks, 1953; Mucina & Rutherford 2006). The link between *V. karroo* encroachment and thicket expansion is its role in facilitating the establishment of pioneer seedlings, leading to development, formation and amalgamation of bush clumps that eventually leads to increases of thicket species and the loss of C4 grasses (O'Connor & Chamane, 2012).

Thicket expansion can be highly variable, context-dependent and can be influenced by multiple interacting site-specific factors that relate to fire frequency, grazing or browsing regimes, climate, soil properties and the functional attributes of each encroaching species (Archer *et al*., 2017). Mean annual precipitation limits woody plant cover up to a mean annual rainfall of just above ~ 650 mm and localized patterns of disturbance such as fire, browsing, and soil properties may prevent the maximum potential for woody thickening being reached (Archer *et al*., 2017). When these constraints on woody thickening are absent, interactions between seasonality, variation between years, intensity and duration of precipitation events then determine the rates and extent of woody plant recruitment and spread (Archer *et al*., 2017). Generally, pioneering species have positive associations with the canopies of the larger trees, benefiting from similar mechanisms to the nurse plant effects of savanna trees on forest species and shrubs (e.g., O'Connor & Chamane, 2012). In some savannas, certain thicket species may encroach through facilitation by acacia trees until a point at which the thicket species are persisting and the acacias die off (e.g., O'Connor & Chamane, 2012).

Precipitation and disturbances in arid or semi-arid savannas can vary in both time and space, helping to explain the variations in observed rates of both savanna thickening and thicket expansion from different locations (Archer *et al*., 2017; Parr *et al*., 2012). The rates of thicket expansion have been found to be highest during the early stages and then either decline or fluctuate as woody density thresholds are reached. Variation in the extent and rate of encroachment is then also mediated by local and regional differences in site specific environmental factors or disturbance regimes (Archer *et al*., 2017). Pioneer species can form clumps that amalgamate to cause a biome shift from savanna to a closed canopy thicket, having important ecosystem level implications that are irreversible over management time scales once the C_4 grass layer is lost (O'Connor *et al.*, 2014; O'Connor & Chamane, 2012).

This biome or 'regime shift' in system state from savanna to thicket as trees shade out the C_4 grass layer is occurring in certain places and becoming an increasingly widespread phenomenon of concern, however the relative extent of thicket formations and savanna thickening have not been estimated or mapped in Southern Africa (Parr *et al.*, 2012). When the regime shift from savanna to thicket occurs, closed habitats contain a higher density of woody thicket species but differ largely in the structure and functional composition of broad-leaved trees, and a reduction of the C_4 grass layer; as well as system properties such as reduced flammability and light availability in the understorey (Parr *et al.*, 2012).

The thicket formation process has been characterised as a form of succession (Jamison-Daniels *et al.*, 2021), with parallels to savanna and forest coexistence as alternative stable states (Charles-Dominique *et al.*, 2018). These states are maintained by feedback thresholds with shade/light and fire as mechanisms, similar to bush encroachment, and once woody cover increases beyond a certain level a biome shift can occur (Charles-Dominique *et al.*, 2018). This regime shift can have cascading functional consequences and implications for biodiversity, agriculture and conservation with the potential loss of specialist savanna species (Parr *et al.*, 2012). Khoza (2021) investigated the increase in abundance, size, diversity and canopy cover of the woody species, their effect on light and the herbaceous layer and identified critical thresholds for vegetation shifts from savanna to closed canopy thicket.

1.2. The encroachment sequence

The process of bush clump formation has been documented in several studies around the world (Archer, 1994; Jamison-Daniels *et al.*, 2021; O'Connor & Chamane, 2012). The general consensus is that the process starts when bush clumps are formed through nucleation after the establishment of savanna species in grassland (Archer, 1994; O'Connor & Chamane, 2012). Kraaij & Ward (2006) show that the success of early tree recruitment and early survival in bush-encroached savanna of South Africa is the most influential process in the course of bush encroachment. This occurred when conditions for the initial stages are triggered by events of unusually high precipitation and/or nutrients (O'Connor & Chamane, 2012). Establishment of savanna trees is linked to rainfall events resulting in episodic recruitment, but the establishment of the thicket pioneers that make use of nurse plants is less rainfall dependent, as shown by Seymour (2006) for *Acacia erioloba* and the bush clump forming species that establish underneath. With increasing clump size formation over time, there is an increase in species richness and diversity (O'Connor & Chamane, 2012). Changes in species composition along the successional gradient affected light intensity, soil moisture, relative humidity and temperature within clumps (Jamison-Daniels *et al*., 2021; Khoza, 2021).

Patterns of compositional variations within clumps are closely related to clump size, as differences resulting from clump formation disappeared as clumps developed (Jamison-Daniels *et al*., 2021; O'Connor & Chamane, 2012). Directional changes of species along the successional gradient or encroachment sequence suggest a deterministic process of succession, which is driven by changes in the local environmental conditions during clump formation and amalgamation (Jamison-Daniels *et al*., 2021). Based on information from previous work in different parts of South Africa (Jamison-Daniels *et al*., 2021; O'Connor & Chamane, 2012; O'Connor & Crow, 1999), the shift from savanna to a closed canopy thicket represents a temporal sequence of encroachment that also corresponds to an increase in canopy cover, and which may or may not involve the formation of bush clumps.

Not all thicket expansion processes involve bush clump formation, however the temporal sequences are qualitatively different in the sizes, canopy area, density and spatial patterning of plants (Parr *et al*., 2012). Where bush clump formation occurs, the general process entails encroaching savanna species establishing and increasing in density in the open grassland without a nurse plant, which in turn facilitates the establishment of other pioneer thicket species under their canopies, which increase in size and eventually form a bush clump. O'Connor & Crow (1999) confirm that a change in system state due to bush encroachment in the Kei Road-Komga region of the Eastern Cape had taken place over a time span of 50 years with specific reference to *V. karroo* causing encroachment via thicket expansion. O'Connor & Crow (1999) report the thicket pioneer species responsible and their characteristics. Early successional stages were characterized by larger *V. karroo* individuals which initiate the recruitment of pioneer thicket species such as *Scutia myrtina* and *Gymnosporia buxifolia*, as these have been documented to be growing under their canopy (O'Connor & Crow, 1999).

Savanna-thicket association is commonly initiated via directed dispersal using animal dispersal vectors, which are integral to some major plant groups in the arid thicket of South Africa (Adie & Yeaton, 2014). There are benefits to thicket species having directed dispersal, such as increased nitrogen availability (which may facilitate establishment, germination and growth of seedlings) and/or reduced water stress; *V. karroo* is highly likely to be acting as a nurse plant that provides such advantages (similar to Pugnaire *et al*., 1996).

The intermediate successional stages are characterized by the thicket pioneer species growing and amalgamating into bush clumps, surrounding the initial nurse plant and possibly starting to compete for space, nutrients and water. As the associated individuals become larger, the initial nurse plant may show signs of stress, such as reduced growth, decreased seed productions or die-back of branches. Charles-Dominique *et al*. (2018) showed how ecological thresholds of canopy closure can separate biomes through species traits. At their research site, the fire suppression threshold (where flammable C4 grass biomass becomes insufficient to carry a fire; Hoffman *et al*., 2012) had a 'leaf area index' (LAI) of 0.5 separating savanna from thicket. They also identified a deep shade threshold (LAI of 1.5) that separated forest from thicket and where grass composition shifted to understorey specialists. Grass species were sorted on this light gradient according to their photosynthetic pathway as C_4 grasses dominate in open canopy and at intermediate levels of shading (LAI of 0.4– 1.5), while C_3 grasses mostly dominate in the deep shade (LAI > 1.5).

Khoza (2021) similarly identified two LAI thresholds in the Eastern Cape relating to transitions in vegetation structure. The first threshold occurred with a canopy cover of ~ 40% (LAI ~ 0.5, light transmittance ~ 75%), where herbaceous species and dominant C_4 grasses started to decline in abundance (Khoza, 2021). The second threshold indicated a species compositional shift between savanna and closed-canopy vegetation states, occurring at ~50% light transmittance (LAI ~ 1 and canopy cover ~70%), at which point the savanna state switched to a thicket with a marked reduction in the occurrence of C_4 grasses (Khoza, 2021).

Late successional stages are characterized by the thicket pioneer species increasing in size, abundance and richness as the clumps increase in size. At this stage, the nurse plant becomes unidentifiable as the founding individual due to the thicket species becoming larger than the original founder, or due to the death of the founder (O'Connor & Chamane, 2012). Another feature of this stage are the effects of increased tree density and canopy-cover on grass composition and especially the dominance by *Panicum maximum* which is a common feature of encroached areas in the Eastern Cape (Mogashoa *et al*., 2021).

1.3. Competition and facilitation structuring woody savanna communities

Much savanna encroachment research has focused on the effects of disturbance (especially fire) and tree-grass interactions, but the role of tree-tree interactions is also important. Both tree-tree (Sea & Hanan, 2012) and tree-grass (Scholes & Archer, 1997) coexistence in savannas has been viewed as a balance, with competition and facilitation of trees and seedlings. Riginos (2009) found that savanna tree saplings experienced stronger competition from grasses than larger trees mostly in terms of growth, but tree growth in both the juvenile (1 to 2.5 m tall) and adult (>2.5 m tall) size-classes were also substantially suppressed. Riginos (2009) demonstrates through her results that both tree-tree and tree-grass competition can substantially reduce the growth and demographic transition probability of trees, regardless of demographic stage, as well as rainfall having a large impact on the responses. Competition interactions between plants have traditionally been viewed to shape community structure (Grime, 1977), however research indicates the importance of both competitive and facilitative mechanisms (Bruno *et al*., 2003). The stress-gradient hypothesis for example predicts an increasing importance of facilitative mechanisms relative to competition along gradients of increasing environmental stress (Dohn *et al*., 2013).

Non-resource-based hypotheses propose that trees are better competitors than grasses, but tree populations are suppressed by mortality or top-kill caused by fire, herbivory and other forms of disturbances as opposed to self-thinning processes (Sea & Hanan, 2012). Tree-tree competition and fire are two key factors that structure semiarid to mesic savannas and thicket clump formation (Calabrese *et al*., 2010). While competition can substantially depress tree density, competition and fire combined interact non-linearly with strong negative consequences for tree density (Calabrese *et al*., 2010). Competition based models generally assume that tree seedling and/or sapling recruitment could be limited by surrounding individuals, but competitive asymmetry can change direction for larger post sapling trees (Scholes & Archer, 1997; Sea & Hanan, 2012). Sankaran *et al*. (2004) provided a useful synthesis of how competition and demographic bottlenecks can be integrated to understand tree-grass coexistence, but do not include any facilitative interactions.

Using data from 850 sites across Africa, Sankaran *et al*. (2005) show that the maximum woody cover in savannas receiving a mean annual precipitation of less than 650 mm is constrained by, and increases linearly with, mean annual precipitation. Sankaran *et al*. (2005) propose that arid and semi-arid savannas may be considered ‘stable’ systems in which water constrains woody cover. Above the mean annual precipitation of ~ 650 mm, savannas become ‘unstable’ systems in which mean annual precipitation is sufficient for woody canopy closure or possibly thicket clump formation and disturbances such as fire, herbivory and soil properties interact to reduce woody cover (Sankaran *et al*., 2005). Mean annual precipitation can mediate tree-tree interactions, with associations shifting qualitatively between 550 and 737 mm to be less competitive (Moustakas *et al*., 2013). Generally, facilitation is a more common interaction between species in more stressful or arid environments, whereas competition dominates most associations in more productive and high precipitation environments (Dohn *et al*., 2017; Moustakas *et al*., 2013).

Dohn *et al*. (2017) examined plant spatial patterns and quantified the degree of competition among woody individuals in semiarid savanna. They found woody plants showed strongly clumped spatial distributions at scales of 1–5 m, with analysis of woody plant growth rates relative to their conspecific and heterospecific neighbours revealing strong competitive interactions at neighbourhood scales of up to 5 m for most woody plant species. Woody plants aggregating in clumps despite significantly decreased growth rates indicate that the spatial distribution may result from dispersal and establishment processes rather than reflect the effects of competitive, density-dependent mortality (Dohn *et al*., 2017). The suppressive effects of woody plants on neighbours also suggest an important role for tree-tree competition in controlling vegetation structure, and they also contribute to the patterns of maximum tree cover across rainfall gradients in African savannas (Dohn *et al*., 2017).

1.4. Facilitation and seed dispersal

Schleicher *et al*. (2011a) tried to disentangle facilitation and seed dispersal from environmental heterogeneity as mechanisms that are responsible for spatial associations of *Vachellia karroo* and *Grewia occidentalis* in a semi-arid savanna in South Africa. Their results showed positive spatial associations of the two species, with no *G. occidentalis* individuals growing under trees of a height less than ~ 1 m (Schleicher *et al*., 2011a). Schleicher *et al*. (2011a) excluded environmental heterogeneity as the sole cause of this positive association, showing that it is due to both directed seed dispersal and facilitation caused by improved water and nitrogen fixation under the host acacia species. Seymour (2006) examined the facilitation of bush clump formation by *Acacia erioloba* and experimentally examined different processes of bush clump formation in *Ziziphus mucronata*, another common thicket species. *Ziziphus mucronata* demography revealed that growing under the *A. erioloba* sub-canopy is important for establishment, with seeds from plants growing in the sub-canopy environment germinating faster than in the inter-canopy (Seymour, 2006).

Abreu *et al*. (2021) showed how isolated trees are able to favour woody encroachment and species turnover by overcoming dispersal limitation and environmental filtering in a savanna released from fire. They found that the effectiveness as a nucleator differed among species, but was unrelated to functional guilds such as habitat preference or dispersal syndrome (Abreu *et al*., 2021). Results suggested that this process first starts when host trees reach a size at which they can act as perches, favouring other bird-dispersed species (Abreu *et al*., 2021). Host trees act as a nurse, which is partially explained by increased soil moisture and decreased daily temperature amplitude, changing the environment in favour of recruits (Abreu *et al*., 2021). Over time, nuclei expand and merge causing savanna specialists to disappear and the savanna changes state (Abreu *et al*., 2021; Parr *et al.,* 2012).

To predict plant community dynamics the seed dispersal and seedling establishment patterns must be understood (Nishi & Tsuyuzaki, 2004). This is especially true in arid or infertile environments where seedling establishment is critical in succession, as seed germination of pioneer species is influenced by micro environments such as temperature, moisture and topography (Nishi & Tsuyuzaki, 2004). There is also strong evidence for interactions between frugivorous birds and trees producing bird dispersed seeds that increase species distributions in harsh environments (Nishi & Tsuyuzaki, 2004). Often these relationships between dispersal and facilitation can go hand-in-hand by overcoming environmental limitations (Abreu *et al*., 2021; Schleicher *et al*., 2011a).

1.5. Tree-tree interactions and nutrient availability

Soil nutrients are essential for plant growth and persistence, and act as a determinant of the vegetation structure, composition and productivity of savannas (Scholes & Archer, 1997). While the nutrient status of the parent material is initially important, biological activities of trees have been shown to create and maintain localised islands of enhanced soil fertility that differ from the inter-canopy spaces (Scholes & Archer, 1997). Nutrients that are added by trees to sub-canopy areas in the form of tree litter and/or animal droppings have also been shown to increase the understory productivity when compared to open grassland (Belsky, 1994). There is ample evidence for the slow process of soil enrichment under tree canopies, which is especially important for areas with nutrient poor sandy soils (Hagos, 2001).

Enrichment of soil nutrients by *V. karroo* includes mostly increased potassium and nitrogen under crowns on red-soils and vertisol (Moyo *et al*., 1998). In semi-arid areas of South Africa, tree crowns have been shown to create or influence the micro-habitat under their canopy, improve the soil physical and nutrient status and reduce evapotranspiration with increasing canopy size (Moyo *et al*., 1998). The general trend of soil nutrient change (accumulation) with woody encroachment has been reported to mainly include increased concentrations of phosphorus, carbon, nitrogen, calcium and magnesium (Mogashoa *et al*., 2021). Evidence for increased nutrient status usually focuses on the total percentage of nitrogen, organic carbon and cations that are exchangeable such as Ca, K, Mg and Na (Hagos, 2001). This is often demonstrated through correlations of total carbon and nitrogen in the soil under tree canopies and the tree girth, which is a measure of age and size (Hagos, 2001). Patterns in soil nutrient status between the understorey of *V. karroo* and inter-canopy areas have been consistently shown, as well as patterns in soil volumetric water content of open areas being greater than *A. karroo* understorey areas (Moyo *et al*., 1998).

Treydte *et al*. (2013) found that large trees changed the relative amount of potassium and nitrogen limitation in grasses under tree canopies. Also, grass leaf potassium and nitrogen contents were shown to be positively correlated with available herbaceous layer biomass across all their sites with potassium showing stronger trends than nitrogen (Treydte *et al*., 2013). The sites with higher biomass corresponded to low herbivore density (including browsers) and showing higher nutrient contents for the herbaceous layer than sites of lower herbaceous biomass, particularly for phosphorus, an often-limiting nutrient in savanna systems (Treydte *et al*., 2013). Belsky (1992a, 1992b) has also shown that leguminous acacia trees can increase the forage quality, yield, tissue nitrogen availability and the amount of nutrients in the herbaceous forage under the canopy, but this in turn is offset by a small drop in the digestibility of the forage as lignin increased from the open grassland to the canopy zone. The effects of bush encroachment on soil nutrients have been found to be very similar, with concentrations of nitrogen, phosphorous, potassium, calcium, and copper increasing from early to late stages of encroachment while concentrations of magnesium and zinc decrease with increasing density and canopy size (Moyo *et al*., 1998; Scholes & Archer, 1997).

1.6. Tree-tree interactions and water availability

Under water stressed conditions, the establishment of many species is restricted to shady areas because of reduced transpiration (Pugnaire *et al*., 2011). Shading by a nurse tree may impose a cost on the photosynthetic performance of individuals in the sub-canopy (Belsky, 1994; Pugnaire *et al*., 2011), but most species from arid environments have photosynthetic optima below the prevailing radiation and many benefit from the decrease in radiation provided by nurse plant canopies.

At higher tree densities or with larger individuals with bigger canopies, however, competition for water can become intense. Bush encroachment and projected increases in drought events are expected to decrease the resilience of water-limited ecosystems and affect their functioning or delivery of ecosystem services, while tree mortality due to water stress and competition can shift savanna ecosystems into alternative states such as thicket (Caldeira *et al.*, 2015). Caldeira *et al.* (2015) used a shrub removal experiment and the co-occurrence of a drought event to show that native bush encroachment and extreme drought synergistically reduced ecosystem transpiration and the resilience of the key tree species, as tree transpiration decreased from 23% to 16%. They found that tree transpiration in encroached sites declined much more than that in sites cleared from pioneer species. Trees in encroached sites also had much lower pre-dawn leaf water potential (−1.41 ± 0.08 MPa) than that of trees in un-encroached sites (−1.18 ± 0.05 Mpa).

Dwyer *et al.* (2010) surveyed and mapped mass mortality of woody stems within *Eucalyptus melanophloia* dominated savanna (Central Queensland, Australia). They found that drought induced stem death was not randomly distributed in space, and identified a positive relationship between survival and living neighbour density indicating a resource patch effect and negative density dependent effects. Dwyer *et al.* (2010) also found strong support for an interaction between microsite quality and neighbourhood stem densities, suggesting that interactions are driven by plant available water and available resources or resource heterogeneity. More generally, positive and negative interactions between neighbouring individuals are considered fundamental processes that interact dynamically with the abiotic environment to determine community structure (Bruno *et al.*, 2003).

The encroachment of woody species into savanna can interfere with the hydrological budget such as the amount of evapotranspiration, runoff by changing soil infiltration rates, deep drainage and moisture recharge below the rhizosphere (Archer *et al.*, 2017). In water limited systems such as semi-arid savannas, run-off is created during rainfall events when precipitation input exceeds the infiltration rate of the soil. Encroachment can change the species associations (competition), infiltration characteristics of the soil through effects on soil quality, microbial biomass, nitrogen mineralization and spatial heterogeneity (Archer *et al.*, 2017).

The proportion of precipitation reaching the ground under canopies is influenced by many factors, such as the intensity and amount of rainfall and the characteristics of the particular tree e.g., tree height, canopy size, architecture, leaf area and even the bark texture (Marañón *et al.*, 2009). It has also been shown that soils under tree canopies potentially receive up to 19 to 24% less rainfall than adjacent open areas (inter-canopy) with northern and eastern aspects (Marañón *et al.*, 2009). *Vachellia karroo* tree crowns have also been found to reduce solar radiation of understorey areas by roughly 88% and rainfall by over 15% (Moyo *et al.*, 1998).

1.7. Research objectives

There has been a limited amount of research on bush clump succession (Jamison-Daniels *et al*., 2021; O'Connor & Chamane, 2012; Scholes & Archer, 1997) and the interspecific interaction mechanisms resulting in spatial patterning during this process in South Africa or elsewhere (Mureva & Ward, 2016; Seymour, 2006). There has also been little research on thicket establishment in these ecosystems that considers the ecological roles of different plants and the abiotic properties of their microsites (Pueyo *et al*., 2016).

Assessments examined multiple plots, looking at what the associations and patterns are of the most common thicket pioneer species, similar to Schleicher *et al*. (2011b) who also looks at tree-tree interactions, changes of woody plant interactions, growth and spatial distributions. This is to look at biotic interactions that have happened during this process, and what has led towards a threshold where thicket species persist, and savanna species die out. Disentangling these abiotic mechanisms, whether its hydrological benefits and/or nutritional improvements may reinforce the spatial patterning in the positive relationship between spatial occurrence of acacia and thicket species (similar to Wiegand & Moloney, 2004).

First assessments included examining where the different species recruit, which species recruit out in the open, under acacias, and which only grow once the thicket clumps are bigger and more established (O'Connor & Chamane, 2012). Later assessments test abiotic mechanisms such as water stress and nutrient availability at different stages of the temporal encroachment sequence coupled with removal experiments to determine the effects of interspecific competition for water more closely.

Endwell farm in the Smaldeel region of the Eastern Cape, South Africa, is a representative example of a place where thicket clump formation has been documented to be occurring. Khoza (2021) has shown that different parts of the farm represent different temporal stages in the woody encroachment sequence and the thicket formation process.

Chapter 2 examines (1) the sequence of species that recruit first and which recruit later by looking at size class distribution of each species at each stage, and (2) whether established individuals of *V. karroo* (and possibly other species later in the sequence) provide establishment sites for thicket pioneers (inferred from associations of larger *V. karroo* with smaller thicket pioneers). The abundance of the different species also tells us which species contribute the most to the thicket expansion phenomenon. This was done by documenting the thicket encroachment sequence and determining which particular species-associations we find in the field from the early to late successional stages using association rule analysis. I predicted that *V. karroo* would be the initiator of thicket clumps as thicket species benefit from *V. karroo* during the early stage of encroachment, as well as through the role of seed dispersal. I expected large *V. karroo* and smaller thicket species in the early stages, and at the later stages large *V. karroo* and large thicket species combinations are expected to be common. Clumps of large thicket species with absent *V. karroo* and the recruitment of thicket climax species during the late successional stages are also expected.

Chapter 3 aims at untangling inter-specific interactions via their effect on soil and foliar nutrients. I examined soil nutrient content under the sub-canopy vs inter-canopy spaces, and leaf nutrients of plants growing in pair-size combinations and control individuals. I expected soil nutrients (N, P, K and C) to increase as plants increase in size and higher under the sub-canopy compared to inter-canopy spaces. Combinations of large *V. karroo* and large *S. myrtina* are expected to compete and have less soil nutrients, compared to large control individuals. Combinations of large *V. karroo* and small *S. myrtina* are expected to have little to no effect on *V. karroo* foliar nutrients but there is benefit to the *S. myrtina.*

Chapter 4 tests for positive and negative effects of pairwise tree interactions on available soil water and plant water potential, by looking at differences between inter-canopy and sub-canopy soil moisture and bulk density. Also, soil water and plant water stress were examined by measuring pre-dawn and mid-day leaf water potential (Ψ) of individuals in different pair-size combinations and individual controls. A selective removal of large *V. karroo* individuals was done and pre-dawn and mid-day leaf Ψ were re-measured to confirm the effects of competition between large individuals and facilitation by large *V. karroo* of the establishment and early growth of *S. myrtina*. I expected higher soil water content under sub-canopies compared to inter-canopy spaces, with lower bulk density under the sub-canopies compared to inter-canopy spaces. I expected that soil water content would be higher, and bulk density lower, under the canopies of larger trees, with the lowest soil water content and highest bulk density under the canopies of large *S. myrtina* and large *V. karroo* pairs. I also expected that growing with medium or large *V. karroo* would lead to less negative water potential in small *S. myrtina* (indicating less water stress), and large *S. myrtina* growing with large *V. karroo* trees would be the most water stressed (most negative leaf Ψ). After removing large *V. karroo* individuals, I expected small *S. myrtina* to show higher water stress (more negative Ψ), similar to that of small *S. myrtina* control individuals. It was also expected that removal of the large *V. karroo* may slightly reduce water stress (less negative Ψ) of large *S. myrtina*.

Chapter 5 synthesizes results and discussions with regards to outcomes, implications, challenges/limitations and recommendations for future research.

CHAPTER 2: The encroachment sequence: patterns and associations between savanna and thicket species

2.1. Abstract

This chapter's main objectives were to describe the thicket encroachment sequence. In particular, I set out to infer the sequence in which different key species recruit and whether recruitment of thicket pioneer species occurs in association with established savanna trees. To do this, I quantified changes in species size class composition, such as which species and size classes are found at the different stages, from early to late succession. I determined the most common associations in terms of species and size-class at different encroachment stages using the novel association rule analysis (ARA) methodology. I found large individuals of *Vachellia karroo* to be central to associations at all stages, with *Scutia myrtina* being the most common thicket pioneer that establishes and grows in association with *V. karroo*. ARA showed *V. karroo* to invade first, providing nucleation sites for thicket clumps with *S. myrtina* being the earliest and most abundant pioneer. Pioneer species are all associated with *V. karroo* in the first stage, while in the later stages thicket species start associating with each other; thus *V. karroo* and *S. myrtina* are of most interest from both an ecological and management point of view.

Keywords: Bush encroachment; Savanna- thicket associations; Spatial ecology; Successional phases; Thicket expansion.

2.2. Introduction

The characterization of a shift from an open savanna to a closed canopy thicket is based on the temporal sequence of encroachment that also corresponds to an increase in woody cover (Jordaan *et al*., 2004; Khoza, 2021). The early phase of this temporal sequence is qualitatively different in the species composition, sizes, canopy area and density of plants as compared to the late successional phase. This is similar to changes in LAI thresholds demonstrated by Charles-Dominique *et al*. (2018) and Khoza (2021). The general process of encroachment in certain regions of the Eastern Cape starts with *Vachellia karroo* individuals establishing in large numbers by themselves in the open grassland (O'Connor, 1995; O'Connor & Crow, 1999). Invasion of *V. karroo* in turn facilitates the establishment of other pioneer thicket species such as *Scutia myrtina* and *Gymnosporia buxifolia* under their canopies, referred to here as the early successional stage (Pugnaire *et al*., 1996). Over time, these pioneer thicket species increase in size and eventually form a bush clump (O'Connor & Crow, 1999).

Vachellia karroo growing with *S. myrtina*, *G. buxifolia* or *Olea europaea* subsp. *africana* are the most commonly occurring species combinations found in what is now classified as Bisho Thornveld (Mucina & Rutherford, 2006; O'Connor & Chamane, 2012). Understanding the interspecific facilitation and competition interactions and how they change and interact over time to affect functioning and resilience of ecosystems will be critical in the prediction of responses to global change and implementation of ecosystem management and mitigation strategies (Aubrey & Reynolds, 2002; O'Connor *et al*., 2014). Classic successional studies use a space-for-time substitution approach to infer the successional sequence from species composition, abundance and size-classes on plots of different ages or stages of encroachment (Le Roux, 1996). Reconstruction of the sequence of bush clump formation has been done by analysing the size-classes within clumps and the species associated with small, medium or large clumps (e.g., Archer 1990, 1995; Jamison-Daniels *et al*., 2021; O'Connor & Chamane, 2012). These studies inferred the 'founder' species initiating the bush clumps as being the individual with the greatest stem diameter, which was assumed to reflect the oldest individual in the clump (O'Connor & Chamane, 2012). Inferring whether bush clump formation represents a successional sequence thus requires not only information on association of species, but also which size-classes.

Common ways in which species associations have been statistically modelled in ecological research. Approaches include both basic association analysis that only deals with the occurrence of one species with another, and more complicated analysis such as principal component analysis, hierarchical cluster analyses or joint species distribution models that take into account both environmental variables and the interactions between certain species (Wang, 2020). However, there are challenges analysing association of multiple individuals of different species in different size-classes, which exceeds the capability of traditional contingency-type analyses (Li, 2017). Association rule analysis (ARA) can be used to overcome these challenges (Hobbs *et al*., 2005; Li, 2017). This type of analysis has only recently been used in ecology, for example to identify indicator species or attributes (Ferrarini & Tomaselli, 2010; Leote *et al*., 2020; Rossi *et al*., 2014). Association rule analysis has been applied to examine associations between species in different forest types (Souza *et al*., 2021) and can provide a novel tool for examining which species in which size-classes commonly occur together throughout the encroachment process, also allowing a description of encroachment species-associations that can be easily repeated (Hobbs *et al*., 2005; Li, 2017).

Association rule analysis is a data mining method known more widely as market basket analysis due to its original application in marketing, retailing, clustering and classification (Li, 2017; Nisbet *et al*., 2018). This method analyses what items are frequently bought together (or between different objects in a dataset, frequent patterns in a transaction database, relational databases or information repository) by generating a set of rules called association rules (Li, 2017). This type of analysis can be applied in an ecological context to provide a basic picture of the interrelation between species-size associations, suggesting interactions between them (Li, 2017; Souza *et al*., 2021).

Application of association rule mining is a two-step approach. The first step is frequent item-set generation, which finds all frequent item-sets with support that is greater or equal to pre-determined minimum support values. The second step is the rule generation, a calculation of support, confidence and lift of all rules (Li, 2017; Nisbet *et al*., 2018). The support value that is computed by the algorithm is the joint probability or relative frequency of co-occurrences of each association rule (Nisbet *et al*., 2018). The algorithm works by first looking for single species that occur frequently and have the minimum support. Then the algorithm finds pairs of species that have the minimum support, so that one of the species in the pair was frequently occurring. This process is repeated with increasingly larger combinations of species until all ‘frequent item-sets’ have been found, those that satisfy the minimum confidence requirements are then reported as association rules (Hobbs *et al*., 2005).

My objectives in this chapter were to document and determine which associations (species, size-class combinations) are most common along a known gradient representing early to late stages of bush clump formation. I used ARA to examine the associations between woody species in different demographic stages along a thicket encroachment gradient to reconstruct not only the sequence at which different species become abundant, but also whether particular species recruit preferentially under established individuals of other species.

Based on research in the same vegetation type (Khoza, 2021; O'Connor & Chamane, 2012), I predicted a process where *V. karroo* establishes on its own in open savanna, then providing establishment sites for thicket pioneers which grow bigger and in turn facilitate further thicket formation. I thus expected my data to show that:

1) Sites representing the early stages are dominated by *V. karroo* with few thicket pioneer individuals. The longer a site has been experiencing woody encroachment, the greater the total number of individuals with a greater number and diversity of thicket species. Early successional sites have *V. karroo* size distribution with many young individuals indicating recruitment. Intermediate sites have various *V. karroo* size-classes, becoming more dominated by larger individuals, while populations of the pioneer species increase in number with a high proportion of young individuals. The oldest sites will be dominated by large individuals of thicket species, some large *V. karroo* but little *V. karroo* recruitment (i.e., few in the small size categories).

2) At the youngest sites, clumps consist of few individuals, mainly *V. karroo* that often occur on their own. At intermediate sites I expect associations between early thicket pioneers in small size classes with large *V. karroo*. In the late stage I expect clumps with more associated individuals, and that involve large *V. karroo* and bigger thicket pioneers; and likely some clumps of just large thicket species but few clumps that include large thicket clumps and small *V. karroo*.

2.3. Methods

2.3.1. Study area

The field site, Endwell farm (32˚44’55”S, 26˚27’19”E), is located 30 km west of Fort Beaufort in the Smaldeel region of the Eastern Cape, South Africa. Land use in the Smaldeel region is predominantly commercial livestock farming (Martens *et al.*, 1996; Smit, 2004). Endwell farm is primarily used for farming cattle, and numbers of small livestock (sheep and goats) have been insignificant over the last 25 years due to the risk of livestock theft. Wild ungulates on the farm include the common duiker (*Sylvicapra grimmia*), impala (*Aepyceros melampus*), kudu (*Tragelaphus strepsiceros*), waterbuck (*Kobus ellipsiprymnus*) and blesbok (*Damaliscus pygargus phillipsi*). Since 1966, fire and other forms of bush control have been limited to cutting or poisoning of selected trees in small areas, such as access points for workers and cattle, or where they threatened structural integrity of nearby fences, buildings and gates.

The Smaldeel region of the Eastern Cape spans approximately 130 km from west to east from Somerset East to Fort Beaufort. This region’s northern boundary spans along the Bosberg, Winterberg, Elandsberg and Amatola mountains, while the southern boundary is the Fish River valley (Martens *et al.*, 1996). The region has distinct wet and dry seasons and receives a mean annual rainfall of between 450 and 750 mm. Rainfall is highest in the summer months peaking in March and October (Martens *et al.*, 1996). The mean annual rainfall on Endwell farm (1927-2021) is 730 mm. The most predominant soil types in this area are poorly drained Glenrosa, Trevanian and Mispah (Martens & Morris, 1994), containing a mixture of clay, sand and loam (Schulze, 2008).

Endwell farm is a semi-arid savanna with a mixture of trees and a discontinuous grass later. The vegetation comprises a mixture of Bedford Dry Grassland and Bisho Thornveld (Mucina & Rutherford, 2006). Bisho Thornveld is characterized by a grass layer with varying densities of acacia dotted with clumps of broad-leaved woody species that are similar to those found in Albany Thicket (Mucina & Rutherford, 2006). The forage quality is very good in this veld type as it has a high protein content that is able to sustain cattle and game farming through the dry season, usually a characteristic of warm fertile savannas (Blackmore *et al.*, 1990). *Vachellia karroo* is the dominant tree species in this area (Acocks, 1953), occurring as isolated individuals originally but with increases in woody density since 1953. Other common species on the farm include *Gymnosporia buxifolia, Brachylaena elliptica, Grewia* *occidentalis, Olea europaea* subsp. *africana*, *Ziziphus mucronata, Searsia longispina* and *Scutia myrtina* (Mucina & Rutherford, 2006).

2.3.2. Sampling site selection

I selected plots for determining the changes in abundance, size classes and association of woody species with time. This was done by selecting plots that showed encroachment for short, intermediate and long periods of time based on sequential aerial imagery dating back to 1949 analysed by Khoza (2021). Khoza (2021) sampled fifty 150 m × 150 m grid cells on the digital aerial images and quantified the change in percent woody cover between the photographs taken at different dates. Plots were chosen on a topographically homogeneous section of the farm, located along the same slope to reduce the effects of and control for altitude and edaphic factors. Khoza (2021) found that total woody canopy cover corresponded to duration of encroachment and that areas with higher woody cover had more large trees and a greater diversity of woody species. Based on Khoza's (2021) analyses, I sampled 15 plots, of which five each represented early (0 to 19% woody canopy cover), intermediate (20 to 59% cover) and late stages (60 to 100% cover) of thicket formation. Early stages had been encroached for ~ 15 years, intermediate stages ~ 30-40 years and late stages are > 50 years old according to aerial photographs (Khoza, 2021).

2.4. Data collection

To document and characterize the thicket encroachment sequence and determine what combinations of species are associated in the field, I recorded all woody individuals in belt transects. In the centre of each 150×150 m plot, I laid out two 100 m long and 5 m wide transects running parallel to one another and 50 m apart. This allowed me to determine the density and size class distributions of different species at different stages of the encroachment gradient.

Each belt transect was mapped using a hand-held GPS and trees were also spray-painted to record the start and end of each transect. To analyse patterns of association, I recorded the species identity and size-class of each woody individual in the transect, as well as the unique clump identification (ID) each belonged to. Individuals that had a canopy overlap of more than 50% with one or more other individuals were considered as being part of the same clump. Individuals with no canopy overlap (or between 1- 49%) were considered to be recruiting on their own and were recorded as being the only individual in their ‘clump’. Each individual was identified and characterized into size-classes < 0.5 m, >0.5- 1 m, >1- 1.5 m, >1.5-2 m, >2-3 m, >3-4 m and > 4 m using a measuring pole.

2.5. Data analysis

Analysis of data was carried out using the statistic software R v.1.0.136 (R Core Team, 2020). Mean total tree density of all species is represented visually to summarise the trend in overall tree numbers using a box-plot, showing the density (all size classes combined) in stages 1 (sites 1-5), 2 (sites 6-10) and 3 (sites 11-15). Differences in density and size-class distributions along the age gradient were examined for the six most commonly occurring species found in each encroachment category (stages 1, 2 and 3 representing early, intermediate and late stages of thicket formation). The size-class distributions were presented as the mean density of each of the size-classes of each species in the five plots in each stage, and species were plotted separately for each stage.

Species size-class associations at each stage, and for all stages combined, were determined using association rule analysis (ARA) using the R package *arules* (Hahsler *et al*., 2005, 2011, 2022). In the case of my data, the ‘transactions’ were the clumps of overlapping individuals, and the ‘items’ were all individuals (classified by species and size-class) in a clump. Raw data was first converted from a data frame (cluster, species-size and quantity columns) into transactional data, called the basket format. The size-classes were reduced from seven to four to simplify the analysis and reduce the number of item categories. The size classes were coded 1 (< 0.5 m), 2 (>0.5 to 1 m), 3 (>1 to 2 m) or 4 (>2 to > 4 m), and ‘items’ analysed were all combinations of species x size-class (thus for example, an individual classed ‘Scutia3’ represented a *Scutia myrtina* in the size class 1-2 m).

The rules were then mined using the *APRIORI* algorithm (Borgelt & Kruse, 2002) to determine minimum and maximum values for support, confidence and lift for each rule (Li, 2017). Values used by the algorithm in R (*ARA* and *APRIORI*) include parameter values that were specified to have a minimum support of 0.01 and minimum confidence of 0.8 (Li, 2017). Summary of quality measures included a graph-based format using the package *arulesViz* (Hahsler *et al*., 2019) to visualize the association rules using vertices and edges (Li, 2017).

Interpretation of each rule consists of two sides, the left-hand side (LHS) which is a category level (such as one item or species) that has high frequency of occurrence, called support; while the right-hand side (RHS) is composed of one or more category levels that is related to the LHS in a frequency called confidence (Souza *et al*., 2021). Each rule indicates which item(s) is frequently associated with what item(s). The LHS and RHS parts of each rule can be interpreted such that the item(s) on the right were frequently associated ("bought along with" in the original application of market basket analysis) with items on the left. The confidence is based on the rule support and informs how frequent the rule is in the dataset (Leote *et al*., 2020; Souza *et al*., 2021). Once the rules have been generated, they are accompanied by three measures: lift, coverage and Fisher's exact test p-value (Souza *et al*., 2021). Lift is a measure of importance, where lift > 1 indicates a greater probability of having item 2 if item 1 is present. Lift is the factor by which the observed co-occurrence of two or more items exceeds the expected probability of the items co-occurring, had they been independent. If lift = 1, that rule's items are independent from one another, while values < 1 indicate a negative relationship between items (Souza *et al*., 2021). The second includes coverage, a measure of a rule's frequency that evaluates the support of the species present in the LHS (Souza *et al*., 2021). The third measure (Fisher's exact test p-value) is the rule's significance, an evaluation of expected frequency or by chance (Hahsler, 2005; Souza *et al*., 2021).

2.6. Results

2.6.1. Trends in woody density, size classes and composition

Trends show that the total tree density (all species and size-classes; Fig. 2.1) increased over time from stage 1 (3014 ha^{-1}) to stage 2 (3722 ha^{-1}) and then stage 3- (4372 ha^{-1}).

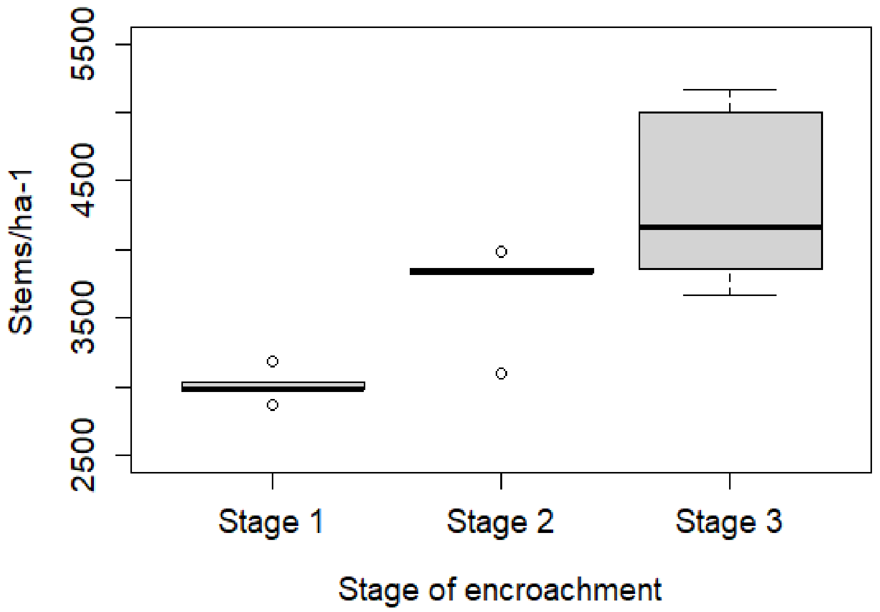

Figure 2.1: Total tree density of all species (all size-classes combined) for each stage of encroachment. Encroachment is classified into stage 1 (0-19% cover), stage 2 (20-39% cover) and stage 3 (40-100% cover), and N = 5 sites per class.

Size-class distributions and density of the six most commonly occurring species throughout the encroachment sequence are shown in Fig. 2.2. *Vachellia karroo* is by far the most commonly occurring and abundant species throughout the encroachment sequence. Both early and intermediate successional stages had *V. karroo* size distributions with many young individuals (size-classes 1 and 2), indicating invasion and recruitment. Intermediate sites had *V. karroo* size-classes becoming dominated by larger individuals, while populations of pioneer species (*S. myrtina*, *G. buxifolia, O. europaea, S. longispina* and *Z. mucronata*) start to increase in number with a high proportion of young individuals (size-classes 1 and 2). The oldest sites were dominated by large individuals of thicket species, some large *V. karroo,* but little evidence of *V. karroo* recruitment (few individuals in size-classes 1 and 2). *Scutia myrtina*, *G. buxifolia, O. europaea, S. longispina* and *Z. mucronata* all show similar patterns, starting to recruit in stage 1 and increasing slightly in stage 2, with a significant increase in recruitment at stage 3 (coinciding with patterns of large *V. karroo* density).

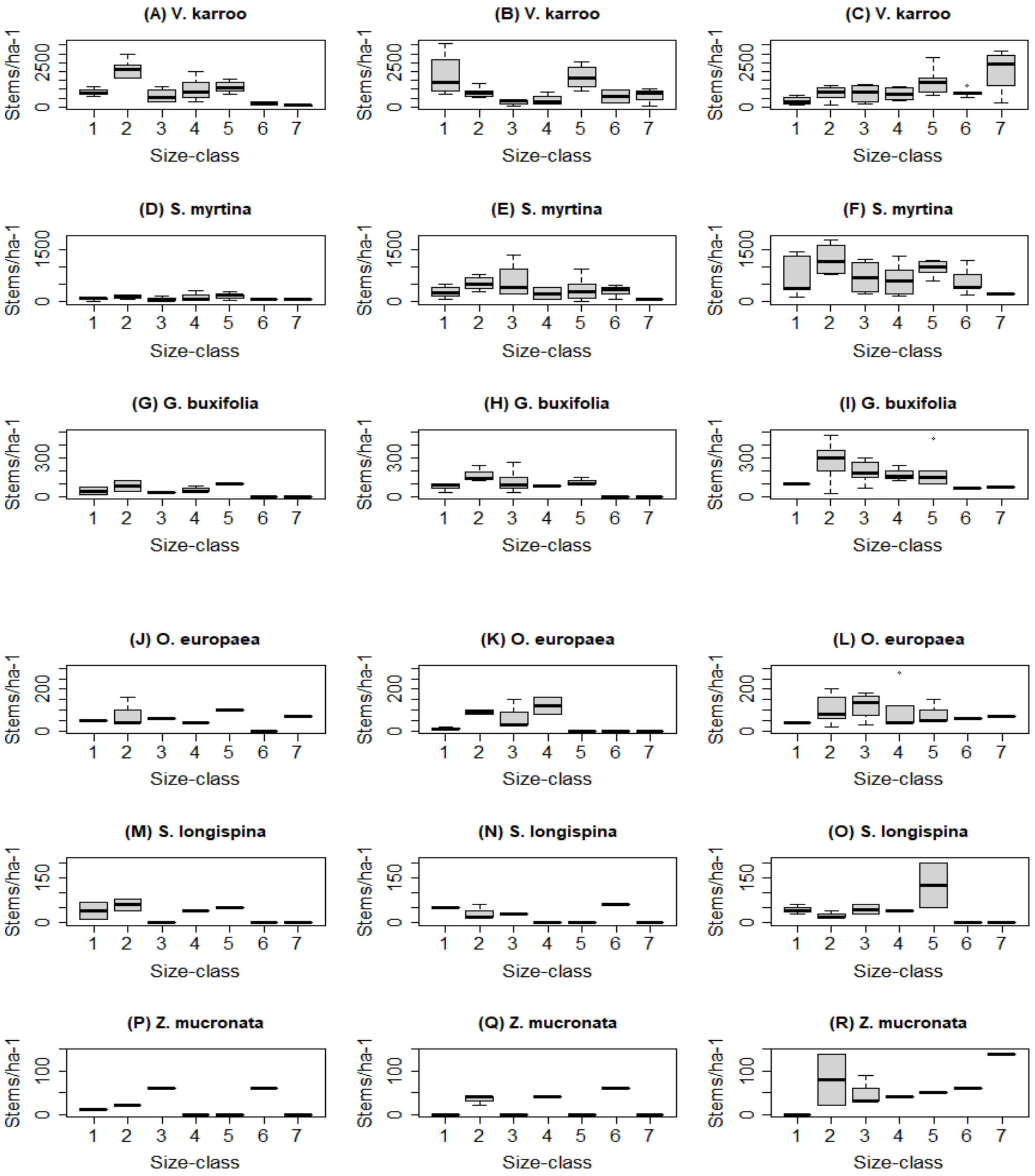

Figure 2.2: Density and size class distribution of the six most commonly occurring species found in the field (*Vachellia karroo*, *Scutia myrtina*, *Gymnosporia buxifolia, Olea europaea, Searsia longispina* and *Ziziphus mucronata*). Panels in columns 1 to 3 represent encroachment stages 1 to 3, respectively. The encroachment sequence is classified into stage 1: 0-19%, stage 2: 20-39% and stage 3: 40-100% woody canopy cover. Size-class categories were classified by height: 1 (< 0.5 m), 2 (> 0.5 to 1 m), 3 (> 1 to 1.5 m), 4 (> 1.5 m to 2 m), 5 (> 2 to 3 m), 6 (> 3 to 4 m) and 7 (> 4 m).

2.6.2. Species-size class associations across the successional gradient

The ‘APRIORI’ algorithm mined a total of 12 association rules for the entire encroachment sequence, with a minimum support of 0.01 and minimum confidence of 0.86. All 12 rules had quality measures of confidence > 0.85, support < 0.06, lift > 4.7 and coverage < 0.07.

All LHS associations (*S. myrtina* in size classes 1-4) point to RHS associations with *V. karroo*, indicating that when *S. myrtina* occurred, there was usually a large (> 2 m high) *V. karroo* present in the same clump. This is consistent with the hypothesis that established *V. karroo* provide a nucleation site for thicket clump formation.

Visualization of association rules using vertices and edges, showed that *V. karroo* was integral to all 12 association rules (Fig. 2.3). The strongest rule (rule1) associates Scutia3 (*S. myrtina*, 1 to 2 m) on the LHS with Acacia4 (*V. karroo*, 2 to > 4 m) on the RHS (confidence = 0.86, coverage = 0.04, lift = 4.75). The second strongest rule (rule2) associates Scutia4 (*S. myrtina*, 2 to > 4 m; LHS), with Acacia4 (*V. karroo*, 2 to > 4 m; RHS; confidence = 0.87, coverage = 0.05, lift = 4.8). All 12 rules (see detailed lists of associated size-classes and statistics in Appendix A, Table S1) show that *S. myrtina* of different size-classes are found in association with a large (> 2m high) *V. karroo*.

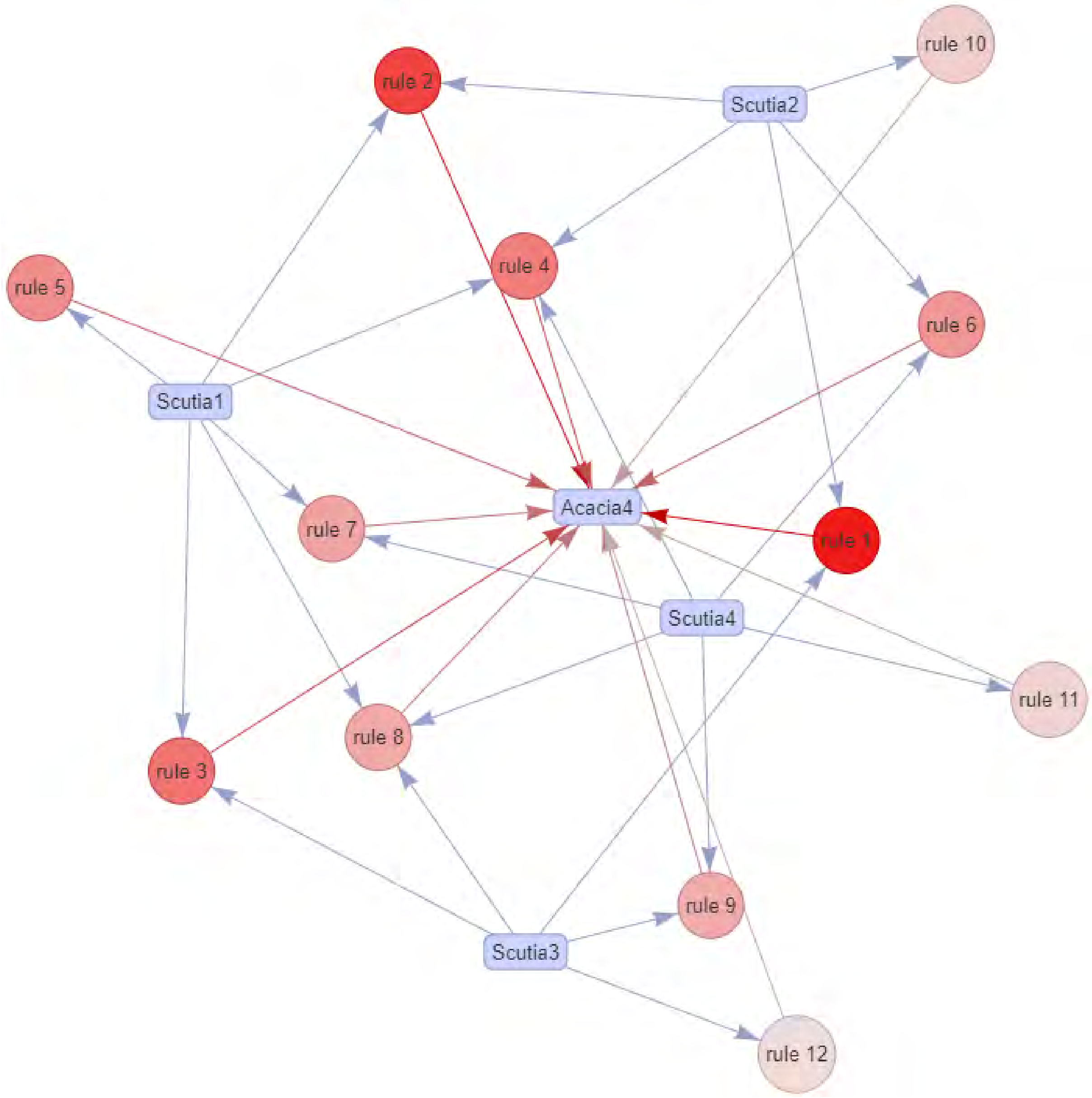

Figure 2.3: Visualization of all 12 association rules between species for the entire encroachment sequence according to the highest lift values. Vertices are labelled with species-size classes (rectangles) and rules are represented as a second set of vertices (circles), while arrows point to the direction of co-occurrence, from the LHS to the RHS species/size class. Size and colour of circles represent confidence of measures: larger circles represent higher support, while darker colours have higher lift. Species returned by the analysis only included Acacia (*Vachellia karroo*), and Scutia (*Scutia myrtina*). All rules have LHS = *S. myrtina,* with RHS pointing to *V. karroo* association. Size-classes classified as 1 (< 0.5 m), 2 (> 0.5 to 1 m), 3 (> 1 to 2 m) or 4 (> 2 to > 4 m). Table S1 shows all rules and their associated statistics.

The algorithm mined a total of 125 association rules for stage 1 (early encroachment), with a minimum support of 0.01 and minimum confidence of 0.8. All 125 rules have quality measures of confidence = 1 (except for rules 11 and 12), lift > 1 and coverage > 0.01. All LHS associations (*S. myrtina*, *G. buxifolia, O. europaea,* and *S. longispina*) point to RHS association with *V. karroo* size-classes 1-4.

Visualization of association rules using vertices and edges, showed that *V. karroo* was integral to all 125 association rules (see Table S2 for details of rules and Fig. 2.4 for the 12 strongest rules according to lift). The strongest rule (rule1) associates Olive3 (*O. europaea*, 1 to 2 m; LHS) with Acacia3 (*V. karroo*, 1 to 2 m; RHS; confidence = 1, coverage = 0.01, lift 2.4). In all 125 rules, an individual *V. karroo* of varying size class had to be present for individual *S. myrtina*, *G. buxifolia, O. europaea,* and *S. longispina* of all size classes to be found.

The algorithm mined a total of 260 association rules for stage 2 (intermediate encroachment), with a minimum support of 0.01 and minimum confidence of 0.8. All 260 rules have quality measures of confidence > 0.9, lift ≥ 1 and coverage of ≥ 0.01.

All LHS associations (*S. myrtina*, *G. buxifolia, O. europaea,* and *S. longispina*) point to RHS association with both *V. karroo* size-classes 1-4 and *S. myrtina* size-classes 1-4. This points to both *V. karroo* and *S. myrtina* being key to clump formation or expansion at this intermediate stage.

Visualization of association rules using vertices and edges, showed that *V. karroo* was integral to all 260 association rules, with both *V. karroo* and *S. myrtina* combinations being integral to association rules (see Table S3 for details of rules and Fig. 2.5 for the 15 strongest rules according to lift).

The algorithm mined a total of 12094 association rules for stage 3 (high encroachment), with a minimum support of 0.01 and minimum confidence of 0.8. All 12094 rules have quality measures of confidence > 0.8, lift > 1.6 and coverage ≥ 0.02.

The LHS associations (*S. myrtina*, *G. buxifolia, O. europaea, S. longispina* and *Z. mucronata* in various size classes), point to RHS association rules with *V. karroo* size-classes 1-4, *S. myrtina* size-classes 1-4, *G. buxifolia* size-classes 2-4 and *O. europaea* size-class 1 and 2. This indicates that at the advanced stage of encroachment, associations become more diverse (see Table S4 for details of rules and Fig. 2.6 for the 12 strongest rules according to lift).

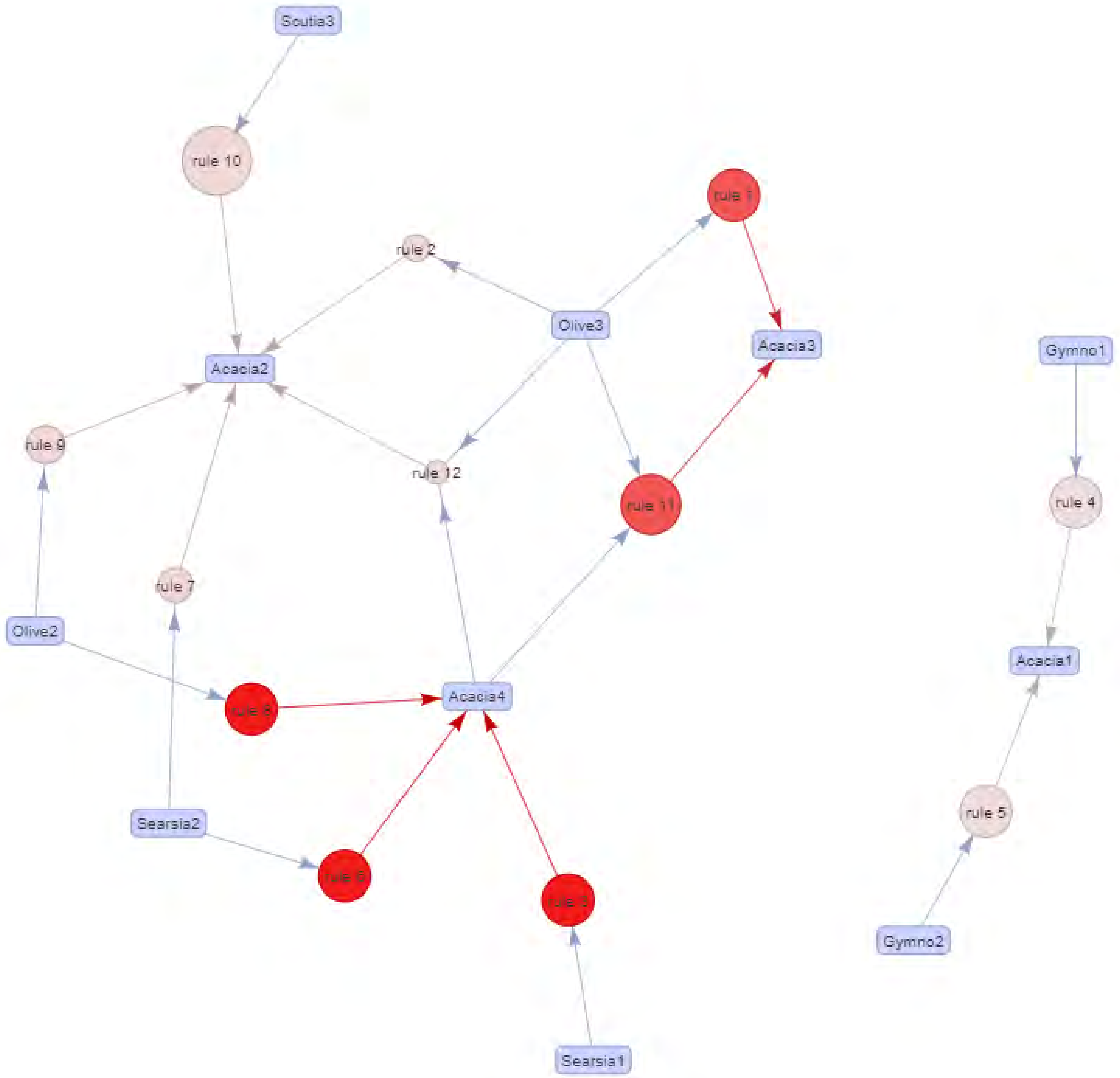

Figure 2.4: Visualization of the top 12 of 125 association rules between species for Stage 1 (early encroachment) according to the highest lift values. Vertices are labelled with species-size classes (rectangles) and rules are represented as a second set of vertices (circles), while arrows point to the direction of co-occurrence, considering the reference species. Size and colour of circles represent confidence of measures: larger circles represent higher support, while darker colours have higher lift. Species returned by the analysis included Acacia (*Vachellia karroo*), Scutia (*Scutia myrtina*), Gymno (*Gymnosporia buxifolia*), Olive (*Olea europaea* ssp. *africana*) and Searsia (*Searsia longispina*). All rules have LHS = *S. myrtina*, *G. buxifolia, O. europaea,* and/or *S. longispina* with RHS pointing to association with *V. karroo* size-classes 1-4. Size-classes classified as 1 (< 0.5 m), 2 (> 0.5 to 1 m), 3 (> 1 to 2 m) or 4 (> 2 to > 4 m). Table S2 shows top 12 rules and their associated statistics.

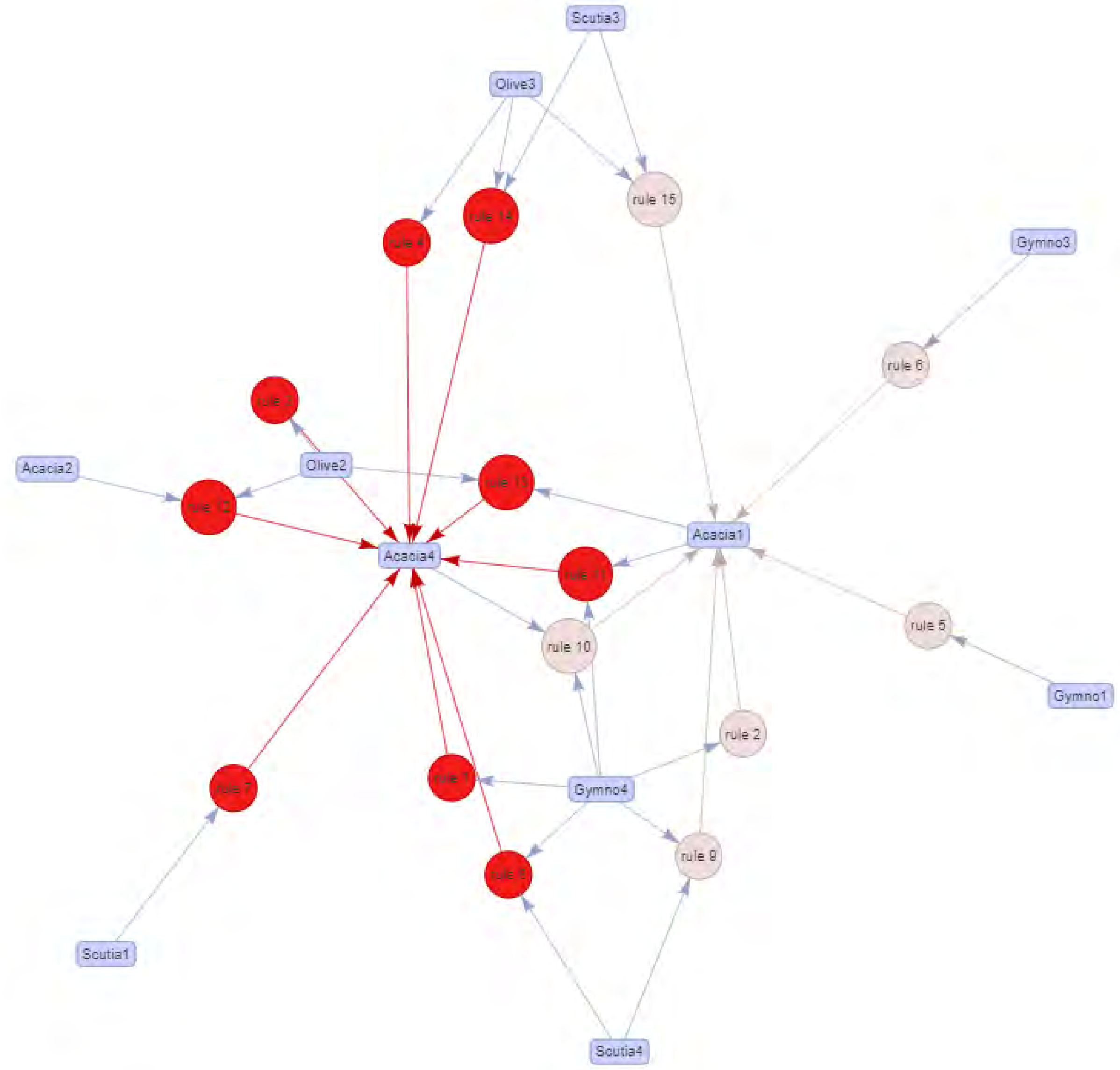

Figure 2.5: Visualization of the top 15 of 260 association rules between species for stage 2 (intermediate encroachment) according to the highest lift values. Vertices are labelled with species-size classes (rectangles) and rules are represented as a second set of vertices (circles), while arrows point to the direction of co-occurrence, considering the reference species. Size and colour of circles represent confidence of measures: larger circles represent higher support, while darker colours have higher lift. Species returned by the analysis included Acacia (*Vachellia karroo*), Scutia (*Scutia myrtina*), Gymno (*Gymnosporia buxifolia*), Olive (*Olea europaea* ssp. *africana*) and Searsia (*Searsia longispina*). All rules have LHS = *S. myrtina*, *G. buxifolia, O. europaea,* and *S. longispina* with RHS pointing to association with both *V. karroo* size-classes 1-4 and *S. myrtina* size-classes 1-4. Size-classes classified as 1 (< 0.5 m), 2 (> 0.5 to 1 m), 3 (> 1 to 2 m) or 4 (> 2 to > 4 m). Table S3 shows the top 15 rules and their associated statistics.

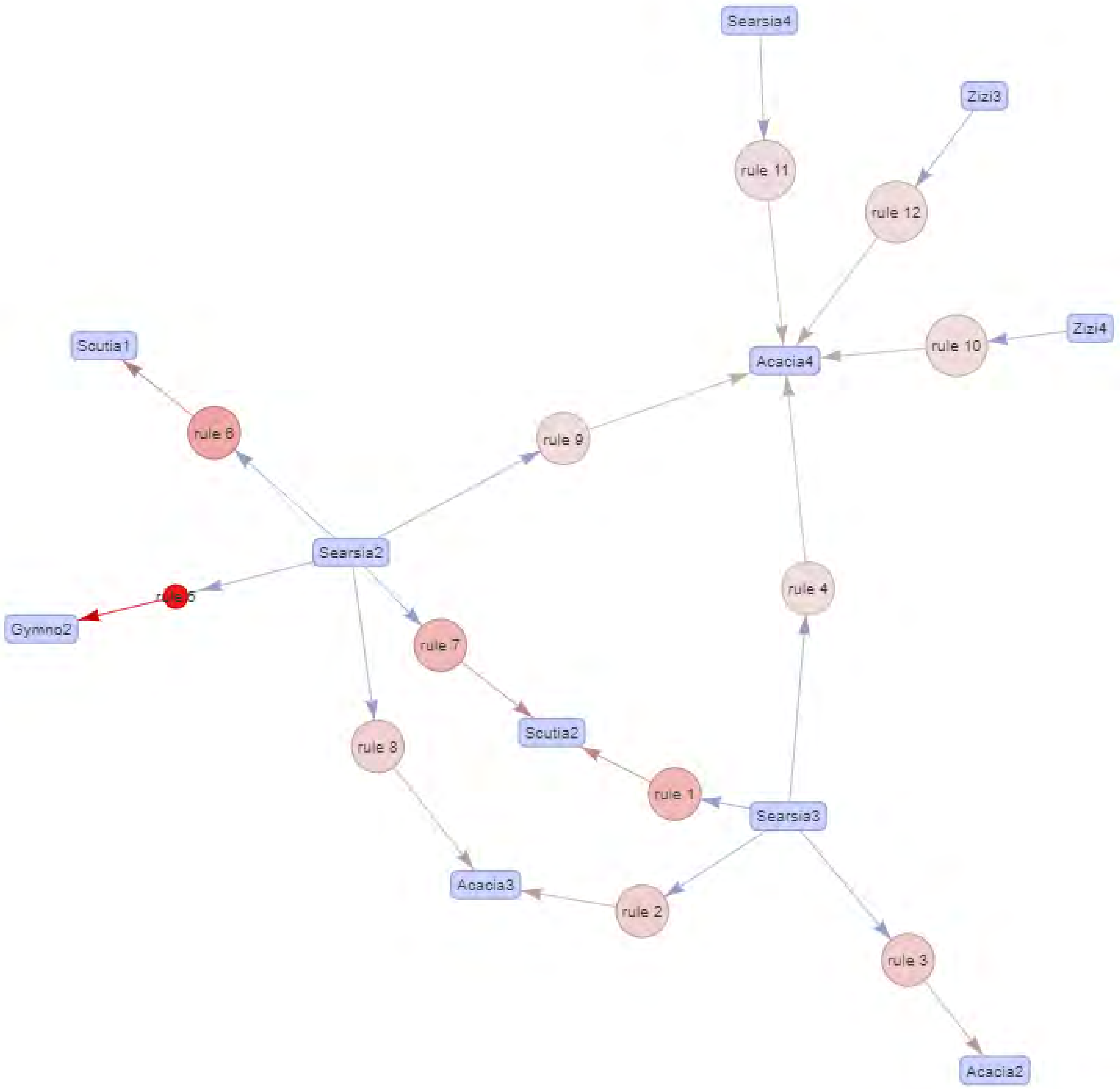

Figure 2.6: Visualization of the top 12 of 12094 association rules between species for stage 3 (high encroachment) according to the highest lift values. Vertices are labelled with species-size classes (rectangles) and rules are represented as a second set of vertices (circles), while arrows point to the direction of co-occurrence, considering the reference species. Size and colour of circles represent confidence of measures: larger circles represent higher support, while darker colours have higher lift. Species returned by the analysis included Acacia (*Vachellia karroo*), Scutia (*Scutia myrtina*), Gymno (*Gymnosporia buxifolia*), Olive (*Olea europaea* ssp. *africana*), Searsia (*Searsia longispina*) and Zizi (*Ziziphus mucronata*). All rules have LHS = *S. myrtina*, *G. buxifolia, O. europaea, S. longispina* and *Z. mucronata*), with RHS pointing to association with *V. karroo* size-classes 1-4, *S. myrtina* size-classes 1-4, *G. buxifolia* size-classes 2-4 and *O. europaea* size-class 1 and 2. Size-classes classified as 1 (< 0.5 m), 2 (> 0.5 to 1 m), 3 (> 1 to 2 m) or 4 (> 2 to > 4 m). Table S4 shows all rules and their associated statistics.

2.7. Discussion

The hypotheses regarding changes in density, composition and size-classes of the common woody species to infer a successional sequence were supported by the data. Sites representing early stages of encroachment were dominated by *V. karroo* with very few thicket pioneer individuals. As expected, the longer a site had been encroached, the greater the number of individuals with a greater diversity of thicket species and increased size-classes. The overall trend of increasing stem density, larger individuals and greater woody species diversity are similar to those reported by Khoza (2021). These findings are similar to other studies with broad-leaved thicket species increasing in density to form bush clumps around species that initially encroach savanna (O'Connor & Chamane, 2012; O'Connor & Crow, 1999; Parr *et al*., 2012). Similar succession patterns of bush clump formation and encroachment have also been documented by Jamison-Daniels *et al*. (2021), O'Connor & Chamane (2012) and Archer, (1994), but they do not consider the abiotic properties of species microsites.

The hypothesis regarding changing species and size-class associations along the encroachment sequence were also supported by the association rule analysis (ARA). In the youngest sites (stage 1), clumps consisted of few individuals, mainly *V. karroo* that occur on their own (no *V. karroo* to other *V. karroo* associations). In the earliest succession stage, *S. myrtina*, *G. buxifolia, O. europaea,* and *S. longispina* were significantly associated with *V. karroo* in order to establish and be present for thicket clump formation to take place. As expected, intermediate sites (stage 2) associations between early thicket pioneers in small size-classes with large *V. karroo* increase. ARA showed in stage 2 that *S. myrtina*, *G. buxifolia, O. europaea,* and *S. longispina* all had to have an association with either *V. karroo* or *S. myrtina* in order to establish and form bush clumps. In the sites with the most advanced encroachment (stage 3), clumps are more diverse and have more associated individuals that involve both large *V. karroo* and bigger thicket pioneers, with some clumps consisting of only thicket species. ARA showed in stage 3 that *S. myrtina*, *G. buxifolia, O. europaea, S. longispina* and *Z. mucronata* could have an association with *V. karroo*, *S. myrtina*, *G. buxifolia* or *O. europaea* in order to establish and contribute to bush clump amalgamation. Similar patterns of bush clump formation are also shown by Jamison-Daniels *et al*. (2021) in a mesic savanna in South Africa.

The utility of the ARA for my approach to sampling the clumps was useful, being relatively easy and considerably less time-consuming compared to the methods used by Archer, (1994), Jamison-Daniels *et al*. (2021) and O'Connor & Chamane (2012), as well as analysing associations. ARA has proven to be a novel, relevant and time efficient method for assessing the successional sequence and association patterns. These methodologies would be useful for comparing and analysing many different forms of bush succession and the clump formation process at different regions and under different conditions.

2.8. Conclusion

Results support the hypothesis in that *V. karroo* invades first and provides nucleation sites for thicket clumps with *S. myrtina* being the earliest and most abundant pioneer. Pioneers are always (or at least in all significant rules) associated with bigger acacias except in the later stages when thicket species start associating with each other. This makes these two species of most interest both from an ecological and management point of view. Thus, these two species were selected for closer investigation of the interactive mechanisms and outcomes in Chapter 3 (focus on nutrients) and 4 (focus on available water).

CHAPTER 3: Inter-specific interactions affect soil and foliar nutrients in woody plants facilitation -and competition at different stages of encroachment

3.1. Abstract

Vachellia karroo is known to act as an initiator of bush clump formation, however the mechanisms which promote preferential recruitment and association with this savanna woody species remain unclear. The main objectives of this study was to examine the effects of pairwise tree interactions between *V. karroo* (a common encroacher) and *Scutia myrtina* (a common thicket pioneer), on soil and leaf nutrient content. This was carried out by determining differences between inter-canopy and sub-canopy soil nutrient content, and the effects of various pair-size combinations and individual controls on plant leaf nutrients. Data was collected on Endwell farm, located in the Smaldeel region of the Eastern Cape, South Africa. Plots (150 m x 150 m) that covered areas with combinations of the co-occurring species were chosen using digital aerial photographs. Pair-size combinations and single control individuals were sampled along two 620 m belt transects (width of 100 m) running parallel to one another within the chosen plots. Results confirmed that pair-size tree interactions affected both soil nutrient and leaf nutrient content. All individuals increased soil K, N and organic C in the sub-canopy. Association with *Vachellia karroo* increased *S. myrtina* foliar N, P and K, while association with *S. myrtina* lowered *V. karroo* foliar N, P and K. Small *S. myrtina* individuals were found to be most facilitated by establishing and growing up next to a large *V. karroo* individual, suggesting that nutrient enrichment may promote the association between savanna trees and thicket pioneers.

Keywords: Leaf nutrient content; Nurse plant; Pair-size combinations; Soil nutrient content; Sub-canopy vs. inter-canopy.

3.2. Introduction

Nutrients are essential for growth and persistence, and are a determinant of vegetation structure, composition and productivity (Smit, 2004). There is a large body of evidence for the process of nutrient enrichment under tree canopies, an important mechanism in areas with nutrient poor soils (Hagos, 2001). Ward *et al.* (2018) showed that soil under savanna trees is nutrient-enriched via positive feedbacks. This creates an 'island of fertility' effect indicated by higher carbon and nitrogen concentrations, with concentrations usually relating to tree size rather than species. De Boever *et al.* (2015) found that scattered trees can function as fertile islands in arid and semiarid environments, increasing organic matter and nutrients in the upper soil layers (first 20 cm) within the canopy radius. Organic matter and total nitrogen (N) increase with tree age below the canopy reaching a maximum threshold after about 75 years (De Boever *et al.*, 2015). Nutrients added by trees to sub-canopy zones in the form of tree litter or animal droppings can also increase understory productivity by fertilizing nutrient limited soils (Belsky, 1994). In an East African savanna, Ludwig *et al.* (2004) showed soil nutrient availability increased with both tree age and size, with nutrients being lowest in open grassland and highest under dead trees. They also found that productivity in open grassland was limited by N, while under trees, the limiting nutrient changed gradually from N-limited to P-limited (Ludwig *et al.*, 2004).

With woody plant encroachment of savanna or grassland, there is an increase in plant-bound carbon due to increasing woodiness of the vegetation (Ward *et al.*, 2018; Weintraub & Schimel, 2005). There are a number of studies showing that large shrubs or trees have much larger effects on soils than smaller shrubs or trees due to greater biomass inputs to the soil (Throop & Archer, 2008; Wiegand *et al.*, 2005). However, it has still not been resolved whether the effect of plant size outweighs that of functional traits such as nitrogen-fixation (Ward *et al.*, 2018). Furthermore, it is unknown if savanna trees create islands of fertility for thicket pioneers that contribute to the formation of closed-canopy thicket clumps.

Several measures of soil and plant fertility can be utilised to understand the effect of large trees on the sub-canopy microclimate. The carbon to nitrogen ratio is relevant to consider in ecological research, and is also an important factor for soil decomposers and is commonly used as an index for decomposition in plant litter and soil (Bell *et al.*, 2014; Manzoni *et al.*, 2008; Ward *et al.*, 2018). Differences in soil carbon to nitrogen ratios can also cause differences in the availability of phosphorus between different species and vegetation types, however the mechanisms are still unclear (Sugihara *et al.*, 2014). The balance and availability of nitrogen and phosphorus can also limit plant growth in almost all terrestrial ecosystems, and variations in the relative abundance as reflected by nitrogen to phosphorus ratios of plant biomass and soil can influence species interactions, vegetation composition and functioning (Güsewell, 2004; Sinclair & Park, 1993). In a study of grasslands, it was found that nitrogen fertilization mainly promoted species with high nitrogen and high nitrogen to phosphorous ratios (except for legumes), whereas phosphorus fertilization mainly promoted the species with the highest phosphorous and lowest nitrogen to phosphorous ratio (Mamolos *et al*., 1995). Legumes that form symbiotic association with nitrogen fixing bacteria are usually favoured by a high availability of phosphorus and low availability of nitrogen (Vitousek & Field, 1999). Nitrogen-fixers on their own also often have higher nitrogen (N: P) ratios than their co-occurring non-fixer species (Güsewell, 2003, 2004).

Research is still limited on the effects of trees on other soil physiochemical properties, especially for minerals such as potassium (K) that are responsible for regulating primary production and even vegetation succession (Sugihara *et al*., 2014). Some studies have found that co-occurring shrubs do not modify soil organic matter, nitrogen and phosphorous, or water content, but they significantly increased the amount of available potassium which has been shown to improve the water-use efficiency of many species under dry conditions (Gómez *et al*., 2005; Sugihara *et al*., 2014). Potassium has also been shown to increase plant resistance to drought, as this is mostly due to osmotic adjustments and reduction in transpiration rates (Egilla *et al*., 2001; Gómez *et al*., 2005). Despite potassium's potential benefit on water-use efficiency, this nutrient has seldom been highlighted when looking for facilitation mechanisms by nurse plants, as the majority of studies focus on the modification of soil nitrogen or phosphorous as the main nutrients through which facilitation occurred (Carrillo-García *et al*., 2000; Gómez *et al*., 2005). Gómez *et al*. (2005) highlight that an increase in soil potassium is an overlooked but relevant soil effect of facilitation in systems where drought is the limiting factor for plant survival.

Vachellia karroo is a commonly occurring savanna woody species that can fix nitrogen, leading to changes in patterns of nutrient cycling, thereby allowing other plants to utilize the higher nitrogen resources found below the canopy (Aubrey & Reynolds, 2002; Kraaij & Ward, 2006; O'Connor & Crow, 1999; Pugnaire *et al*., 1996). O'Connor & Chamane (2012) and Jamison-Daniels *et al*. (2021) both identify *V. karroo* as a nucleator species for the formation of bush clumps. It has also been shown that *V. karroo* nutrient enrichment mostly includes increased potassium and nitrogen under crowns (Moyo *et al*., 1998). Evidence for increased nutrient status usually focuses on the total percentage of nitrogen, organic carbon and exchangeable cations such as Ca, K, Mg and Na (Hagos, 2001). The aim of this study was to examine soil nutrient content in the sub-canopy vs inter-canopy zones, and leaf nutrients of plants growing in different pair-size combinations and as individual controls. This was done to determine if there are nurse plant effects between the two focus species, *Vachellia karroo* and *Scutia myrtina* (a commonly occurring pioneer species). The objectives were to examine the effects of the interactions between *S. myrtina* and *V. karroo* of different size combinations and control individuals to test facilitation-competition interactions, outlined in detail below (see Fig. 3.1).

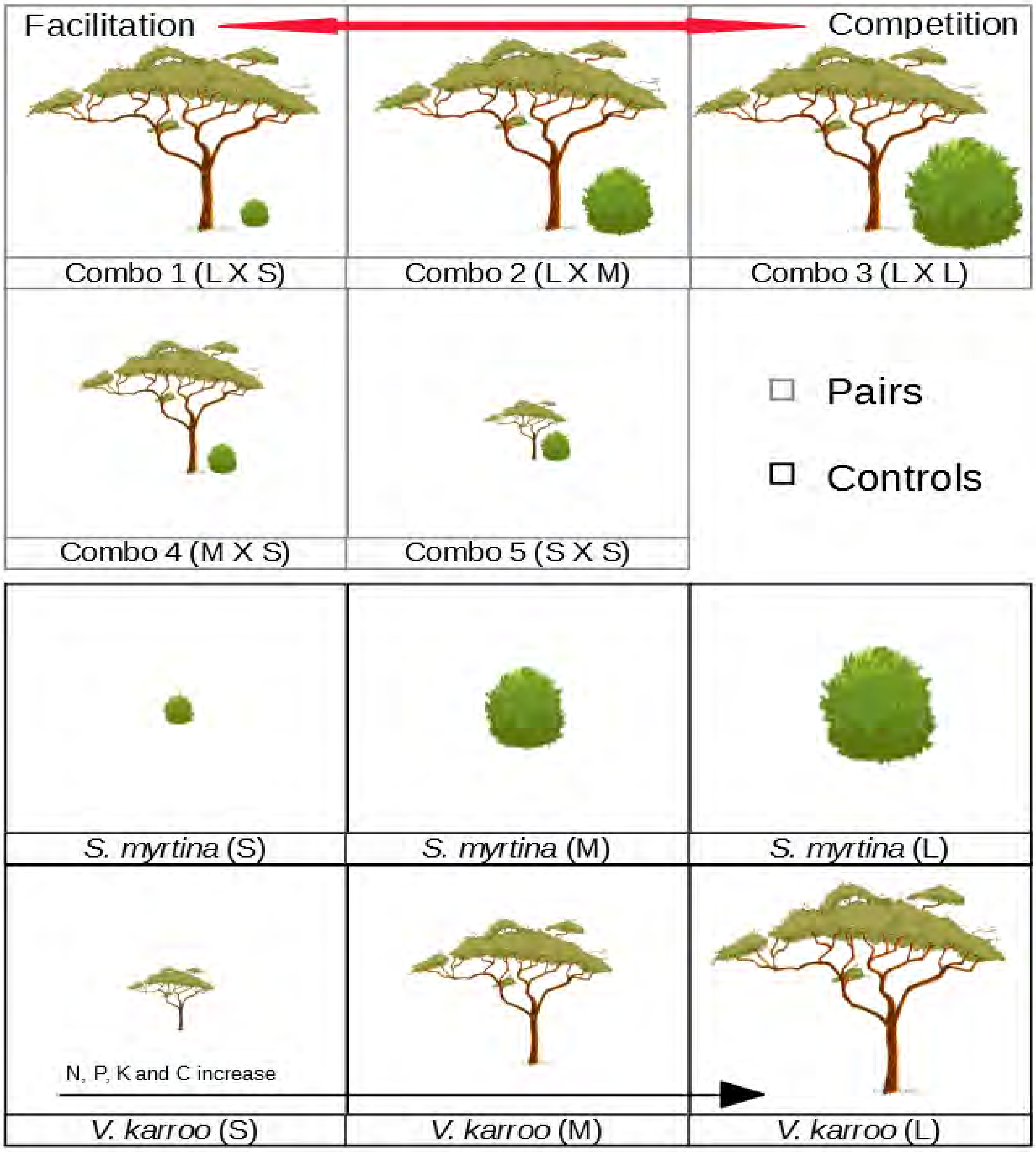

Figure 3.1: Species pair-size combinations (top 1-5), controls (bottom 1-6) and the most important predicted nutrient trends for soil and foliar nutrients (N, C, P and K); between pair-combinations and control individuals. The red arrow (top) indicates a shift from facilitation to competition. The black arrow (bottom) indicates predicted nutrient increases from left to right. Letters in parentheses show: L = large, M = medium and S = small size-classes.

3.2.1. Soil nutrient content (inter- vs. sub-canopy)

I expected soil nutrients such as N, P, K and C to increase as plants increase in size, and to be higher in the sub-canopy compared to inter-canopy zone. This may be due to organic litter inputs and other biophysical processes taking place under tree canopies over time, such as N-fixation. I also expected that soil nutrients under the canopy of large *V. karroo* would be the same irrespective of whether it was growing with a small *S. myrtina* or not, due to minimal effects of small shrubs on larger trees. Combinations of large *V. karroo* and large *S. myrtina* are expected to compete and therefore have less soil nutrients, decreased N, P, and K compared to large control individuals growing alone.

3.2.2. Soil nutrient ratios

I expected an increase in the soil C:N ratio throughout the encroachment process, with large-large combinations having a higher C:N than small-small combinations (Weintraub & Schimel, 2005; Ward *et al.*, 2018). I expected nitrogen limitation (low N:P) during early succession (small-small combinations), shifting to phosphorous limitation (high N:P) during late successional stages (large-large combinations) similar to Mamolos *et al.* (1995) and Vitousek & Field (1999).

3.2.3. Soil nutrient content RII (inter- vs. sub-canopy)

Both *S. myrtina* and *V. karroo* size are expected to have a significant effect on the magnitude of the difference in soil nutrients (measured as the Relative Interaction Intensity - RII) in the sub- vs. inter-canopy. It is expected that the RII of large-large combinations will be more positive, indicating that sub-canopy > inter-canopy nutrient content, compared to small-small combinations. RII will be more positive as individuals increase in size, due to higher probability of large individuals acting as nurse plants.

3.2.4. Leaf nutrient content

I expected foliar nutrients such as N, P and K to increase as *V. karroo* plants increase in size due to N-fixation and the island of fertility effects around *V. karroo* individuals. Combinations of large *V. karroo* and small *S. myrtina* that occur during early succession are expected to have little to no effect on *V. karroo* foliar nutrients but a beneficial effect on the *S. myrtina* (higher N, P and K compared to small control individuals), due to the above-mentioned effects of *V. karroo*. However, combinations of large *V. karroo* and large *S. myrtina* occurring during late succession are expected to have a competitive negative effect on both species but a more strongly negative impact on the *V. karroo* (decreased N, P and K compared to large control individuals).

3.3. Methods

The field site, Endwell farm (32˚44'55"S, 26˚27'19"E) is located in the Smaldeel region of the Eastern Cape, South Africa. Historically the land has been used for cattle farming and does include low numbers of game. Endwell has excluded small livestock such as sheep and goats for more than 25 years, and since 1966, fire and other forms of large-scale bush control have been excluded. Vegetation is classified to be a mixture of Bedford Dry Grassland and Bisho Thornveld (Mucina & Rutherford, 2006), characterized by a grass layer with varying densities of acacia dotted with clumps of broad-leaved woody species that are similar to Albany thicket (Mucina & Rutherford, 2006). The most predominant soil types in this area include poorly drained Glenrosa, Trevanian and Mispah (Martens & Morris, 1994) containing a mixture of clay, sand and loam (Schulze, 2008).

3.3.1. Site and pair selection

Fifty potential plots had been identified using digital aerial photographs dating back to 1949 that were used to determine the percentage cover change for each plot. I chose two plots (150 m x 150 m), located along the same slope to control for altitude and edaphic factors. The plots were close to a road, and represented differences in the percentage cover change from large-large to small-small combinations of focal species (from 0- 19% to 60- 100% cover). Thus these two plots covered areas with combinations of co-occurring species that also corresponded to an increase in woody cover. Species pair-size combinations were then chosen along two 620 m x 100 m belt transects, parallel to one another, on each side of the road running through the chosen plots (see Fig. 3.2).

Figure 3.2: Study site. Blocks represent two 620 m x 100 m belt transects, on each side of a road. Transects are not drawn to scale.

Pair- associations utilized size-class combination pairs that were based on the commonly observed associations in the field (chapter 2), these included small (< 0.5 to 1 m), medium (> 2 to 3 m) and large (> 3 m) size-classes for both focus species to get an idea where the positive and negative interspecific effects for nutrients were greatest. Species pair-size combinations (see Fig. 3.1) included five different combinations, *V. karroo* (large) with *S. myrtina* (small, medium and large), representing *S. myrtina* establishing and growing up next to a large *V. karroo* individual. Also, pair-size combinations of *S. myrtina* (small) with *V. karroo* (small and medium) are included, representing *S. myrtina* establishing in association with other size-classes of *V. karroo*. This allowed an examination of the effects of large *V. karroo* on nutrient status of different size classes of *S. myrtina*, while small *S. myrtina* with each size-class of *V. karroo* shows us at what size *V. karroo* affects nutrient status. The controls consisted of single individuals of each species in the three target size-classes (small, medium and large).

When choosing species pairs for each pair-replication, all pioneer individuals had a canopy overlap of 100% with the nurse plant. When selecting species pairs, I avoided the presence of other individuals (of the same and different species) within a 2 m radius of each focal individual's canopy in order to eliminate the effects of other neighbouring individuals. Smaller non-focal plants < 50 cm were ignored as species pairs completely by themselves were hard to find. As each pair or single control individual was identified, their species ID and GPS coordinates were taken, followed by tagging with an aluminium tag of unique ID to each individual with wire and marked with spray-paint on the stem. The individual or individuals then had their height and canopy diameter (along two perpendicular axes) recorded in cm.

3.3.2. Soil nutrient sampling and analyses

Soil sampling included first removing the leaf litter or surface debris. Samples were then taken using a hand auger to get a sample of the first 10 cm of soil from three locations under the canopy, within 30-50 cm from the base of the focal plant (in a triangle around the stem). The three sub-samples were pooled in a brown paper sample bag. Two soil bags (each comprised of three pooled sub-samples) were taken per pair or individual, one bag (three sub-samples) under the canopy and another (three sub-samples) in the inter-canopy. Soil samples in the inter-canopy were taken outside the zone of influence, which is defined as half the canopy diameter + 1 m from the edge of the canopy.

Soil fertility was analysed on a volume rather than a mass basis to enable the conversion of the results to a mass basis (Manson & Roberts, 2000). All samples were first oven-dried then crushed and passed through a 1 mm sieve. All material that was coarser than 1 mm such as stones was discarded (Manson & Roberts, 2000).

Total C and N were analysed by the automated Dumas dry combustion method using a LECO CNS 2000 (Leco Corporation, Michigan, USA; Matejovic, 1996). This method involved weighing samples into a ceramic crucible to which 0.5 g of vanadium pentoxide was added as a combustion catalyst. The crucible was then introduced into a horizontal furnace and burned in a stream of oxygen at 1350˚C. The gases produced were passed through two infra-red cells where the carbon (as CO_2), and nitrogen were determined (as N_2) in the thermal conductivity cell (Manson & Roberts, 2000). To measure the total extractable phosphorus, the Ambic-2 extracting solution was used. It consisted of 0.25 M NH_4CO_3 + 0.01 M Na_2EDTA + 0.01 M NH_4F + 0.05 g L-1 Superfloc (N100), adjusted to pH 8 with a concentrated ammonia solution. 25 ml of this solution was added to 2.5 ml soil, and the suspension was stirred at 400 r.p.m. for 10 min using a multiple stirrer. The extracts were filtered using Whatman No. 1 paper. Phosphorus was determined on a 2 ml aliquot of filtrate using a modification of the Murphy & Riley (1962) molybdenum blue procedure (Hunter, 1974; Manson & Roberts, 2000). Ca, Mg, Zinc, Cu and Mn were then determined by atomic absorption. Soil nutrient analysis focussed on C, N, P and K.

3.3.3. Leaf nutrient sampling and analyses

Leaf samples for measurement of nutrients were taken from leaves of similar size from branch tips all over the focus individual and pooled to reduce sample variation. This consisted of one small paper bag filled with leaves from each focus plant in the pair-combinations and single control individuals of each size-class. Leaf material was dried and milled to pass through a 0.84 mm sieve. Sub-samples were then dry-ashed overnight and taken up in 1 M HCl. The P concentration was determined colorimetrically by the same method used for soil extracts, while K, Na, Ca, Mg, Cu, Mn, Fe, Al and Zn were determined by atomic absorption (Manson & Roberts, 2000). Nitrogen was determined before ashing, using near-infrared reflectance (Eckard *et al*., 1988), and by the automated Dumas dry combustion method using a LECO CNS 2000 (Leco Corporation, Michigan, USA; Matejovic, 1996; Manson & Roberts, 2000).

All soil and leaf samples were analysed by the KwaZulu-Natal Department of Agriculture and Rural Development, which performs analyses on soil, plant tissues and water samples (Manson & Roberts, 2000). This particular laboratory performs the analyses by using the rapid procedures described by Hunter (1975) and Farina (1981). Results for foliar leaf analysis focus on N, P and K.

3.4. Data analysis

To quantify the direction and size of the effect of interspecific interactions on soil nutrients the Relative Interaction Intensity (RII) index was calculated (Armas *et al*., 2004), using inter-canopy vs. sub-canopy soil nutrients to show the relative interaction effects (similar to Liua *et al*., 2020). Here X = is either C, N, P or K.

$\text{RII} = (X_1 – X_2) / (X_1 + X_2)$, where

X_1 represents the soil nutrients in the sub-canopy, and X_2 represents soil nutrients in the inter-canopy (similar to Fagundes *et al*., 2018).

In this study, the response variables are not a measure of plant performance, rather a soil characteristic, thus interpretation of RII values are that when RII > 0, then the nutrient in question is greater in the sub-canopy than the inter-canopy.

Analysis of data was carried out using the statistical software R v.1.0.136 (R Core Team, 2020). Shapiro-Wilks normality test (package "stats", functions: "shapiro.test") was done for each of the soil nutrient predictor variables (P, K, Ca, Mg, Zn, Mn, Cu, C, N) to determine if the data was normally distributed. Two-sample paired *t*-tests were performed (package "stats", functions: "t-test") to visualise differences in each nutrient variable and determine if there are general differences in soil fertility between inter-canopy and sub-canopy zones.

C:N and N:P ratios were calculated using sub-canopy soil data and all mass (%) concentrations were converted to a volume basis (mg/L). Linear models (package "stats", functions: "lm") were then performed to test for differences in each nutrient ratio (C:N or N:P) between species size (*V. karroo* size or *S. myrtina* size as a predictor variable). Tukey's honest significance difference test (package "stats", functions: "TukeyHSD") was then performed on each linear model to determine where the significant difference had occurred, if applicable.

To compare the RII (inter-canopy vs. sub-canopy) between all pairwise combinations and test whether the effects of variable pair-size combinations on soil nutrient content differ significantly; linear models were performed to test for differences between species-pair combinations and each soil nutrient RII (inter-canopy vs. sub-canopy). Each linear model was constructed with two predictor variables (*V. karroo* size and *S. myrtina* size with an included interaction) as a function of each soil nutrient RII response variables (similar to Winter, 2013). The R function aov(lm) was then used and a Tukey's HSD was performed on each linear model to determine where the significant difference had occurred, if applicable.

Shapiro-Wilks normality test was done for each of the foliar nutrient (N, P & K) / predictor variables to determine if the data was normally distributed. To test whether the effects of variable pair-size combinations on leaf nutrients differ significantly, linear models were performed to test for differences in each foliar nutrient between the different species-size combinations. Each linear model was constructed by either focussing on *V. karroo* or *S. myrtina* leaf nutrients as a function of a specific pair-size combination. Linear models constructed by focussing on *V. karroo* leaf nutrients show the effect of *S. myrtina* size (large *V. karroo* N, P, or K as a function of *S. myrtina size*) and the effect of *V. karroo* size (*V. karroo* N, P, or K as a function of *V. karroo* size * presence/ absence of small *S. myrtina*). Linear models constructed by focussing on *S. myrtina* leaf nutrients show the effect of *V. karroo* size (small *S. myrtina* N, P, or K as a function of *V. karroo* size) and the effect of *S. myrtina* size (*S. myrtina* N, P, or K as a function of *S. myrtina* size * presence/ absence of large *V. karroo*). Table 3.1 provides a summary of linear model construction. Tukey's HSD were then performed on each of the linear model to determine where the significant differences had occurred, where applicable.

Table 3.1: Linear model summary of foliar nutrients. Linear models constructed by focusing on *V. karroo* leaf nutrients show the effect of *S. myrtina* size and the effect of *V. karroo* size. Linear models constructed by focusing on *S. myrtina* leaf nutrients show the effect of *V. karroo* size and the effect of *S. myrtina* size.

Focusing on *V. karroo*	**Linear model formula**
Effect of *S. myrtina* size	Large *V. karroo* [foliar nutrient] ~ *S. myrtina* size
Effect of *V. karroo* size	*V. karroo* [foliar nutrient] ~ V. *karroo* size * p/a with small *S. myrtina*
Focusing on *S. myrtina*	**Linear model formula**
Effect of *V. karroo* size	Small *S. myrtina* [foliar nutrient] ~ *V. karroo size*
Effect of *S. myrtina* size	*S. myrtina* [foliar nutrient] ~ *S. myrtina* size * p/a with large *V. karroo*

3.5. Results

3.5.1. Soil nutrient content (inter- vs. sub-canopy)

Soil P showed no significant difference between inter- vs. sub-canopy zones (t = -0.81, df = 43, p = 0.42; Fig. 3.3A). Soil K was higher in the inter-canopy compared to the sub-canopy (t = 2.1303, df = 43, p = 0.03; Fig. 3.3B). Soil N was significantly higher in the sub-canopy compared to the inter-canopy (t = -3.68, df = 43, p = 0.0006; Fig. 3.3C). Soil organic C was significantly higher in the sub-canopy compared to the inter-canopy (t = -6.11, df = 43, p = 2.45e-07; Fig. 3.3D). Results for all soil nutrients are included in Appendix B, Table S5.

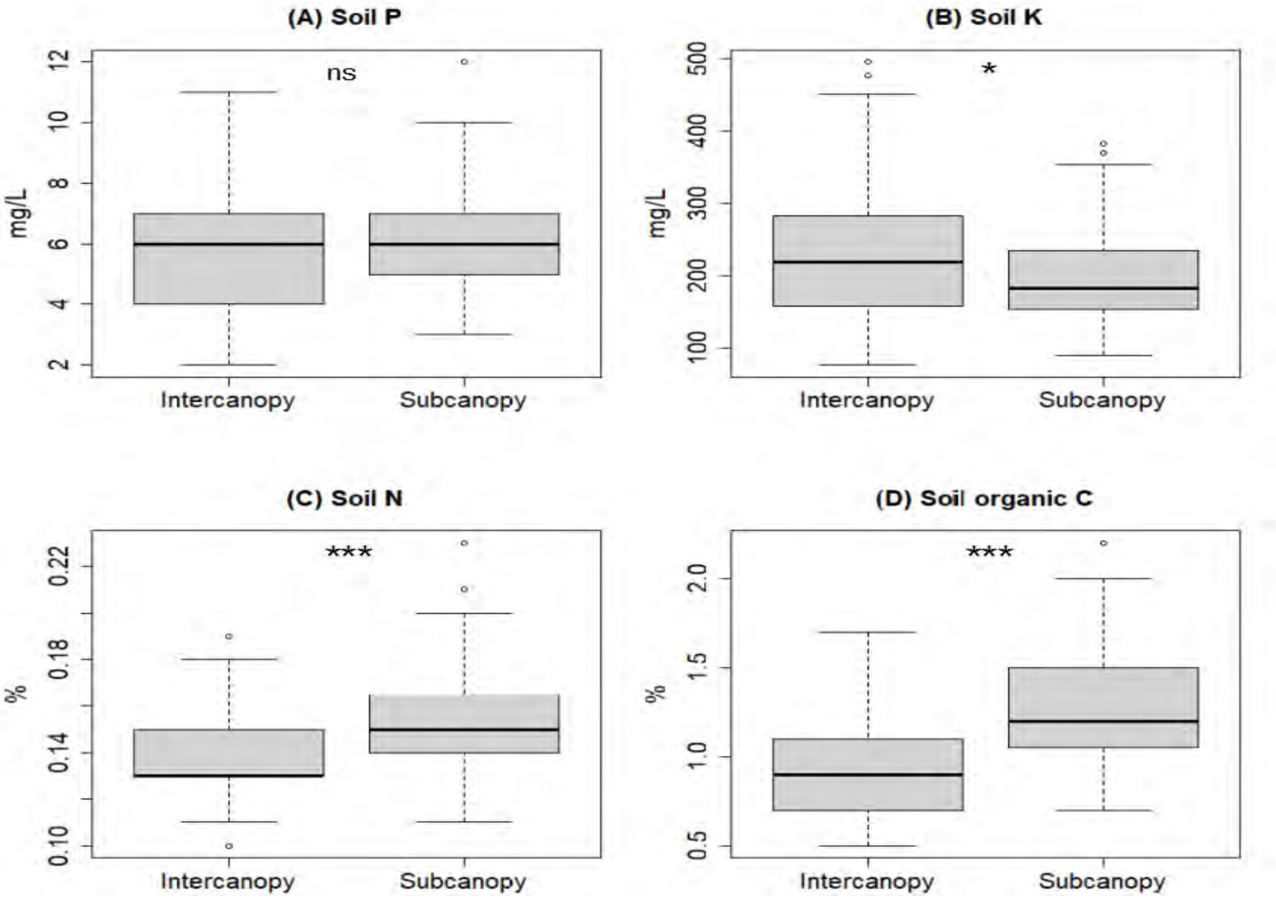

Figure 3.3: Inter-canopy vs sub-canopy soil values of (A) soil P, (B) soil K, (C) soil N, and (D) soil organic C. Significance levels indicate: * = p < 0.05, ** = p < 0.01, *** = p < 0.001.

3.5.2. Soil nutrient ratios

Neither *V. karroo* nor *S. myrtina*, in pairwise combinations or as individual controls, had significant effects on sub-canopy soil C:N (*F* (10,33) = 1.73, *p* = 0.11; Fig. 3.4A- C). N:P (*F* (10,33) = 1.85, *p* = 0.08; Fig. 3.4D- F).

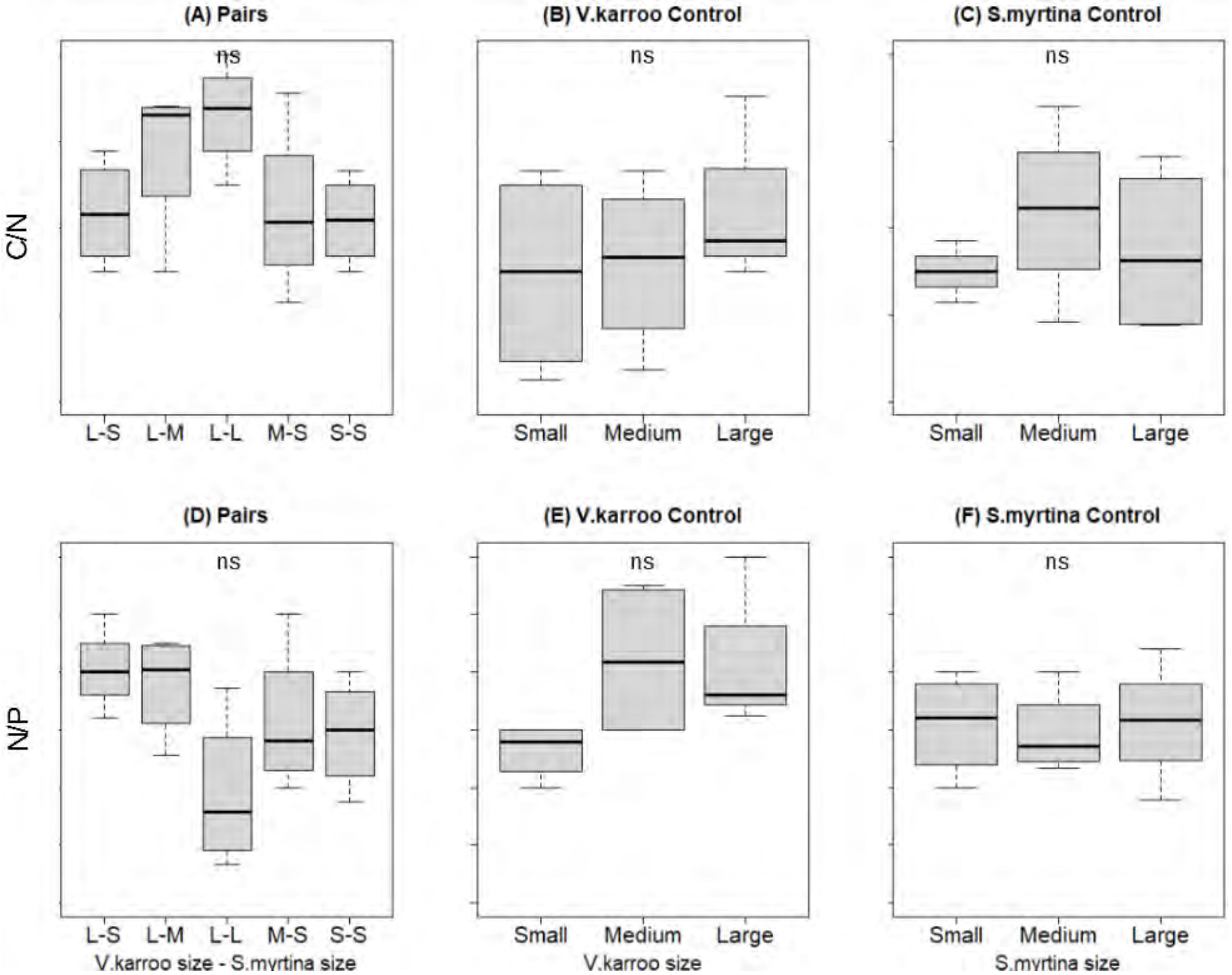

Figure 3.4**:** Soil C:N for (A) paired combinations, (B) *V. karroo* controls and (C) *S. myrtina* controls; and soil N:P for (D) paired combinations, (E) *V. karroo* controls and (F) *S. myrtina* controls. Ratios were calculated from volumetric concentrations (mg.L^{-1}).

3.5.3. Soil nutrient content RII (inter- vs. sub-canopy)

The size of *V. karroo* or *S. myrtina* had no significant effect on the magnitude of the difference in soil N (measured as the RII) in the sub- vs. inter-canopy (*F* (10,33) = 1.07, *p* = 0.4; Fig. 3.5A- C). *Vachellia karroo* size had a significant effect on the magnitude of the difference in soil P (measured as the RII) in the sub- vs. inter-canopy (*F* (10,33) = 3.68, *p* = 0.002; Fig. 3.5D- F); specifically large *V. karroo* controls had significantly higher soil P (*p* = 0.0003). Large *V. karroo* controls were significantly different from medium *V. karroo* controls (Tukey's HSD: *p* = 0.03). Neither *V. karroo* size nor *S. myrtina* size had a significant effect on the magnitude of the difference in soil K (measured as the RII) in the sub- vs. inter-canopy (*F* (10,33) = 1.43, *p* = 0.2; Fig. 3.5G- I). *Scutia myrtina* size had a significant effect on the magnitude of the difference in soil organic C (measured as the RII) in the sub- vs. inter-canopy (*F* (10,33) = 3.16, *p* = 0.006; Fig. 3.5J- L). Specifically medium *S. myrtina* with large *V. karroo* had significantly higher soil organic C (*p* = 0.001). Medium *S. myrtina* with large *V. karroo* are significantly higher (Tukey's HSD: *p* = 0.02), and small *S. myrtina* with medium *V. karroo* are significantly lower in soil organic C (Tukey's HSD: *p* = 0.0004).

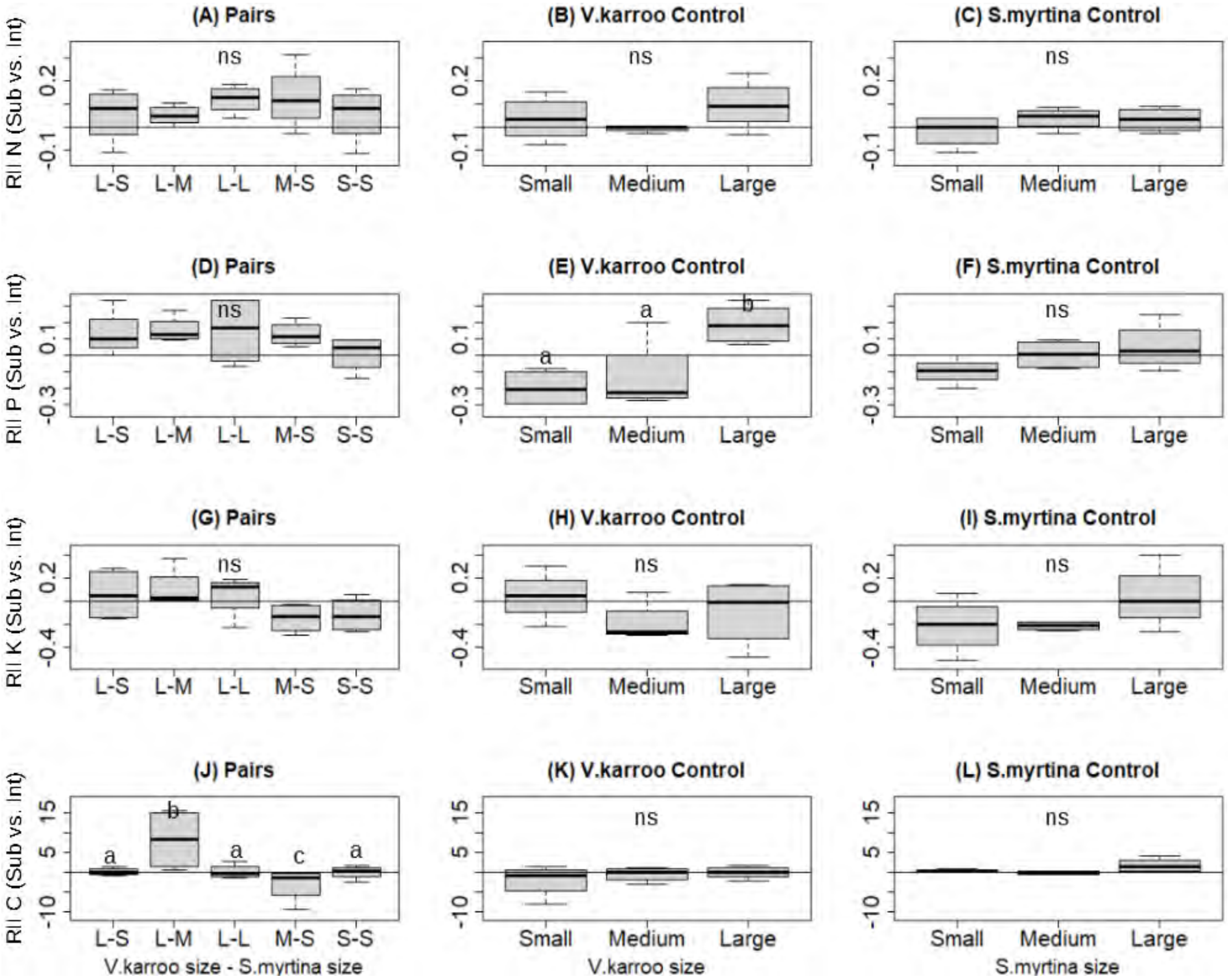

Figure 3.5**:** Effect size of trees (RII of inter-canopy vs. sub-canopy) on soil N, for (A) pairs, (B) *V. karroo* controls and (C) *S. myrtina* controls; soil P, for (D) pairs, (E) *V. karroo* controls and (F) *S. myrtina* controls; soil K, for (G) pairs, (H) *V. karroo* controls and (I) *S. myrtina* controls; soil organic C, for (J) pairs, (K) *V. karroo* controls and (L) *S. myrtina* controls. The horizontal line in each plot indicates RII = 0. Positive values (above the line) indicate that nutrient concentrations in the sub-canopy > inter-canopy, and vice versa. Letters indicate significant differences based on Tukey's HSD. Analysis were done on means assuming a normal distribution, however, the graph shows medians and quartiles to better illustrate the distribution and range of the data.

3.5.4. Leaf nutrient content

Scutia myrtina size had significant effects on large *V. karroo* foliar N (*F* (3,36) = 6.45, *p* = 0.001; Fig. 3.6A), P (*F* (3,36) = 6.44, *p* = <0.001; Fig. 3.6B) and K (*F* (3,36) = 6.83, *p* = 0.0009; Fig. 3.6C). Small *S. myrtina* in association with large *V. karroo* resulted in significantly lower *V. karroo* foliar N (*p* = 0.05), P (*p* = 0.01) and K (*p* = 0.006). Large *V. karroo* without any size *S. myrtina* had significantly higher foliar N (Tukey's HSD: *p = 0.0006*) and P (Tukey's HSD: *p* = 0.0009) than large *V. karroo* with any size *S. myrtina*. Large *V. karroo* foliar K was also significantly lowered by small *S. myrtina* (Tukey's HSD: *p* = 0.03). All leaf nutrients are included in Appendix B, Table S6.

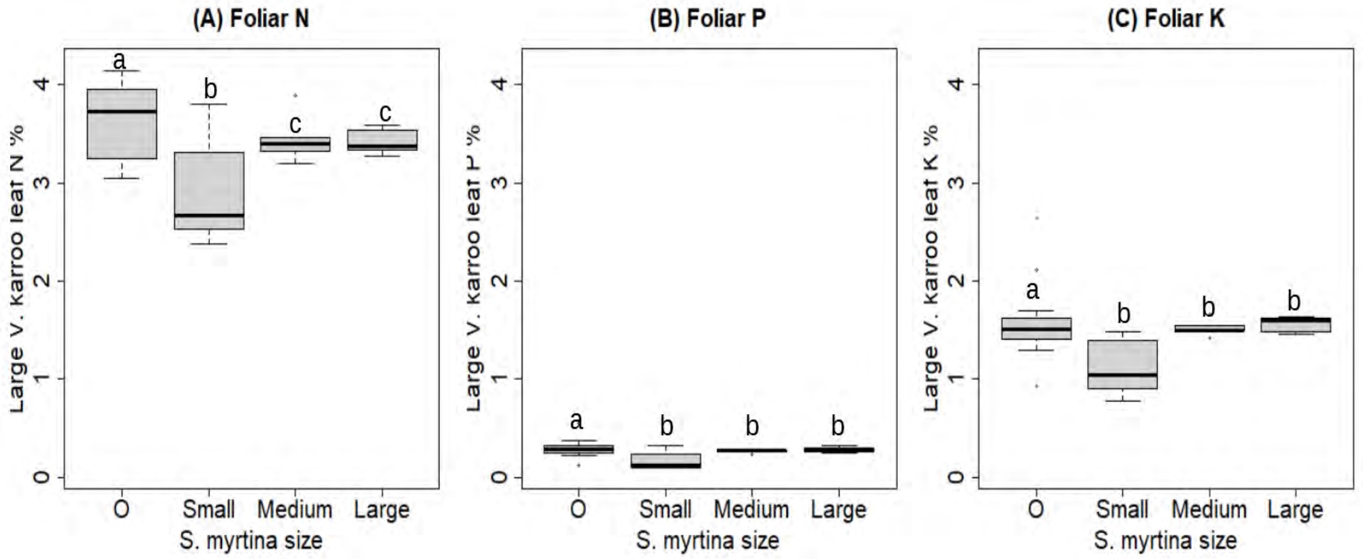

Figure 3.6: Effect of *S. myrtina* size (small, medium and large) on large *V. karroo* ('O' indicates large *V. karroo* control without an *S. myrtina* partner) for foliar (A) nitrogen, (B) phosphorous and (C) potassium. Different letters indicate a significant difference based on Tukey's HSD.

Vachellia karroo size had significant effects on small *S. myrtina* foliar N (*F* (3,36) = 43.47, *p* = 0.0004; Fig. 3.7, A), P (*F* (3,36) = 42.16, *p* = 0.0001; Fig. 3.7, B) and K (*F* (3,36) = 20.71, *p* = 0.001; Fig. 3.7, C). Specifically small (*p* = 0.0001) and medium *V. Karroo* (*p* = 0.0001) increased small *S. myrtina* foliar N, P and K. Small *S. myrtina* foliar N, P and K was significantly higher with both small *V. karroo* (Tukey's HSD: *p* = 0.0001), and medium *V. karroo* (Tukey's HSD: *p* = 0.0001).

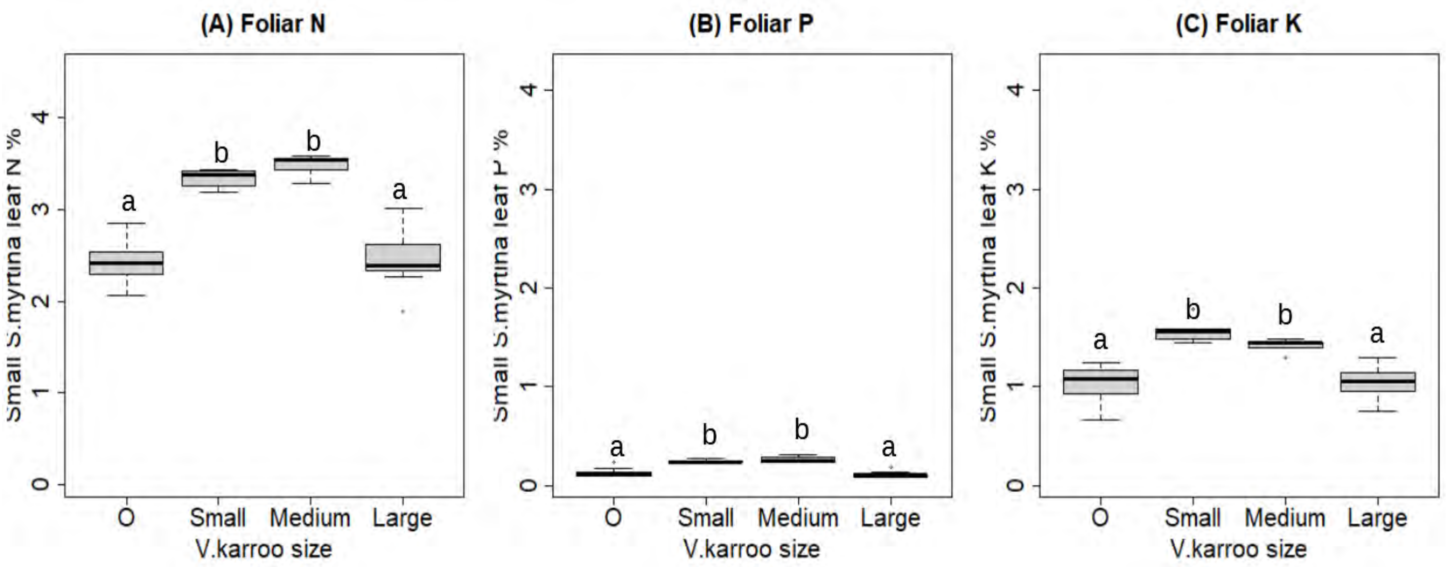

Figure 3.7: Effect of *V. karroo* size (small, medium and large) on small *S. myrtina* (and small *S. myrtina* control 'O' without a *V. karroo* partner) for foliar (A) nitrogen, (B) phosphorous and (C) potassium.

Small *S. myrtina* had significant negative effects on *V. karroo* foliar N (*F* (5,34) = 9.48, *p* = < 0.0001; Fig. 3.8, A), P (*F* (5,34) = 11.27, *p* = < 0.0001; Fig. 3.8, B) and K (*F* (5,34) = 9.91, *p* = < 0.0001; Fig. 3.8, C). Specifically, small (*p* = 0.0002) and medium *V. karroo* (*p* = 0.0003) were negatively affected by small *S. myrtina* partners. Small *S. myrtina* partners decreased both *s*mall (Tukey's HSD: *p* = 0.002) and medium *V. karroo* foliar N (Tukey's HSD: *p* = 0.004)*,* P (Tukey's HSD: *p* = 0.0004) and K (Tukey's HSD: *p* = 0.001).

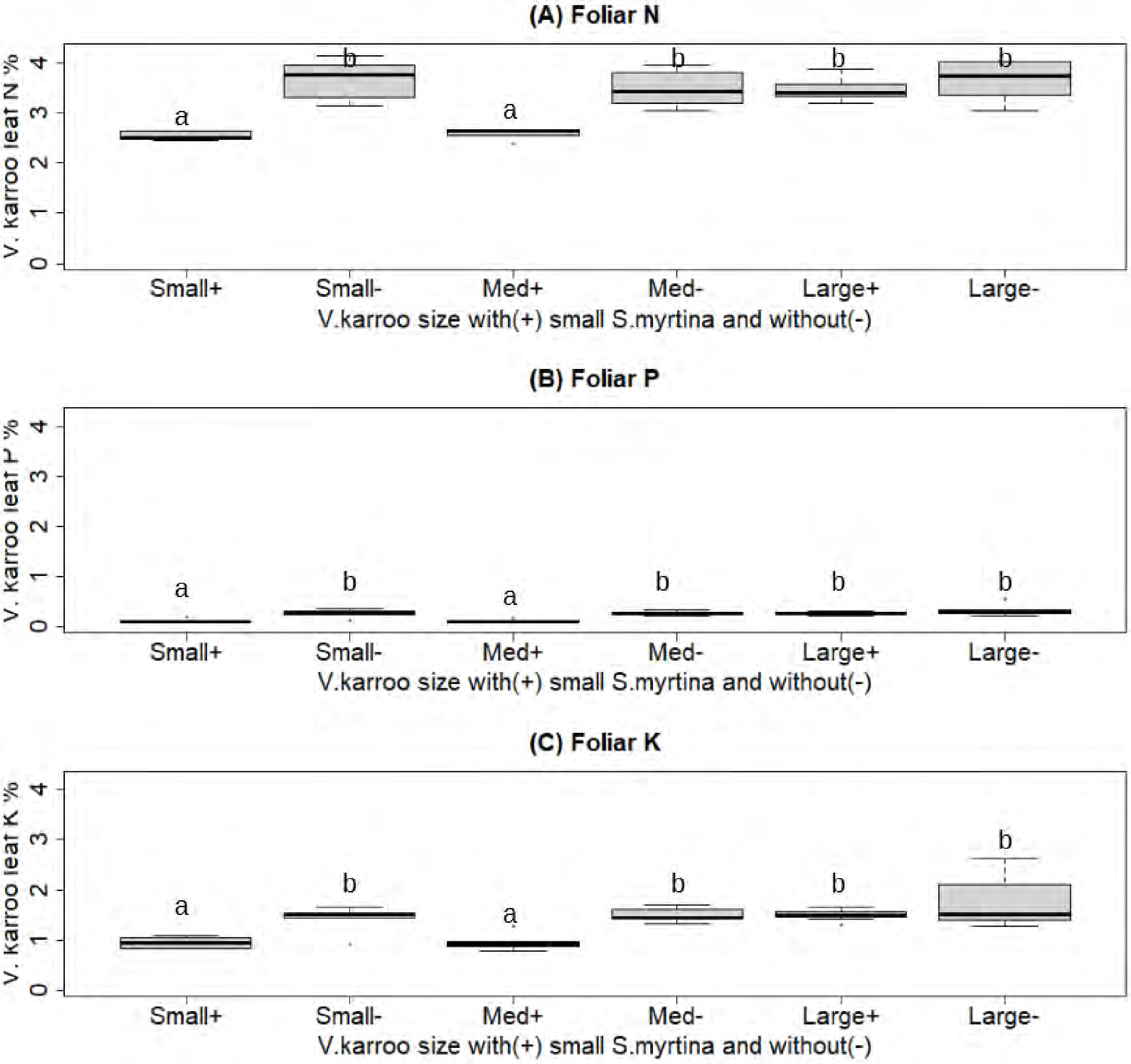

Figure 3.8: *V. karroo* size with (+) small *S. myrtina* and without (-) for (A) leaf N, (B) leaf P and (C) leaf K. When letters are similar, there is no significant difference (Tukey's HSD). Med = medium.

Large *V. karroo* had significant positive effects on *S. myrtina* foliar N (F (5,34) = 11.3, p = < 0.0001; Fig. 3.9, A), P (F (5,34) = 7.53, p = < 0.0001; Fig. 3.9, B) and K (F (5,34) = 8.47, p = < 0.0001; Fig. 3.9, C). Small *S. myrtina* foliar N (p = < 0.0001), P (p = < 0.0001) and K (p = < 0.0001) was significantly increased by large *V. karroo* associations. Small *S. myrtina* foliar N (Tukey's HSD: p = 0.0005), P (Tukey's HSD: p = 0.0001) and K (Tukey's HSD: p = 0.0009) was significantly increased by large *V. karroo* partners.

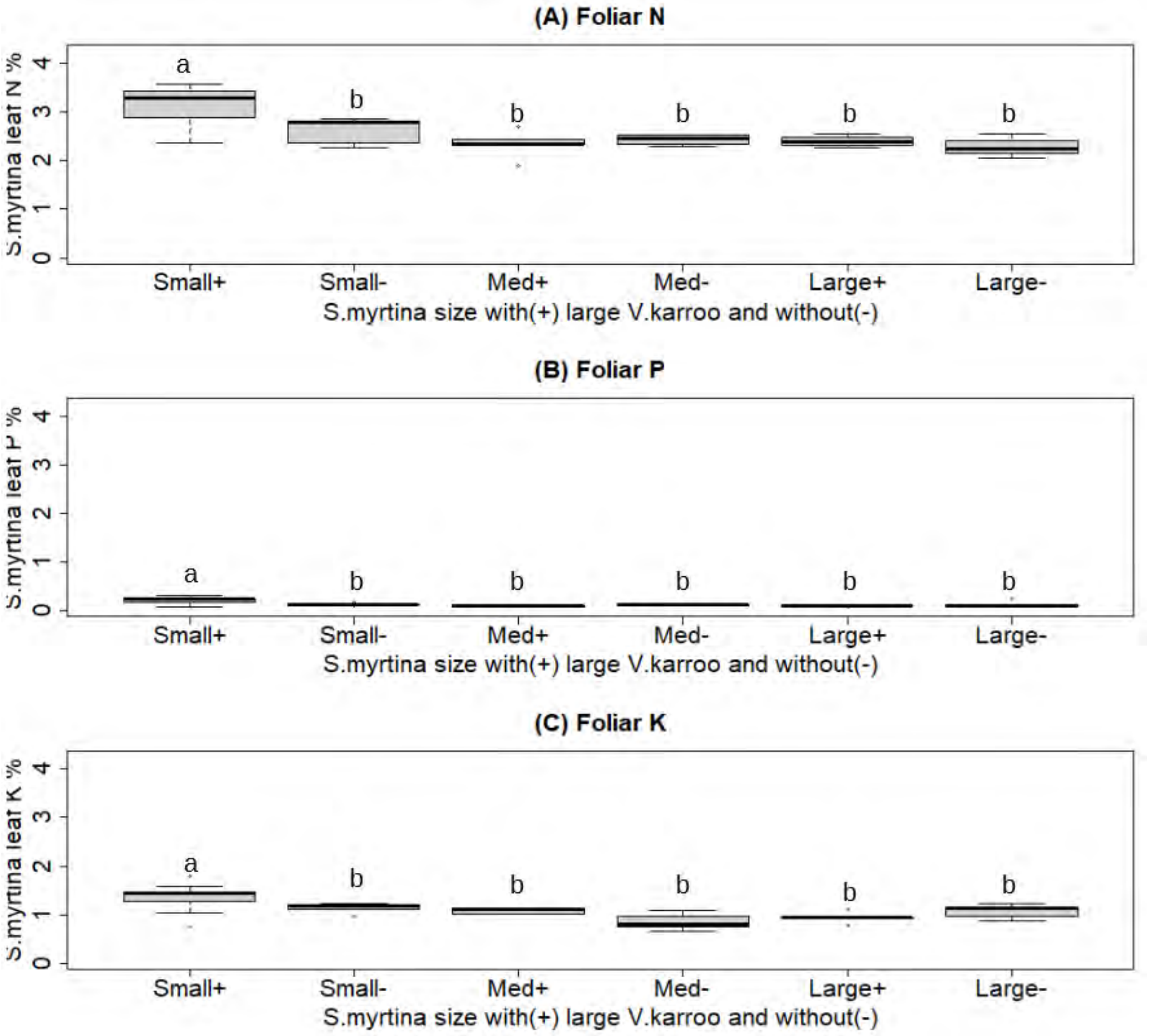

Figure 3.9: *S. myrtina* size with (+) large *V. karroo* and without (-) for (A) leaf N, (B) leaf P and (C) leaf K. When letters are similar, there is no significant difference (Tukey's HSD). Med = medium.

3.6. Discussion

3.6.1. Soil nutrient content (inter- vs. sub-canopy)

As expected, both *V. karroo* and *S. myrtina* individuals, as well as pair-combinations of the two, significantly increased soil C, N and K in the sub-canopy. This could be due to organic litter inputs and other biophysical processes taking place under tree canopies over time, such as N-fixation or the island of fertility effects (Hagos, 2001; Moyo *et al*., 1998). *Vachellia karroo* is a legume with symbiotic nitrogen-fixing bacteria, which are favoured by a high availability of phosphorus and low availability of nitrogen (Vitousek & Field, 1999). Interestingly, soil N was not significantly affected by pair-size associations contrary to my hypothesis that it would increase with *V. karroo* size and decrease when large *S. myrtina* is present compared to control individuals. This could possibly be due to high N demand or competition for N between woody species (Vitousek & Field, 1999). K is an overlooked soil mineral in studies on facilitation in systems where drought can limit plant survival (Gómez *et al*., 2005). K is also shown to be responsible for regulating primary production and vegetation succession (Sugihara *et al*., 2014). Other studies on species-pair combinations on K have shown facilitation through mechanisms of improved water-use efficiency or increased plant resistance to drought due to osmotic adjustments and reduction in transpiration rates (Carrillo-García *et al*., 2000; Egilla *et al*., 2001; Gómez *et al*., 2005).

3.6.2. Soil nutrient ratios

There were no significant effects of individual trees of either species or pair-size combinations on either C:N or N:P ratios in soils. However, soil C:N was highest and N:P was lowest in the sub-canopy of large *V. karroo* and large *S. myrtina* pair-size combinations. These results suggest that large *V. karroo* and large *S. myrtina* pair-size combinations have the ability to transform nutrient ratios over time, which may favour a biome shift from savanna to thicket, through enhancing the bush clump formation process through soil enrichment.

3.6.3. Soil nutrient content RII (inter- vs. sub-canopy)

Soil nutrient content expressed as RII (inter- vs. sub-canopy) showed that large *V. karroo* had a significant effect on the magnitude of the difference in soil P, relative to the inter-canopy zone. Likewise, *S. myrtina* size had a significant effect on the magnitude of the difference in soil organic C, relative to the inter-canopy zone. Specifically, small *S. myrtina* with medium *V. karroo* pairs were significantly lower in soil organic C expressed as RII compared to medium *S. myrtina* with large *V. karroo* pairs. Pair-size tree interactions most likely affected soil P and organic C through the island of fertility effects around focus individuals over time (Hagos, 2001; Moyo *et al*., 1998).

3.6.4. Leaf nutrient content

The effect of different pair-associations on plant leaf nutrients showed that *S. myrtina* in association with large *V. karroo* resulted in significantly lower *V. karroo* foliar nutrients. Specifically, large *V. karroo* controls had significantly higher foliar N, P and K compared to large *V. karroo* growing with any size partner of *S. myrtina* under the canopy. *V. karroo* increased *S. myrtina* foliar nutrients. Specifically, small and medium *V. karroo* increased small *S. myrtina* foliar N, P and K. As expected, large *V. karroo* was generally the same irrespective of whether it was growing with a small *S. myrtina* or not. However, small *S. myrtina* partners decreased both small and medium *V. karroo* foliar N, P and K. Conversely, small *S. myrtina* foliar N, P and K was significantly increased by growing in association with large *V. karroo* partners. These findings may in part explain the shift from facilitation in early stages of clump formation (large-small combinations) to competition during later stages of encroachment (large-large combinations), with regard to species-association mechanisms affecting foliar nutrients.

3.7. Conclusion

My results confirmed that pairwise tree interactions affected both soil nutrient and leaf nutrient content. All trees, regardless of species or size class, increased soil K, N and organic C in their sub-canopy spaces. Association with *V. karroo* increased *S. myrtina* foliar N, P and K. In contrast, association with *S. myrtina* lowered *V. karroo* foliar N, P and K. Small *S. myrtina* was found to be most facilitated by establishing and growing up next to a large *V. karroo* individual, through mechanisms affecting soil and foliar nutrients. *Scutia myrtina* establishing in association with smaller size classes of *V. karroo* showed no significant effects of facilitation. Results are similar to other studies that focus on the modification of soil phosphorous as the main nutrient facilitation mechanism (Carrillo-García *et al*., 2000; Gómez *et al*., 2005). Results highlight high soil potassium under large *V. karroo*, often an overlooked but relevant soil effect on facilitation in systems where drought is the limiting factor for plant survival (Gómez *et al*., 2005). Increased nutrients are an important factor that allows *V. karroo* as a nucleator species for the formation of bush clumps, which agrees with conclusions by O'Connor & Chamane (2012) and Jamison-Daniels *et al*. (2021) in similar contexts.

CHAPTER 4: The effects of interspecific tree associations and selective removal on soil and plant water dynamics

4.1. Abstract

The main objectives of this chapter were to test for positive and negative effects of pairwise tree interactions between a common, encroaching savanna tree, *Vachellia karroo*, and a thicket pioneer *Scutia myrtina*, on available soil water and plant water potential (Ψ). I measured differences in soil moisture and bulk density between inter-canopy and sub-canopy zones. I examined the effect of pairwise associations and controls on plant water stress (pre-dawn and mid-day leaf Ψ) and conducted a selective removal of large *V. karroo* individuals from pairs to confirm the negative and positive effects of pairwise interactions on plant available water and water stress. Data was collected on Endwell farm which is located in the Smaldeel region of the Eastern Cape, South Africa. Two 50 m x 50 m plots that covered areas with combinations of the co-occurring species were chosen using digital aerial photographs, that also corresponded to an increase in woody cover from small-small to large-large combinations of focal species (along the gradient from early to advanced thicket formation). Pair-size combinations were then sampled along one 620 m x 100 m belt transect running through each of the two chosen plots. Similar to other studies, my results confirmed positive and negative effects of pairwise tree interactions. Small *S. myrtina* was most facilitated by establishing and growing up next to a large *V. karroo* individual, through mechanisms affecting soil water content, bulk density and leaf Ψ. *Scutia myrtina* growing in association with medium or small size classes of *V. karroo* showed no significant effects of facilitation, while large *S. myrtina* showed evidence of competitive interactions for water with *V. karroo*.

Keywords: Bulk density; Leaf Ψ; Nurse effects; Pair-size combinations; Removal experiment; Soil water content.

4.2. Introduction

In the savanna biome, structural variation created by a discontinuous canopy cover results in spatial variations of resources and microclimatic conditions (Holdo & Mack, 2014). This spatial heterogeneity can in turn influence biophysical processes and structure vegetation dynamics of semiarid ecosystems (Sankaran *et al*., 2008). Due to significant heterogeneity, savannas are classified as 'source-sink' systems that typically have patches of vegetation, and new seedlings often establish near plant canopies (Pueyo *et al*., 2016). Canopy cover can mediate water availability through its temperature effects (reduced transpiration and evaporation), in turn changing soil properties such as soil moisture content and bulk density (Holdo & Mack, 2014). Tree canopies have been shown to mainly influence microclimate through shading mechanisms that affects incoming radiation and thermal stress, or precipitation that is affected by interception or concentration by the canopy (Holdo & Mack, 2014). According to Bora *et al*. (2021), the canopies of encroaching tree species and co-occurring species have different influences on understory herbaceous vegetation, with encroaching tree species having a greater ability to acquire and compete for resources. Temperature fluctuations are also moderated under sub-canopies, shown to have cooling effects of ~2 °C on mean soil temperatures in a South African savanna (Holdo & Mack, 2014). In these water-limited systems, tree canopies can intercept water during small rainfall events and a substantial amount may then be lost through evaporation prior to reaching the soil surface (Rodriguez-Iturbe *et al*., 1999). Facilitation by overstory trees however, has been reported to be greater and lasts longer during drier years, with host trees serving as a hydrologic buffer against inter-annual precipitation variability (Barron-Gafford *et al*., 2021).

Variations in available resources have fundamental effects in structuring recruitment and can be caused by differences in soil bulk density, a driver of both nutrient and soil moisture dynamics (Holdo & Mack, 2014). Bulk density directly affects soil water content, one of the most important hydrological components and it can account for spatial and temporal variability, in that it best characterizes the spatiotemporal availability of water for plant establishment (Pueyo *et al*., 2016). In South African savannas there is usually lower soil bulk density and higher porosity in the first 10 cm of soil under tree canopies relative to inter-canopy spaces, due to less thermal stress and a build-up of organic matter (Aweto & Dikinya, 2003; Holdo & Mack, 2014). Canopy throughfall or litterfall may also increase soil nutrients and decrease bulk density near large trees and may explain higher understorey productivity (Marañón *et al*., 2009). A higher concentration of tree roots near the base of the trees also has the effect of loosening the soil and reducing soil bulk density (Holdo & Mack, 2014). Soils in the inter-canopy spaces are usually drier as they are exposed to direct solar radiation and higher temperatures, which accelerates thermally induced soil organic matter decomposition. This also results in organic matter and clay colloids shrinking and makes the soil more compact, increasing surface run-off and erosion due to reduced permeability and infiltration capacity (Holdo & Mack, 2014).

Plants are able to modify and mediate their physical environment through biological interactions and improve the physical conditions for growth, such as water stress or improved water relations and availability of both nutrients and water (Pugnaire *et al.*, 1996). Pugnaire *et al.* (1996) found that plants by themselves and pairs of woody species all had the same pre-dawn water potential (Ψ), a measure of water available to the plant in the soil, but those growing in association with woody partners all had significantly more negative midday water potential Ψ, which reflects water stress. To study the beneficial effects of tree-shrub interactions, Barnes & Archer (1999) sampled pre-dawn and mid-day leaf Ψ of plants and showed that differences were most pronounced during drought conditions and after overstory removal. They found that the effect of overstory nurse plants on understory shrubs was either neutral or positive in all cases, while simultaneously the woody understory species had strong negative effects on the growth and reproduction of all the nurse plants. Zou *et al.* (2005) also examined plant–soil water relations in the context of a selective removal study on different soil types in a subtropical savanna parkland. Their results confirm that there is soil moisture redistribution by hydraulic lift in savanna woody patches, occurring with greater frequency and magnitude on deeper soils compared to shallow soils, and that it is also temporally dynamic in savanna tree–shrub communities (Zou *et al.*, 2005). Interactions between the large nurse individuals and understory shrubs in woody patches was found to be positive, negative or neutral over annual cycles, and/or reflect variability in precipitation (Zou *et al.*, 2005). Selective removal of large nurse plants reduced the frequency of hydraulic lift, and removal of the large nurse plant from clusters decreased leaf water potential during periods when hydraulic lift would take place (Zou *et al.*, 2005).

Vachellia karroo is one of the most prolific and widespread encroaching species in Eastern Cape savannas and has been shown to act as nucleator species for both thicket clump expansion and savanna thickening (Balfour & Midgley, 2008; Parr *et al.*, 2012). Both of these bush dynamics may be undesirable from both socio-economic and conservation viewpoints (Sankaran *et al.*, 2004, 2005), and have various impacts on ecosystem services, functioning or productivity (Scholes & Archer, 1997; O'Connor *et al*., 2014). Shade provided by the canopy of a large *V. karroo* may protect pioneer *S. myrtina* seedlings and saplings from temperature extremes, thermal stress and water loss through transpiration and reduce photoinhibition (Pugnaire *et al*., 2011). However, the mechanisms of these processes are still unclear, these can also be described as non-resource factors (Fig. 4.1). Hydraulic lift is a commonly reported mechanism for facilitation through an increase in soil moisture under nurse plant canopies in dry environments (Fig. 4.1) and could also possibly play a role in facilitation of pioneer species during early succession, although not considered in this study (Pugnaire *et al*., 2011). Facilitation works on the passive movement of water pulled up through deeper plant roots to the surface driven by water potential gradients (Pugnaire *et al*., 2011). However, in combination with competition, there have been reports of lower soil moisture levels in the understorey of large *V. karroo* and other large woody species compared with open areas, due to greater aboveground and below-ground competition for water (Moyo *et al*., 1998).

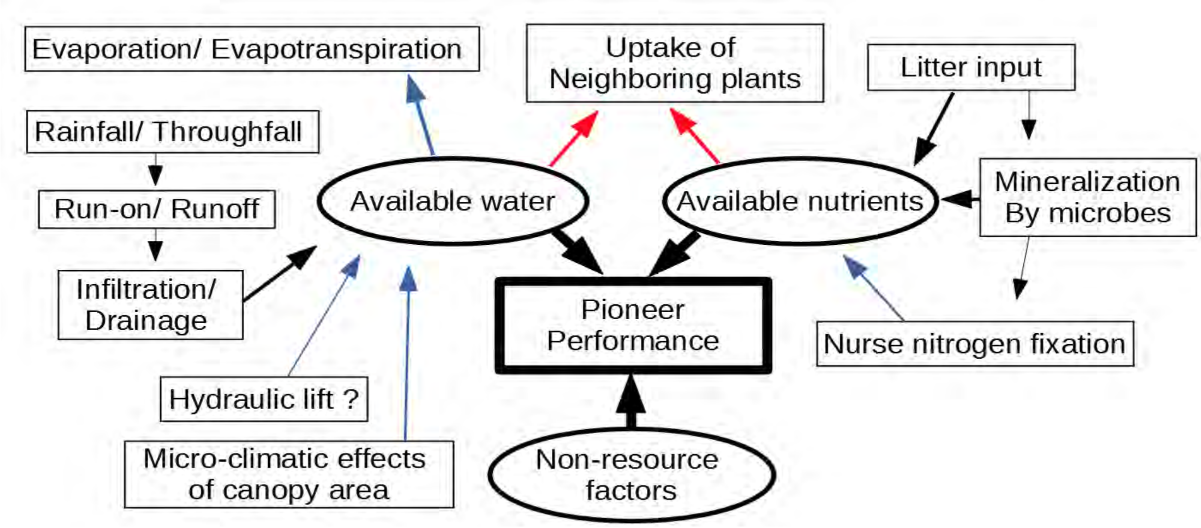

Figure 4.1: The most relevant factors related to thicket pioneer growth, survival and nurse plant effects in semi-arid source-sink ecosystems, adapted from Pueyo *et al*. (2016). Red arrows represent competitive mechanisms while blue arrows represent facilitative mechanisms influencing plant growth and survival. Black arrows show general trends.

The objectives of this study were to test for positive and negative effects of pairwise tree interactions on available soil water and plant water potential. Specifically, I looked for evidence of competition and/or facilitation between a common encroacher, *Vachellia karroo*, and *Scutia myrtina*, a thicket pioneer. This was done by first examining the differences between inter-canopy and sub-canopy soil moisture and bulk density between pair-size combinations and individual controls. Second, I examined the effect of savanna and thicket pioneer associations on soil water and plant water stress by measuring the pre-dawn and mid-day leaf Ψ. Finally, a selective removal of large *V. karroo* individuals was done and pre-dawn and mid-day leaf Ψ was measured to confirm the effects of competition between large individuals and facilitation by large *V. karroo* of the establishment and early growth of *S. myrtina*. To distinguish between dispersal and facilitation as reasons for the association, removing the large *V. karroo* primarily demonstrated the facilitation effects expressed as increased water stress. I outline the hypotheses in more detail below for each of these sections.

4.2.1. Soil: sub-canopy vs. inter-canopy

I expected higher soil water content in the sub-canopy of both paired and single *Vachellia karroo* and *Scutia myrtina* trees compared to inter-canopy spaces due to shade provided by the canopy*,* protecting soil from temperature extremes or thermal stress and water loss though evaporation (Pugnaire *et al*., 2011). I also predicted that there would be lower bulk density under the sub-canopies compared to inter-canopy spaces, due to higher resources and the effects of plants roots on soil properties (Barron-Gafford *et al*., 2021; Holdo & Mack, 2014). I expected that larger trees (controls or pairs with one or two large individuals) would have higher soil water content and lower bulk density in the sub-canopy than pairs and individuals of smaller trees only. This would be due to effects of canopy area effects, as well as the physical effects of roots and organic input into the soil increasing over time and as trees increase in size (Pugnaire *et al*., 1996). It was also expected that the lowest soil water content and highest bulk density would be under the canopies of large *S. myrtina* and large *V. karroo* pairs, due to competition and higher water use as trees increase in size, combined with the above-mentioned effects.

4.2.2. Leaf Ψ of S. myrtina and V. karroo

I expected that the effect of a small *S. myrtina* on small, medium or large *V. karroo* would be small, resulting in no, or a slight, increase in *V. karroo* water stress due to competition of small woody understory species for resources such as water demonstrated by Barnes & Archer (1999). I also expected that growing with any size of *V. karroo* would lead to less negative water potential in small *S. myrtina* (indicating less water stress). This is due to facilitation through micro-climatic conditions created by the canopy, such as protection from thermal stress, reduction in photoinhibition or water loss though evaporation and evapotranspiration (Pugnaire *et al*., 2011). Generally, facilitation was expected during smaller juvenile stages of *S. myrtina* recruitment and establishment, with a switch to competition being the predominant process when *S. myrtina* is mature. Thus, it was also expected that large *S. myrtina* growing with large *V. karroo* trees would be the most water stressed (most negative leaf Ψ), as associations with large *V. karroo* trees are hypothesized only to be beneficial for small or medium *S. myrtina*, and large *V. karroo* and large *S. myrtina* are expected to compete strongly for water. Large *V. karroo* was expected to have the most beneficial effect on small *S. myrtina* (least water stressed under large *V. karroo*, compared to medium or small *V. karroo*). This is due to the larger *V. karroo* trees having greater micro-climatic effects (larger canopy area), thus larger controls are expected to have less negative Ψ compared to smaller control individuals, also due to larger rooting systems and greater access to resources.

4.2.3. Removal of large V. karroo

After removing large *V. karroo* individuals from pairs with small *S. myrtina* individuals, I expected the small *S. myrtina* to show higher water stress (more negative Ψ) due to higher temperatures and evapotranspiration without the nurse canopy, similar to that of small *S. myrtina* control individuals in the previous experiment. Small *S. myrtina* were expected to show greater water stress compared to medium or large *S. myrtina* individuals after the removal of large *V. karroo* partners, due to their smaller and shallower rooting systems that are not yet fully established. It was also expected that in pairs of large *V. karroo* and large *S. myrtina*, which are typically found during the late successional stage, removal of the large *V. karroo* would slightly reduce water stress of the large *S. myrtina* due to competition and demand for resources.

4.3. Methods

4.3.1. Study area and sampling design

Data was collected on Endwell farm (32˚44'55"S, 26˚27'19"E), located in the Smaldeel region of the Eastern Cape, South Africa. The land has been used for cattle farming with low numbers of game. Fire and other forms of large-scale bush control have been excluded since 1966. Vegetation is classified to be a mixture of Bedford Dry Grassland and Bisho Thornveld (Mucina & Rutherford, 2006). The most predominant soil types in this area include poorly drained Glenrosa, Trevanian and Mispah (Martens & Morris, 1994; Schulze, 2008). Two plots that cover areas with prevalent combinations of co-occurring focus species were chosen from a total of 50 potential plots that had been identified using digital aerial photographs (Khoza, 2021). These two plots were chosen on a specific region of the farm, also located along the same slope to control for altitude and edaphic factors. The two chosen plots were close to a road and 650 m apart. Plots represented differences in the percentage cover change from large-large to small-small combinations of focus species. Species pair-size combinations were then chosen along a 620 m x 100 m belt transect running through each plot.

Measurements of soil water and bulk density, and of pre-dawn and mid-day leaf water potential, were performed on pairwise combinations of *V. karroo* and *S myrtina* representative of commonly observed associations in the field (see Chapter 2). Details of the study site and the stages of the thicket formation process are described in Chapter 2. Associations comprised large *V. karroo* with small, medium and large *S. myrtina* (representing the different stages of *S. myrtina* establishing under the canopy of large *V. karroo* individuals), as well as small *S. myrtina* with small and medium *V. karroo* (representing *S. myrtina* establishing in association with other size classes of *V. karroo*). Combinations where *S. myrtina* was larger than *V. karroo* (indicating that *V. karroo* established in association with an established *S. myrtina*) were rarely observed in the field (Chapter 2) and thus not included in this study.

For the removal experiment, I selected species-pairs of large *V. karroo* associated with either small, medium or large *S. myrtina* and removed the large *V. karroo* in half of the selected pairs. This sequence of pair-size combinations represents how a large *V. karroo* can affect *S. myrtina* establishment in the process of thicket formation. Presence of other individuals were avoided when choosing species-pairs within each focus individuals' canopy in order to eliminate the effects of other neighbouring individuals. Species identity and GPS coordinates of selected pairs of interest were taken, and each individual tree received an aluminium tag of unique ID. Tree height and overall dimensions of the canopy area were recorded in order to classify individuals into large, medium, and small size-classes or pair-size combinations.

4.3.2. Soil: sub-canopy vs. inter-canopy

I compared soil water content and bulk density in the inter- and sub-canopy, based on pair-size combinations described above. Samples for measurements of soil water content and bulk density were collected from the inter-canopy and sub-canopy of all 40 pairs and 48 single control individuals (N = 8). Bulk density was sampled and measured using the core method (Manson & Roberts, 2000). Gravimetric soil moisture content was quantified by taking 50 g soil samples from beneath the canopy of each of the focus individuals and adjacent inter-canopy spaces, disturbing the sample structure as little as possible (Campbell & Henshall, 2001; Dirksen, 1999). Samples were taken in the same locations of soil fertility samples (under the canopy within 30-50 cm from the base of the focus individual, in the inter-canopy outside the zone of influence, being half the canopy diameter + 1 m from the edge of the canopy). A single sample was taken from the sub-canopy and inter-canopy of each pair or control individual. Each sample was placed into a paper bag, labelled, placed in a sealable bag and then stored in a cooler box. Samples were placed in the oven on the same day to dry at 105°C for 24 h after the wet weight was recorded. Dry weight was recorded after there was no longer any loss of mass. Bulk density by the core method was calculated by using the formula: Db = (ODW- RF – CW)/ [CV – (RF/PD)], where, Db = Bulk density of < 2 mm soil particles at sampled field water state (g.cm^3); ODW = Oven-dry weight; RF = Weight of rock fragments; CW = Empty core weight; CV = Core volume; PD = Density of rock fragments (Manson and Roberts 2000). Bulk density is reported to the nearest 0.01 g cm^3.

The direction and size of the effect of interspecific interactions on soil water was quantified using the Relative Interaction Intensity (RII) index (Armas *et al*., 2004). The RII was calculated from inter-canopy vs. sub-canopy response variables (soil water content and bulk density) to show whether soil water content (SWC) or bulk density (BD) increased or decreased due to the presence or size of the trees (i.e., in the sub-canopy, similar to Liua *et al*., 2020). This index was specifically developed for plant interactions and measures the RII with strong mathematical and statistical properties, which can be applied to any form of interaction such as competitive exclusion or symbiosis (Armas *et al*., 2004). RII can also be scaled up to measure multispecific interactions at the community level (Armas *et al*., 2004). This index traditionally measures interactions of two plants growing in proximity to one another, but in this study, we investigated the effect of plant presence on soil properties. Here X = is either SWC or BD. RII = $(X_1 – X_2) / (X_1 + X_2)$, where X_1 represents the soil properties in the sub-canopy, and X_2 represents soil properties in the inter-canopy (similar to Fagundes *et al.*, 2018; see also Chapter 3). RII values > 1 indicate that SWC or BD is greater in the sub-canopy.

4.3.3. Leaf Ψ of S. myrtina and V. karroo

To compare pre-dawn and mid-day leaf Ψ of *V. karroo* and *S. myrtina* individuals growing in different pair-size combinations and individual controls, I used the same pairs as in the comparison of soil water content and bulk density. Pairs comprised large *V. karroo* with small, medium and large *S. myrtina*, as well as small *S. myrtina* with small and medium *V. karroo*. Leaf Ψ was measured for each individual in the eight pair-replicates of each size-class combination and control (40 pairs and 48 controls) totalling 128 individual measurements for both pre-dawn and mid-day leaf Ψ. Pre-dawn and mid-day leaf water potentials were measured with a Scholander pressure chamber. In order to reduce the variation in data between days, measurements of leaf water potential were randomized for different treatment size-combinations in time. Measurements were taken as close together in time as possible in order to collect the data under similar conditions such as time of day, temperature and relative humidity (Wright *et al.*, 2015). Measurements of mid-day leaf Ψ were made between 11 am and 3 pm, while pre-dawn leaf Ψ was measured between 2 am and 6 am. During all measurements, leaves were wrapped in sealable plastic bags immediately after cutting and transferred to the pressure chamber as quickly as possible (Wright *et al.*, 2015). Mid-day leaf Ψ was first measured in December 2020 (29/11/2020 to 02/12/2020), but these values are not included in the statistical analysis as measurements are too far apart in time. However, these 2020 measurements were extremely similar to 2021 measurements. Each individual was measured for pre-dawn leaf Ψ on February 2021 (23/02/2021 to 26/02/2021) in conjunction with a second sampling of measurements for mid-day leaf Ψ (23/02/2021 to 26/02/2021).

4.3.4. Removal of large V. karroo

Selective removal focused on the effect of large *V. karroo* on all three size-classes of *S. myrtina* by removing large *V. karroo* individuals from half the pair-combinations and comparing the water potential of *S. myrtina* before and after removal of the large *V. karroo*. Selective removal only utilized large *V. karroo* size-class combinations and included completely separate pairs specifically chosen for removal. The same size-class combinations included *V. karroo* (large) with *S. myrtina* (small, medium and large), representing the different stages of *S. myrtina* establishment under a large *V. karroo* sub-canopy. When finding and selecting the new pairs for the selective removal, for each pair-replication, all pioneer individuals had a canopy overlap of 100% with the large *V. karroo* partner. Each size-class combination was replicated five times totalling 15 removal pairs. Pre-dawn and mid-day leaf Ψ of each of the 30 individuals in the removal pairs was measured before removal. After all leaf water potential measurements had been taken, the 15 large *V. karroo* individuals were cut down with a chainsaw and dragged 2 m from the base, which was then painted with Picloram (pyridine carboxylic acid) as potassium salt (a herbicide called 'BROWSER')**,** that works through cut surfaces in order to kill the nurse tree completely. Pre-dawn and mid-day leaf Ψ of the 15 *S. myrtina* individuals remaining were re-sampled after the removal.

Scutia myrtina individuals in the removal-pair combination**s** had measurements of both mid-day (23/02/2021) and pre-dawn (24/02/2021) leaf Ψ taken before removal, all within one day during February 2021. Measurement of mid-day (22/04/2021) and pre-dawn (23/04/2021) leaf Ψ was repeated on the 15 remaining *S. myrtina* individuals after two months of removal of the large *V. karroo* partners. To compare *S. myrtina* leaf Ψ with and without large *V. karroo,* and determine the effect of a large nurse *V. karroo* on different sizes of the target species *S. myrtina* leaf Ψ, the pairwise Relative Interaction Intensity (RII) index was calculated (Armas *et al*., 2004). Linear models were constructed using RII for mid-day and pre-dawn leaf Ψ as a function of *S. myrtina* size. RII: X = S. *myrtina* leaf Ψ (kPa), RII = (X with neighbour – X without neighbour) / (X with neighbour + X without neighbour), where X with neighbour and X without neighbour are the values of the target Ψ variable measured in the presence and absence of a large nurse *V. karroo* (similar to Noumi, 2020). The interpretation of values is that if RII > 0 there was positive interactions and if RII < 0 they were negative. Thus, if leaf Ψ with large *V. karroo* present < leaf Ψ with *V. karroo* removed, this would indicate that removal resulted in increased water stress in *S. myrtina* (leaf Ψ becomes more negative) and the presence of *V. karroo* had a positive effect on *S. myrtina* leaf Ψ.

4.4. Data analysis

4.4.1. Soil: sub-canopy vs. inter-canopy

Analysis of soil data was carried out using the statistical software R v.1.0.136 (R Core Team, 2020). Shapiro-Wilks normality test was done for both SWC and BD to determine if the data is normally distributed. Paired Welch's two-sample *t*-tests were performed (package "stats", functions: "t-test") to test for differences in soil water content and bulk density between inter-canopy and sub-canopy spaces. To compare the RII (inter-canopy vs. sub-canopy) between all pairwise combinations and test whether the effects of variable pair-size combinations on both SWC and BD differ significantly, linear models (LM's) were performed. Linear models were used to test for differences between species-pair combinations and both SWC and BD RII (inter-canopy vs. sub-canopy). Each linear model was constructed with response variables (SWC or BD RII) as a function of two predictor variables, *V. karroo* size and *S. myrtina* size (Winter, 2013). The formula is thus: response variable ~ predictor variable 1 + predictor variable 2 + ε (Winter, 2013). Tukey's honest significance difference test was performed on each LM to determine where the significant difference had occurred, if applicable.

4.4.2. Leaf Ψ of S. myrtina and V. karroo

Shapiro-Wilks normality test was done for each response variable (*V. karroo* Ψ and *S. myrtina* Ψ) and confirmed that data was normally distributed. To test whether the effects of variable pair-size combinations on either pre-dawn leaf Ψ or mid-day leaf Ψ differ significantly, LM's were performed. Each LM was constructed by either focussing on *V. karroo* or *S. myrtina* leaf Ψ as a function of a specific pair-size combination, see Table 4.1. Tukey's HSD were then performed on each of the LM's to determine where the significant differences occurred.

Table 4.1: Leaf Ψ linear model (LM) summary. Linear models focusing on *V. karroo* leaf Ψ show the effect of *S. myrtina* size and the effect of *V. karroo* size in the presence or absence of small *S. myrtina*. Linear models focusing on *S. myrtina* leaf Ψ show the effect of *V. karroo* size and the effect of *S. myrtina* size in the presence or absence of large *V. karroo*.

Focusing on *V. karroo*	**Linear model formula**
Effect of *S. myrtina* size	Large *V. karroo Ψ ~ S. myrtina* size
Effect of *V. karroo* size	*V. karroo* Ψ ~ V. *karroo* size * p/a small *S. myrtina*
Focusing on *S. myrtina*	**Linear model formula**
Effect of *V. karroo* size	Small *S. myrtina Ψ ~ V. karroo size*
Effect of *S. myrtina* size	*S. myrtina Ψ ~ S. myrtina* size * p/a large *V. karroo*

4.4.3. Removal of large V. karroo

Shapiro-Wilks normality test was done on mid-day and pre-dawn leaf water potentials (kPa) measured before and after removal to confirm that data was normally distributed. To determine the effects of *S. myrtina* size and removal of large *V. karroo* on *S. myrtina* (small, medium and large) leaf Ψ, linear mixed effects analyses were performed (see Winter, 2013). R software package ‘lme4’ (Bates *et al*., 2012) and the function ‘lmer’ (effectively repeated measures models) were used to construct models with pre/post removal and *S. myrtina* size as fixed effects and individual tree ID as the random effect for mid-day and pre-dawn *S. myrtina* leaf Ψ. The null model had pre/post removal and *S. myrtina* size as fixed effects and tree ID as the random effect. Residual plots of this ‘full’ model were visually inspected and did not reveal any obvious deviations from homoscedasticity or normality. To test for significance of pre/post removal and *S. myrtina* size, two reduced models were created, each leaving out the variable of interest, and compared to the null model using the ‘anova’ function as a likelihood ratio test (Winter, 2013). Thus, p-values were obtained by likelihood ratio tests between the ‘full’ model (with the variable in question) against the model without the variable in question.

4.5. Results

4.5.1. Soil: sub-canopy vs. inter-canopy

Soil water content was significantly higher in the sub-canopy compared to the inter-canopy (t = -2.24, df = 43, p = 0.03; Fig. 4.2A). Bulk density was significantly higher in the inter-canopy compared to under the sub-canopy (t = 4.9917, df = 43, p = < 0.0001; Fig. 4.2B).

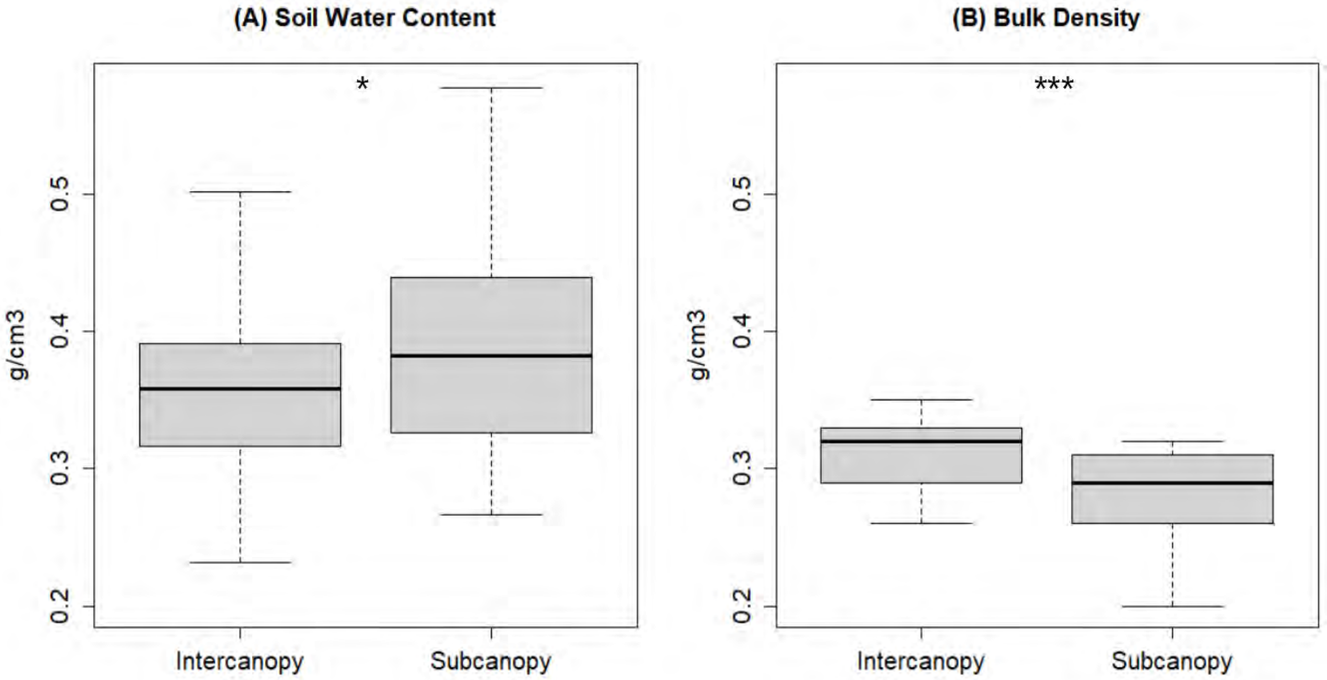

Figure 4.2. Inter vs sub-canopy soil water content (A) and bulk density (B) for all pairs combined. Significance levels indicated with: * = p < 0.05, ** = p < 0.01, *** = p < 0.001.

Neither *V. karroo* size nor *S. myrtina* size had a significant effect on the magnitude of the difference in soil water content (measured as the RII) in the sub- or inter-canopy (F (10,33) = 0.87, p = 0.56; Fig. 4.3A–C). Similarly, neither *V. karroo* size nor *S. myrtina* size had a significant effect on the magnitude of the difference in bulk density (measured as the RII) in the sub- or inter-canopy (F (10,33) = 1.35, p = 0.24; Fig. 4.3D–F).

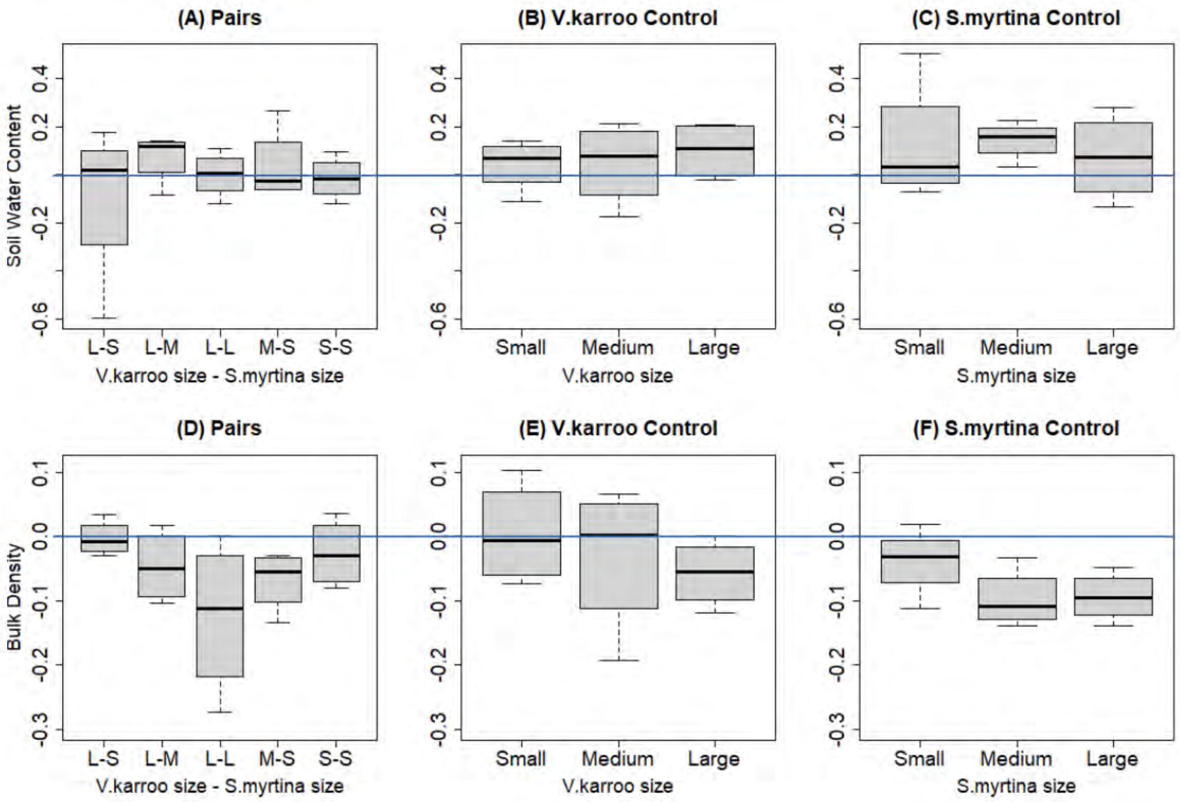

Figure 4.3: Effect size of trees on soil water content (RII of inter-canopy vs. sub-canopy) for (A) paired combinations, (B) *V. karroo* controls and (C) *S. myrtina* controls; and bulk density (RII of inter-canopy vs. sub-canopy) for (D) paired combinations, (E) *V. karroo* controls and (F) *S. myrtina* controls. The blue horizontal lines indicate where RII = 0. Positive values (above the blue line) indicate that SWC in the sub-canopy > inter-canopy, and vice versa.

4.5.2. Leaf Ψ of S. myrtina and V. karroo

Scutia myrtina size had no significant effect on large *V. karroo* pre-dawn leaf Ψ ($F = 1.94$, $p = 0.14$; Fig. 4.4A). However, *S. myrtina* size had a slightly significant negative effect on large *V. karroo* mid-day leaf Ψ ($F = 3.79$, $p = 0.02$; Fig. 4.4B). The presence of *S. myrtina* of any size reduced mid-day water leaf Ψ (more water stress) of large *V. karroo* compared to large *V. karroo* control individuals with no *S. myrtina* partners.

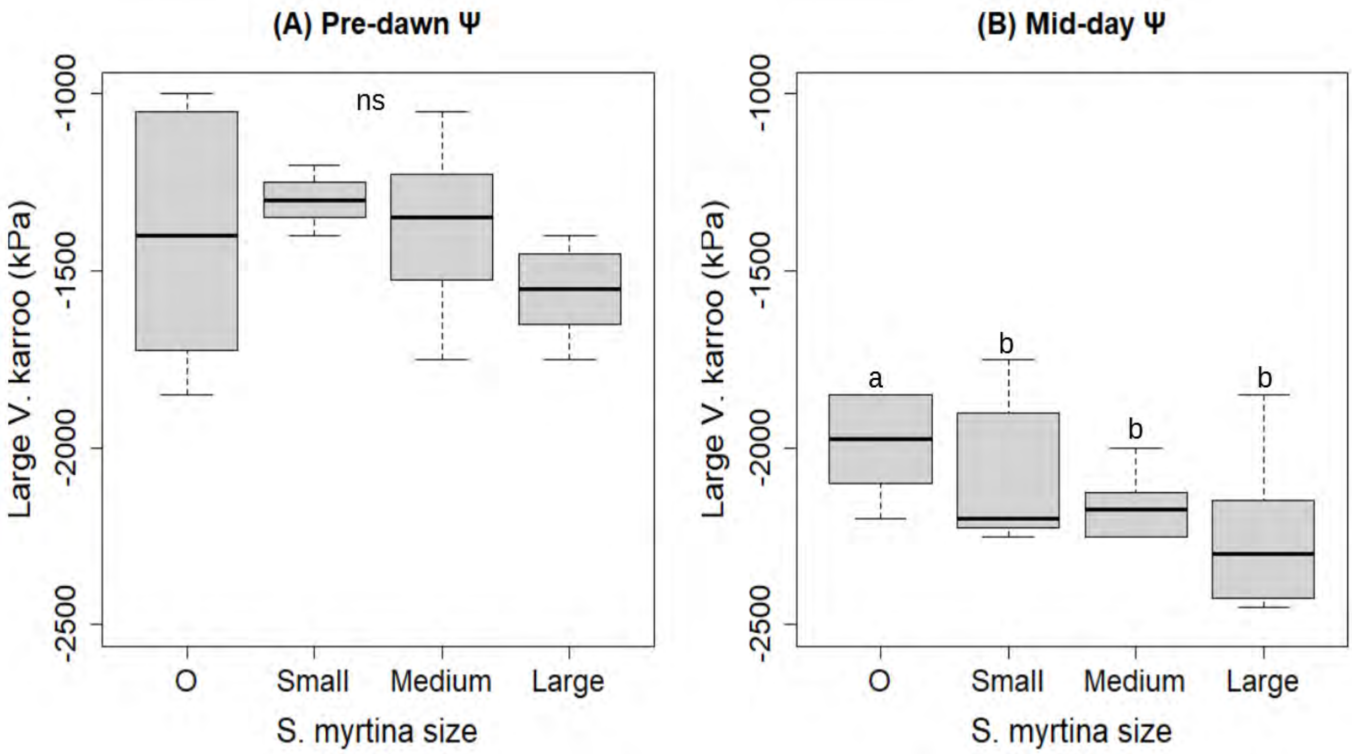

Figure 4.4: Large *V. karroo* leaf Ψ (control individual with no partner 'O') with increasing *S. myrtina* size-combinations (small, medium and large) for both (A) pre-dawn and (B) mid-day leaf Ψ. The more negative the kPa value, the more water stressed the individuals. When post hoc letters are the same there is no significant difference.

The presence of small *S. myrtina* had no significant effect on *V. karroo* pre-dawn leaf Ψ (F (5,42) = 3.7, p = 0.007; Fig. 4.5A). The presence of small *S. myrtina* did not lead to greater water stress, and had no significant effect on *V. karroo* mid-day leaf Ψ (F (5,42) = 2.46, p = 0.06; Fig. 4.5B).

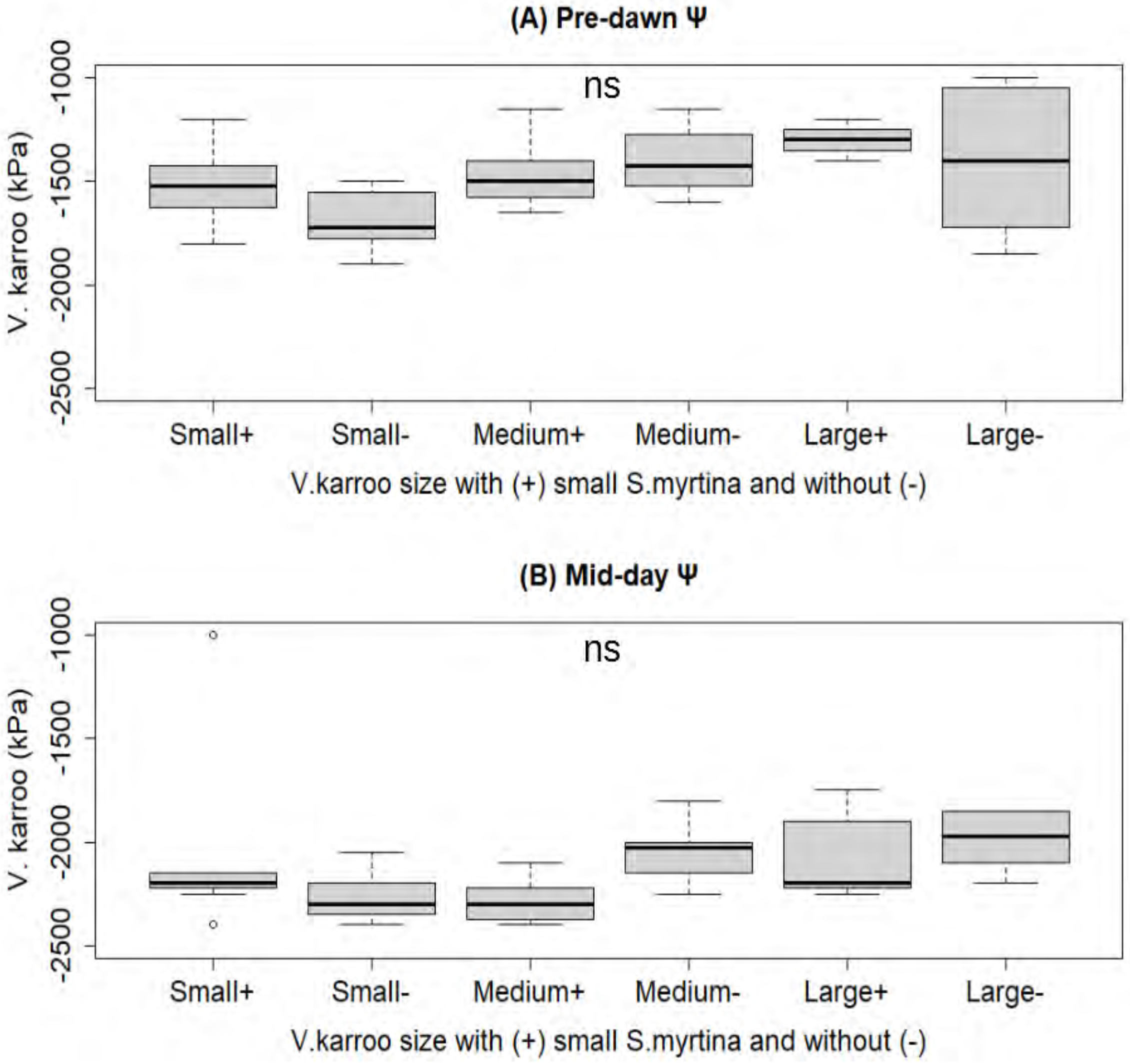

Figure 4.5: Leaf Ψ of small, medium and large *V. karroo* growing in association with (+) small *S. myrtina* and without (-) for both (A) pre-dawn and (B) mid-day leaf Ψ.

Vachellia karroo size had a significant effect on small *S. myrtina* pre-dawn leaf Ψ ($F = 3.6$, $p = 0.02$; Fig. 4.6A). Specifically, small *S. myrtina* with a large *V. karroo* partner had less negative pre-dawn leaf Ψ than *S. myrtina* controls or *S. myrtina* growing with small or medium *V. karroo*. *Vachellia karroo* size had a significant positive effect on small *S. myrtina* mid-day leaf Ψ ($F = 7.05$, $p = 0.001$; Fig. 4.6B), specifically small *S. myrtina* growing with a small or large *V. karroo* partner had less negative mid-day Ψ while small *S. myrtina* with a medium *V. karroo* partner did not significantly differ from any of the combinations or control.

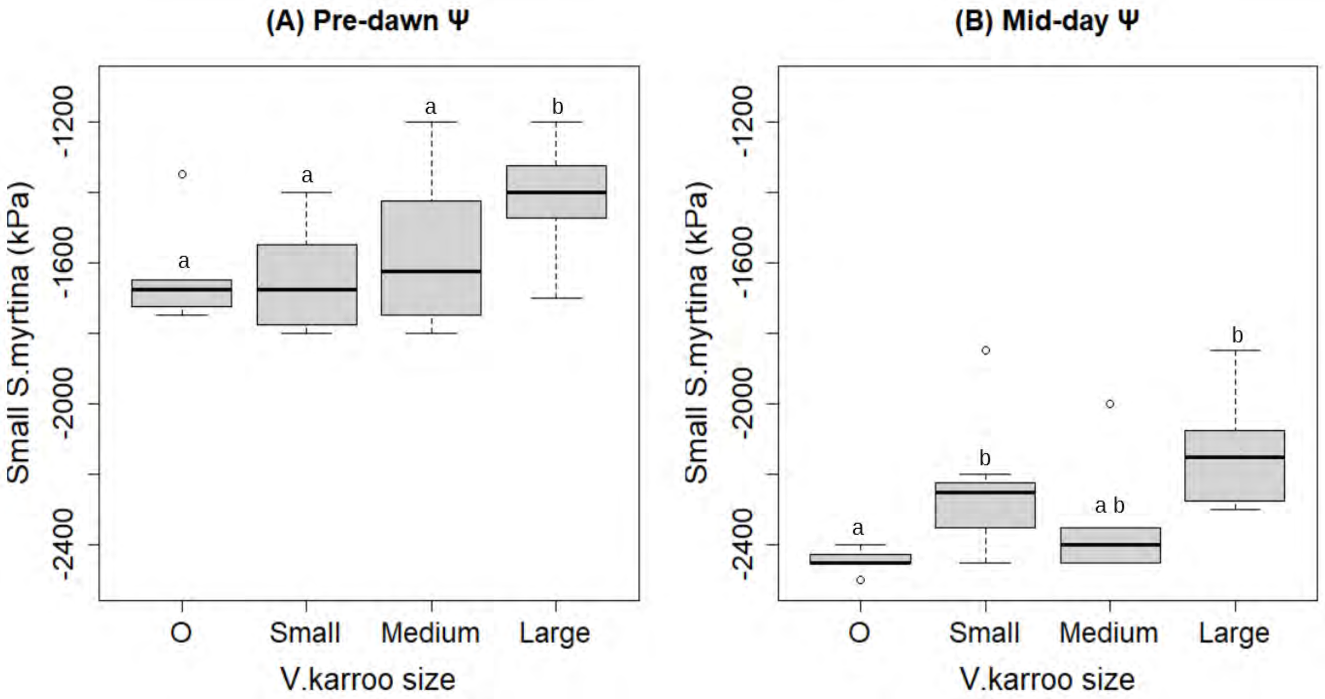

Figure 4.6**:** Small *S. myrtina* leaf Ψ (control individual with no partner 'O') with increasing *V. karroo* size-combinations (small, medium and large) for both (A) pre-dawn and (B) mid-day leaf Ψ. When post hoc letters are similar there is no significant difference.

The presence of large *V. karroo* had a significant effect on *S. myrtina* pre-dawn leaf Ψ (*F* (5,42) = 4.82, *p* = 0.001; Fig. 4.7A). Small *S. myrtina* growing with large *V. karroo* had the least negative pre-dawn Ψ, reflecting higher soil water availability. Large *V. karroo* also had a significant positive effect on *S. myrtina* mid-day leaf Ψ (*F* = 6.82, *p* = 0.0005; Fig. 4.7B), specifically on small *S. myrtina.*

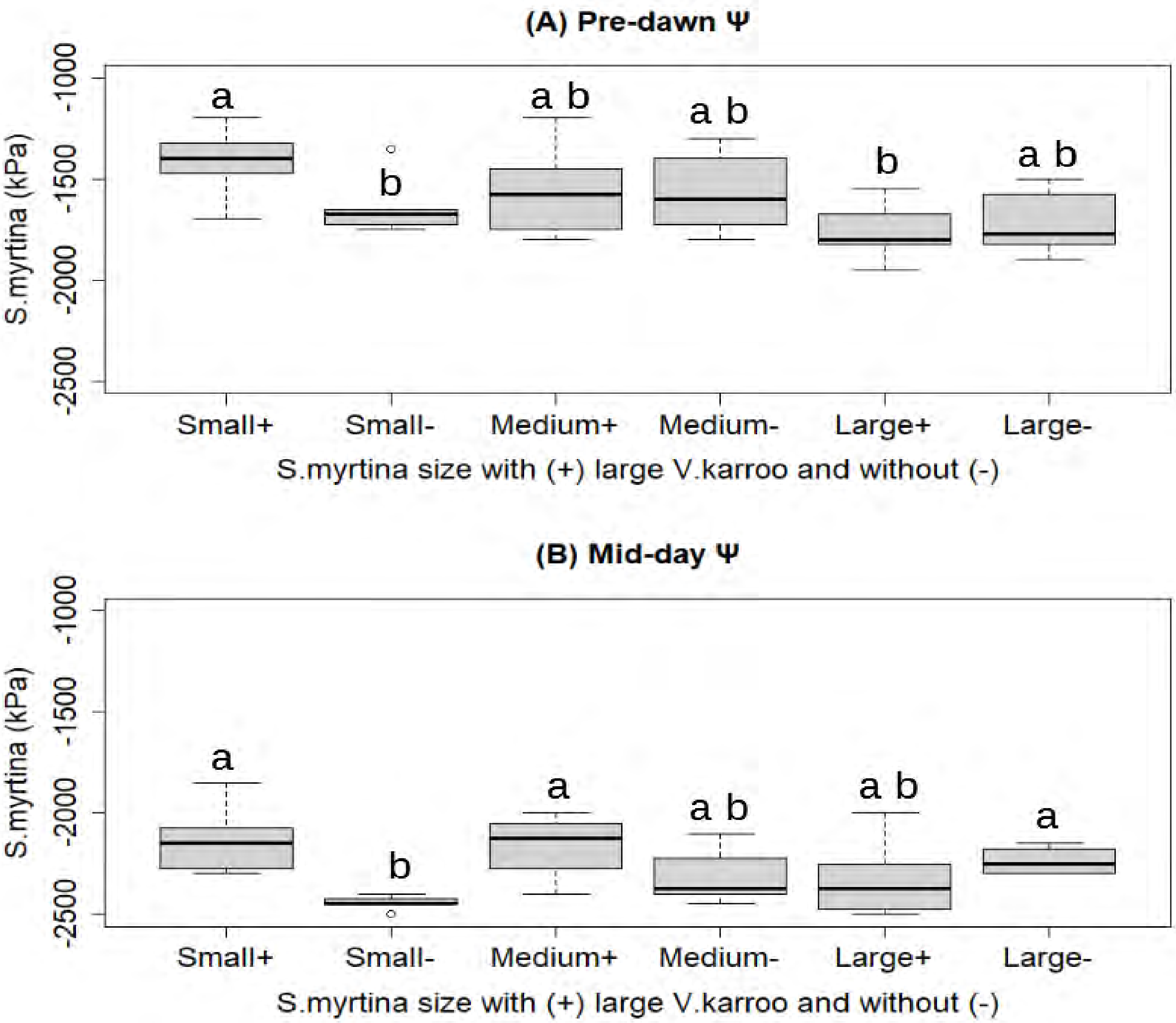

Figure 4.7: *Scutia myrtina* leaf Ψ (small, medium and large) with (+) large *V. karroo* and without (-) for both (A) pre-dawn and (B) mid-day leaf Ψ. Similar letters indicate no significant difference.

4.5.3. Removal of large V. karroo

There were no significant effects of removal of large *V. karroo* (χ^2 (1) = 0.31, $p = 0.57$) or *S. myrtina* size (χ^2 (2) = 5.52, $p = 0.06$; Fig. 4.8, A) on *S. myrtina* pre-dawn leaf Ψ. There was also no significant interaction between removal and *S. myrtina* size (χ^2 (2) = 1.03, $p = 0.59$). There were no significant effects of *V. karroo* removal on *S. myrtina* mid-day leaf Ψ (χ^2 (1) = 1.91, $p = 0.16$). The size of *S. myrtina* significantly affected mid-day leaf Ψ (χ^2 (2) = 20.28, $p = < 0.0001$; Fig. 4.8, B) with smaller *S. myrtina* showing less water stress irrespective of removal treatment. There was no significant interaction between removal and *S. myrtina* size (χ^2 (2) = 5.25, $p = 0.07$).

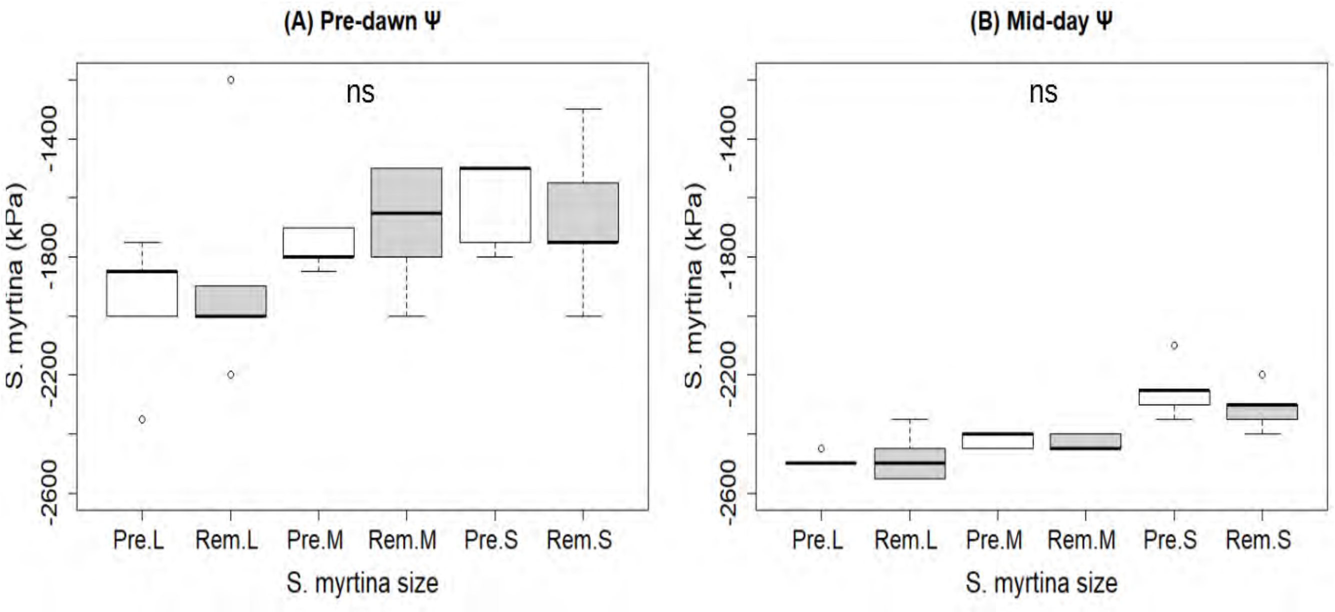

Figure 4.8: *Scutia myrtina* (small, medium and large) leaf Ψ before vs. after removal of large *V. karroo* for both (A) pre-dawn and (B) mid-day leaf Ψ. Pre = before removal, Rem = after removal. L = large, M = medium, S = small.

Scutia myrtina size had no significant effect on the magnitude of the difference in pre-dawn leaf Ψ (expressed as the RII) in the same individuals before and after removal (*F* (2,12) = 0.42, *p* = 0.66; Fig. 4.9, A). *Scutia myrtina* size also had no significant effect on the magnitude of the difference in mid-day leaf Ψ RII before and after removal (*F* (2,12) = 2.88, *p* = 0.09; Fig. 4.9, B). RII overall is close to zero (with some variation) but in the case of mid-day RII, it is slightly < 0 for all individuals indicating that large *V. karroo* caused slightly more water stress as reflected in more negative *S. myrtina* mid-day leaf Ψ.

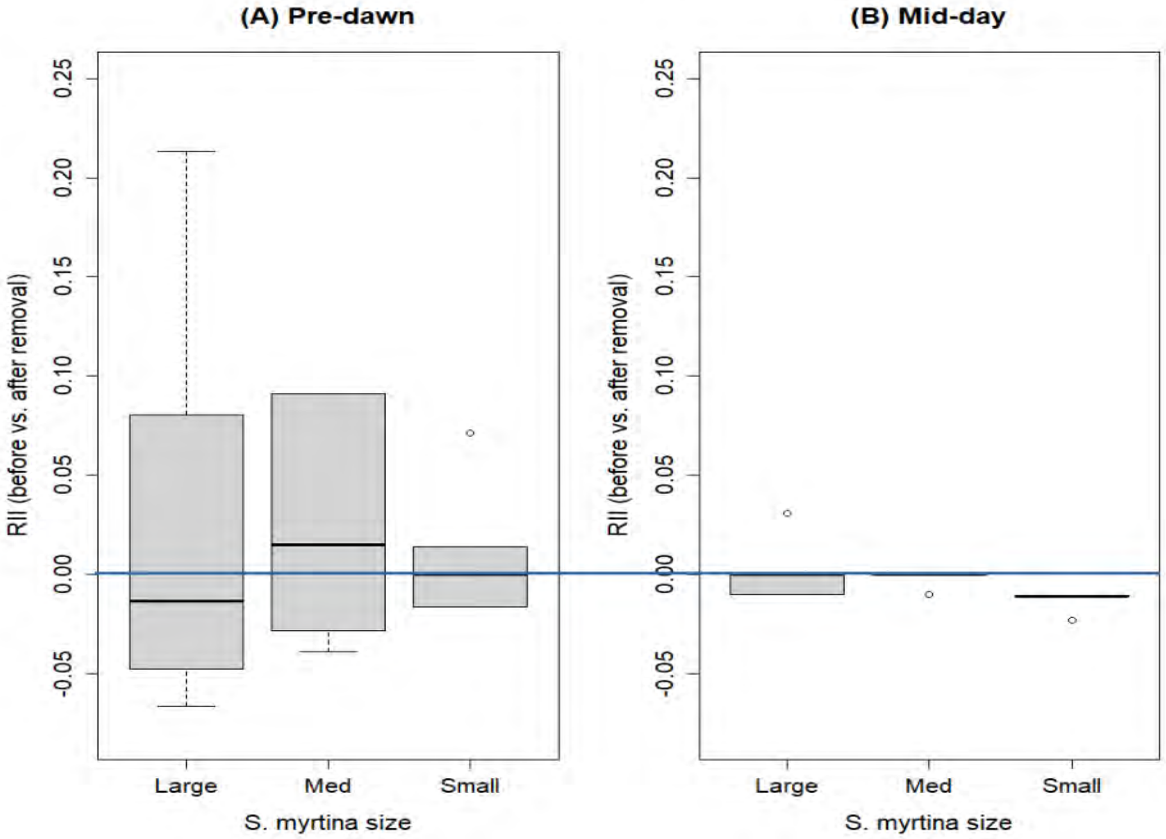

Figure 4.9: Effect size of the removal of large *V. karroo* on *S. myrtina* leaf Ψ (RII of before vs. after removal) for both (A) pre-dawn leaf Ψ and (B) mid-day leaf Ψ. Interpretation of RII values can be when RII < 0, Ψ became more negative after removal (more water stressed).

4.6. Discussion

4.6.1. Soil: sub-canopy vs. inter-canopy

I found that the presence of the different combinations of *Scutia myrtina* and *Vachellia karroo* individuals affected bulk density and available water content. I found significant differences between inter-canopy and sub-canopy soil moisture and bulk density between different pair-size combinations and individual controls of each species. As predicted, soil water content was higher under sub-canopies compared to inter-canopy spaces for both paired and single control individuals, providing evidence for micro-climactic effects of tree canopies on soil properties such as water content (Holdo & Mack, 2014; Pugnaire *et al.*, 2011; Rodriguez-Iturbe *et al.*, 1999). My results also confirmed lower bulk density under tree canopies compared to inter-canopy spaces for both paired and control individuals, showing that both tree canopy effects and roots are able to change soil properties under sub-canopy areas

(Aweto & Dikinya, 2003; Holdo & Mack, 2014; Pugnaire *et al.*, 2011). Contrary to my predictions, neither *V. karroo* size nor *S. myrtina* size had a significant effect on the magnitude of the difference in soil water content (SWC) or bulk density (BD) measured as RII.

It was expected that soil water content would be lowest under the sub-canopies of large *S. myrtina* and large *V. karroo* pairs due to high rates of transpiration as trees increase in size, however soil water content showed no significant differences between large-large and small-small pair-size combinations, possibly due to high precipitation a week before sampling. It was also expected that the highest bulk density would be under the sub-canopies of large *S. myrtina* and large *V. karroo* pairs, although there were no significant differences between large-large and small-small pair-size combinations, possibly also due to high precipitation. Large-large combinations had the most negative mean value (measured as RII) of all pair-size combinations, implying that pairs of large individuals had the greatest effects on soil bulk density, possibly due to having the greatest zone of influence.

4.6.2. Leaf Ψ of S. myrtina and V. karroo

My results confirmed that differences in species-size combinations can affect plant available water, as expressed by significant differences in both pre-dawn and mid-day leaf Ψ for both *V. karroo* and *S. myrtina* individuals growing in pair-size associations. Pre-dawn leaf Ψ is a reflection of available soil water at the depth at which the individual plant accesses their water resources. The effects of different species-size combinations on available soil water (pre-dawn leaf Ψ) showed that *S. myrtina* size had no effect on *V. karroo* soil water availability. However, *V. karroo* size did have effects on available soil water to small *S. myrtina*, but not medium or large. Small *S. myrtina* had the highest soil water availability when growing under a large *V. karroo,* showing the direct benefit for establishing under *V. karroo* (similar to Barnes & Archer, 1999).

Mid-day leaf Ψ is a measure of water stress, which can reflect competition for water resources (Barnes & Archer, 1999). Presence of *S. myrtina* had negative effects on *V. karroo* mid-day leaf Ψ (more water stress when growing with *S. myrtina*), and *V. karroo* had positive effects on small *S. myrtina* mid-day leaf Ψ (least water stress when growing under large *V. karroo*, compared to medium or small). Large *V. karroo* control individuals were found to have lower mid-day leaf Ψ (less stressed) compared to large *V. karroo* growing with any size combination of *S. myrtina.* Results also confirmed that small *S. myrtina* without a *V. karroo* partner was more water stressed (higher mid-day leaf Ψ) compared to a small *S. myrtina* with *V. karroo* partner. These effects could be due to micro-climatic conditions created by canopy area, such as protection from thermal stress, reduction in photoinhibition or water loss though evaporation and evapotranspiration (Pugnaire *et al*., 2011). It was hypothesized that large *S. myrtina* growing with large *V. karroo* would be the most water stressed (most negative mid-day leaf Ψ) pair-size combination, however there was no significant differences found between large-large and large-small combinations; yet some of the most negative leaf Ψ sample values were found in large-large combinations. These results may have been more conclusive under drier conditions, or by using larger sample sizes by making use of more replications of target pair-size combinations. Overall, the effect of species-size associations on soil water resource availability revealed by the pre-dawn leaf Ψ translated into water stress differences in mid-day leaf Ψ, especially positive and negative effects of pairwise tree interactions between small *S. myrtina* associations with large *V. karroo*.

4.6.3. Removal of large V. karroo

There were no significant effects of removing large *V. karroo* individuals on *S. myrtina* mid-day or pre-dawn leaf Ψ. However, *S. myrtina* size was highly related to mid-day leaf Ψ, with larger trees being more water stressed than smaller trees. The RII overall was close to zero (with some variation) indicating that associations are neutral interactions, but mid-day RII vales were slightly < 0 for all individuals indicating that *V. karroo* had a slight positive effect on *S. myrtina* mid-day leaf Ψ (facilitation). This suggests that *V. karroo* had provided canopy protection, possibly through shading mechanisms that affected incoming radiation and thermal stress (see Holdo & Mack, 2014). Selective removal to examine the effect of large *V. karroo* on the leaf water potential of *S. myrtina* only used five replicates of the three pair-size classes; increasing the number of replicates might have revealed a clearer distinction.

4.7. Conclusion

My results confirmed that small *S. myrtina* was facilitated by establishing and growing in the sub-canopy of large *V. karroo* individuals, through mechanisms affecting soil water content, bulk density and leaf Ψ. The effects of *S. myrtina* establishing in association with other size classes of *V. karroo* were less evident. This result emphasises the role of established *V. karroo* trees in facilitating the establishment of thicket pioneers. Results agree with other accounts of *V. karroo* being one of the most prolific and widespread encroaching species that acts as a nucleator species for thicket clump expansion, bush clump formation and savanna thickening (similar to Balfour & Midgley, 2008; Parr *et al*., 2012). After recruitment and establishment of thicket species there is a switch to competition and eventual mortality of the *V. karroo* host, mechanisms underlying this process could be studied further in future research. However, large *S. myrtina* showed significant competitive interactions with *V. karroo*.

Chapter 5: Discussion

This research set out to disentangle species-associations and their effects throughout bush clump succession. To this end, I examined where thicket pioneers recruit and the abiotic properties of their microsites that affect water stress and nutrient availability at different stages of the temporal encroachment sequence. This was to understand the associations, patterns and interactions during this process; reinforcing short- and long-term effects of associations (competitive and facilitative mechanisms) that ultimately modify the environment to cause a biome shift.

5.1. Species associations and the encroachment sequence

Vachellia karroo was the most commonly occurring and abundant species throughout the encroachment sequence (Chapter 2), recruiting and increasing in abundance in stages 1 and 2 without any facilitation by established trees. In contrast, the other woody species (such as *Scutia myrtina*, *Gymnosporia buxifolia*, *Olea europaea* ssp. *africana*, *Searsia longispina* and *Ziziphus mucronata*) recruited mainly in stages 2 and 3 and were strongly associated with the sub-canopy of large *V. karroo*. In the latest stages of encroachment, these thicket pioneer species also recruited in association with other thicket species, increasing in density to form a closed canopy state. Species-associations showed that *V. karroo* dominated the large majority of all species-associations, particularly in the earlier stages. However, associations with *V. karroo* became less prominent in stage 3 due to associations between other species, and possibly mortality of old *V. karroo* individuals in mature clumps. Association rule analysis further revealed *V. karroo* to invade first and provide nucleation sites for thicket clumps as it dominates most species-associations in the first stages, with *S. myrtina* being the earliest and most abundant pioneer. These two species are thus of most interest from both an ecological and management point of view, being key to initiating and facilitating a biome shift from open savanna to a closed canopy thicket.

Here, there is a link to trends in woody vegetation structure, composition, and its effects on light transmittance and the herbaceous layer, thereby confirming Khoza's (2021) LAI thresholds at Endwell farm relating to transitions in vegetation structure. The first threshold with a canopy cover of ~ 40% (LAI < ~ 0.5, light transmittance ~ 75%) occurs at stage 2, where thicket species start to recruit more and dominant C4 grasses start to decline in abundance as they start to get shaded out. The second threshold occurs at stage 3 and indicates a species compositional shift between savanna and closed-canopy vegetation states. This second threshold occurs ~50% light transmittance (LAI ~ 1 and canopy cover ~70%) at which point thicket species are starting to amalgamate and savanna switches to a thicket with a marked increase in thicket species-associations and density causing a complete reduction in C_4 grasses (Khoza, 2021).

My findings include a predictable sequence of bush clump formation, allowing managers to anticipate the progression and outcome of bush encroachment and planning interventions, such as removal or thinning of *V. karroo* or *S. myrtina* during early stages to maintain an open system state. Specifically, action must be taken between the transition from stage 1 to stage 2. Optimally, this should be done before the first LAI threshold gets reached at stage 2 (canopy cover of ~ 40%), otherwise thicket species start to recruit more rapidly, and the dominant C_4 grasses start to decline in abundance as they start to get shaded out, making management exponentially harder. It is vital that action must be taken before the second LAI threshold is reached (canopy cover ~70%), as at this point there is a serious reduction in C_4 grasses and changes in vegetation composition (change in system state) that is most likely irreversible.

Tree species traits change once the second threshold is reached, altering the disturbance regimes as densities of relatively fire-intolerant, more shade-tolerant, broad-leaved thicket species increase (Khoza, 2021; Parr *et al*., 2012). Charles-Dominique *et al*. (2018) also showed how ecological thresholds of canopy closure separate biomes through species traits that separated forest from thicket and savanna and where grass composition shifted. These changes in species composition shift the fire suppression threshold (where flammable C4 grass biomass becomes insufficient to carry a fire; Hoffman *et al*., 2012) rendering fire much less effective as a management tool to reduce tree cover.

5.2. Changing interactions along the encroachment gradient

Association-interactions between *Vachellia karroo* and *Scutia myrtina* was found to affect soil and foliar nutrient status (chapter 3), with higher plant leaf nutrients and sub-canopy soil nutrients under trees, particularly under combinations with large *V. karroo*. There was also evidence for a shift from facilitation of small young pioneers during early stages, to competition of large adults during late stages, similar to findings by Barnes & Archer (1999). Soil C:N ratios were highest, and N:P lowest, under the sub-canopy of large *V. karroo* and large *S. myrtina* pair-size combinations. Soil nutrient content (inter- vs. sub-canopy) also showed that *V. karroo* size had a significant effect on the magnitude of the difference in soil P. Pair-associations showed that all *S. myrtina* size-classes lowered *V. karroo* foliar N, P and K. Simultaneously, small *S. myrtina* foliar N, P and K was significantly increased by growing in association with large *V. karroo* partners. Generally, *S. myrtina* was found to be most facilitated by establishing and growing next to a large *V. karroo* individual, through mechanisms affecting soil and foliar nutrients.

These results are similar to Carrillo-García *et al.* (2000) and Gómez *et al.* (2005) that focus on the modification of soil phosphorous and potassium as the main nutrient facilitation mechanism in systems where drought is the limiting factor for plant survival. However, *S. myrtina* establishing in association with other size classes of *V. karroo* were much less facilitated, showing no effect (possibly due to competition). The key finding includes an increase in localized nutrient enrichment under tree canopies providing facilitation and directly affecting ecosystem services, functioning and productivity as density increases (Scholes & Archer, 1997; O'Connor *et al*., 2014). Leguminous acacia trees have been shown to increase the forage quality, yield, tissue nitrogen and the amount of nutrients in herbaceous sub-canopy forage, however digestibility decreases as lignin increases (Belsky, 1992b). These changes in soil nutrients are likely to lead to long term effects that can persist, even after bush clearing for some time, due to slow nutrient cycling during dry periods (Schmidt *et al*., 2010). Implications for management could include feedbacks involving disturbances such as fire or herbivory (higher stocking rates of grazers and browsers), two key factors that structure semiarid to mesic savannas and thicket clump formation (Calabrese *et al*., 2010). Measures to reduce bush density (fire, browsing or other forms of clearing) are recommended at earlier stages (before stage 2) in the encroachment process to reduce the 'legacy effects' of species on soil nutrients (Schmidt *et al*., 2010).

5.3. Interactions on available plant water

Vachellia karroo ameliorated thicket pioneer species growing under their canopy through increased soil and plant water status (Chapter 4). Removal of large *V. karroo* individuals also showed slight indications of nurse plants effects on pioneer species. Combinations of *S. myrtina* and *V. karroo* individuals affected both bulk density and available water content between inter-canopy and sub-canopy spaces, also between different pair-size combinations and individual controls of each species. Soil water availability gets less under large *V. karroo* individuals, with large-large combinations having the least amount of soil water content of all pair-size combinations, implying that pairs of large individuals had the greatest effects on soil moisture availability. Increased soil water content under large *V. karroo* is likely to enhance seedling establishment, reinforcing the association-pattern of bush clump formation in Chapter 2. This is a particularly important mechanism for establishment in arid or infertile environments where seedling establishment is critical in bush clump succession, as seed germination of pioneer species is influenced by the micro environments provided by larger trees (Belsky, 1994; Nishi & Tsuyuzaki, 2004; Pugnaire *et al.*, 2011). Here, relationships between dispersal and facilitation may go hand-in-hand by overcoming environmental limitations (Abreu *et al.*, 2021; Schleicher *et al.*, 2011a, 2011b).

Differences in species-size combinations also affected both pre-dawn and mid-day leaf Ψ for both *V. karroo* and *S. myrtina* individuals. The effects on available soil water (pre-dawn leaf Ψ, a proxy for soil water content) showed that large *V. karroo* facilitated small *S. myrtina*, these combinations had high soil water and low Ψ, demonstrating the direct benefit of establishing under a *V. karroo* sub-canopy. Associations with *S. myrtina*, however, had negative effects on *V. karroo* (more water stress when growing with *S. myrtina*), while *V. karroo* had positive effects on small *S. myrtina* (least water stress when growing under large *V. karroo*). Species-size association effects revealed by pre-dawn leaf Ψ, translated into water stress differences in mid-day leaf Ψ, especially positive and negative effects of pairwise tree-tree interactions between small *S. myrtina* associations with *V. karroo*. There were, surprisingly, no differences between pre-/post removal of large *V. karroo* individuals in pair-size associations, possibly due to low replications and high precipitation events leading up to when data was collected. Analysis showed that large *V. karroo* had a slight positive/ facilitative effect on sub-canopy *S. myrtina* mid-day leaf Ψ. These results emphasise the role of established *V. karroo* trees in facilitating the establishment of thicket pioneers, acting as a nucleation species for thicket clump expansion and bush clump formation through altering both nutrient and water availability.

Similar results have been found in other systems such as in Brazilian savanna (Cerrado) with neotropical savanna trees, where both water use and water status are regulated by a combination of plant physiological and architectural traits that affect microsite conditions (Bucci *et al.*, 2005). There are also similarities to sapling survival in wet–dry savanna in northern Australia, where canopy cover causes variation in microclimate (temperature and vapor pressure deficit), light environment and pre-dawn leaf Ψ (Duff *et al.*, 1997). Decreases in canopy cover caused decreased soil moisture, while increases in canopy cover increased pre-dawn water potential, which were independent of rainfall (Duff *et al.*, 1997). The interaction between both increased nutrients and water could have serious consequences for multiple systems in the face of drought and climate change. For example Lohmann *et al.*

(2012) parameterized an ecohydrological savanna model that assessed impacts of climate change on a semi-arid African savanna, focusing on effects of temperature and CO_2 level increase in combination with changes in inter- and intra-annual precipitation on the long-term dynamics of savanna trees. Their main findings showed that the capacity of the savanna to sustain livestock was strongly reduced by climate change, due to decreased precipitation, higher interannual variation of temperature increase that led to severe losses of the biomass of perennial grasses. According to Lohmann *et al*. (2012), semi-arid rangelands will be at lower risk of shrub encroachment in spite of elevated levels of atmospheric CO_2 due to drought sensitivity of germination and establishment of the encroaching species. However, farm managers of semi-arid rangelands are advised to focus on grass species as indicators for rangeland degradation (Lohmann *et al*., 2012), specifically before stage 2 of encroachment where C_4 species start to decline drastically (Khoza, 2021).

5.4. Implications

It has been shown that some thicket pioneers benefit from savanna trees having positive associations (Moyo *et al*., 1998; O’Connor & Chamane, 2012; Scholes & Archer, 1997). Implications of water status and altered soil conditions are likely to have longer term effects, such as recruitment and increased species richness and diversity as clumps get larger through facilitation (O’Connor & Chamane, 2012), causing changes in vegetation structure and spatial ecology when densities reach their limits through competition (e.g., Mureva & Ward, 2016). Encroachment into savanna can further interfere with the hydrological budget, changing the amount of evapotranspiration, runoff, soil infiltration rates, deep drainage and moisture recharge (Archer *et al*., 2017). Management strategies should focus on intervention of invading species during early stages before there is a shift in abiotic microsite properties, species richness and diversity.

It has been proposed that there is a switch from facilitation to competition during later stages of encroachment. The stress gradient hypothesis predicts a higher frequency of facilitative interactions as resource limitation or other stress increases (O'Brien *et al*., 2017). In a similar semi-arid savanna system, the switch from facilitation to competition was in response to rainfall quantity (O'Brien *et al*., 2017). The magnitude of the switch depended on the amount of direct sunlight, as the hotter (drier) aspects under nurse plants showed competitive effects, while cooler (more shaded) aspects still showed facilitation interactions (O'Brien *et al*., 2017). In this study, small *S. myrtina* was most facilitated by establishing and growing under the sub-canopy of large *V. karroo*. Large *S. myrtina* showed significant competitive interactions with *V. karroo*, highlighting that after recruitment and establishment of thicket species there is a switch to competition and the *V. karroo* eventually die out. Competitive mechanisms of nutrients and water stress underlying the process of bush clump succession could be studied further in future conservation research.

Many areas that have woody encroachment do not experience thicket formation. The extent of thicket expansion (savanna thickening) in South Africa has not been estimated or mapped, a potentially key area of future research. Determining where thicket clump formation occurs and where it does not (and underlying mechanisms in different contexts) or whether local mean annual precipitation would alter this dynamic, could also be a key area of future research. Market basket analysis is a technique not commonly used and is still novel in ecology or for analysing plant relationships, exploration and further development of this approach may also be a useful tool in future research, overcoming challenges which exceeds the capability of traditional contingency-type analyses (Li, 2017). Endwell farm is a semi-arid savanna with a mean annual rainfall of 730 mm, suggesting that there is the climatic potential for woody canopy closure suitable for a vegetation shift from savanna to thicket vegetation indicated through bush clump formation. The applicability of these results extends to other arid and semi-arid savanna systems that have similar invading or encroaching species, high enough precipitation to allow for canopy closure and the lack of disturbances such as fire or herbivory.

Outcomes of these results for management may indicate a biome switch from open savanna to a closed canopy thicket if left unchecked, affecting ecosystem functioning, services and productivity. Findings show how interactions of species-associations cause spatial patterning, as well as, how interactions and outcomes change over time (competition and facilitation); critical for future management of the species responsible, while considering the ecological roles and the abiotic properties of their micro-sites. Thus, determining how and when these species co-occur (and effects of interactions) is critical to understanding, predicting past/ future patterns and changes in community biodiversity, nutrient distribution and available water resources.

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Appendix A

Table S1 [suppl.]; Fig. 2.3: inspection of all 12 association rules between species (all stages combined) according to the highest lift values.

Rule	LHS	-	RHS	Support	Confidence	Coverage	Lift
1	Scutia3	=>	Acacia4	0.04	0.86	0.04	4.75
2	Scutia4	=>	Acacia4	0.04	0.87	0.05	4.8
3	Scutia2	=>	Acacia4	0.05	0.87	0.06	4.82
4	Scutia1	=>	Acacia4	0.05	0.92	0.06	5.09
5	Scutia3, Scutia4	=>	Acacia4	0.02	0.9	0.02	4.97
6	Scutia2, Scutia3	=>	Acacia4	0.01	0.97	0.01	5.39
7	Scutia1, Scutia3	=>	Acacia4	0.02	0.93	0.02	5.16
8	Scutia2, Scutia4	=>	Acacia4	0.02	0.91	0.02	5.07
9	Scutia1, Scutia4	=>	Acacia4	0.02	0.9	0.02	5
10	Scutia1, Scutia2	=>	Acacia4	0.01	0.96	0.01	5.29
11	Scutia1, Scutia3, Scutia4	=>	Acacia4	0.01	0.9	0.01	4.99
12	Scutia1, Scutia2,Scutia4	=>	Acacia4	0.01	0.93	0.01	5.13

Table S2 [suppl.]; Fig. 2.4: inspection of the top 12 of 125 association rules between species for Stage 1 (early encroachment) according to the highest lift values.

Rule	**LHS**	**-**	**RHS**	**Support**	**Confidence**	**Coverage**	**Lift**
1	Olive3	=>	Acacia3	0.01	1	0.01	2.4
2	Olive3	=>	Acacia2	0.01	1	0.01	1.35
3	Searsia1	=>	Acacia4	0.01	1	0.01	2.74
4	Gymno1	=>	Acacia1	0.01	1	0.01	1.3
5	Gymno2	=>	Acacia1	0.02	1	0.02	1.3
6	Searsia2	=>	Acacia4	0.02	1	0.02	2.74
7	Searsia2	=>	Acacia2	0.02	1	0.02	1.35
8	Olive2	=>	Acacia4	0.02	1	0.02	2.74
9	Olive2	=>	Acacia2	0.02	1	0.02	1.35
10	Scutia3	=>	Acacia2	0.05	1	0.05	1.35
11	Scutia4	=>	Acacia2	0.06	0.94	0.06	1.28
12	Scutia1	=>	Acacia4	0.07	0.95	0.07	2.61

Table S3 [suppl.]; Fig. 2.5: inspection of the top 15 of 260 association rules between species for Stage 2 (intermediate encroachment) according to the highest lift values.

Rule	**LHS**	**-**	**RHS**	**Support**	**Confidence**	**Coverage**	**Lift**
1	Gymno4	=>	Acacia4	0.03	0.93	0.01	3
2	Gymno4	=>	Acacia4	0.01	1	0.01	3.78
3	Gymno4	=>	Acacia1	0.01	1	0.01	1
4	Olive2	=>	Acacia4	0.01	1	0.01	3.78
5	Olive3	=>	Acacia4	0.02	1	0.02	3.78
6	Gymno1	=>	Acacia3	0.03	1	0.03	1.04
7	Gymno3	=>	Acacia1	0.03	1	0.03	1.06
8	Gymno2	=>	Acacia2	0.04	0.94	1	1
9	Scutia3	=>	Acacia4	0.06	0.92	0.06	3.48
10	Scutia4	=>	Acacia4	0.07	0.96	0.07	3.65
11	Scutia1	=>	Acacia4	0.09	1	1	3.78
12	Gymno4, Scuti4	=>	Acacia4	0.01	1	0.01	3.78
13	Gymno4, Scuti4	=>	Acacia3	0.01	1	0.01	1.06
14	Acacia4, Gymo3	=>	Acacia3	0.01	1	0.01	1.06
15	Acacia1, Gymo2	=>	Acacia4	0.01	1	0.01	3.78

Table S4 [suppl.]; Fig. 2.6: inspection of the top 12 of 12094 association rules between species for Stage 3 (late encroachment) according to the highest lift values.

Rule	**LHS**	**-**	**RHS**	**Support**	**Confidence**	**Coverage**	**Lift**
1	Searsia3	=>	Scutia2	0.02	1	0.02	2.66
2	Searsia3	=>	Acacia3	0.02	1	0.02	1.97
3	Searsia3	=>	Acacia2	0.02	1	0.02	2.08
4	Searsia3	=>	Acacia4	0.02	1	0.02	1.63
5	Searsia2	=>	Gymno2	0.02	1	0.02	5.53
6	Searsia2	=>	Scutia1	0.02	1	0.02	3.13
7	Searsia2	=>	Scutia2	0.02	1	0.02	2.66
8	Searsia2	=>	Acacia3	0.02	1	0.02	1.97
9	Searsia2	=>	Acacia4	0.02	1	0.02	1.63
10	Zizi4	=>	Acacia4	0.02	1	0.02	1.63
11	Searsia4	=>	Acacia4	0.02	1	0.02	1.63
12	Zizi3	=>	Acacia4	0.04	1	0.04	1.63

Appendix B

Table S5. Results for all soil analysis showing linear models and Tukey’s HSD for each nutrient. Significant combination column has control abbreviated as ‘C’.

Soil fertility: Effect of *S. myrtina* size

Linear Model		**Output**			**Tukey’s HSD**		
Nutrient	***p***	**Significant size**	***t***	***p***	**Significant combination**	**diff**	***p***
N %	0.26						
P mg/L	0.09	Medium	2.32	0.02			
K mg/L	0.93						
Org.C %	0.05	Medium	2.38	0.02	Medium-large C	0.56	0.04
Ca mg/L	0.13	Small	-1.89	0.06			
Mg mg/L	0.27						
pH (KCL)	0.67						
Zn mg/L	0.67						
Mn mg/L	0.23						
Cu mg/L	<0.00	Small	-3.81	<0.00	Small-control	-4.06	<0.00
		Medium			Medium-control	-4.06	0.07

Soil fertility: Effect of *V. karroo* size

Linear Model		**Output**			**Tukey’s HSD**		
Nutrient	***p***	**Significant size**	***t***	***p***	**Significant combination**	**diff**	***p***
N %	0.10	Small	2.66	0.01			
		Medium C	2.15	0.03			
P mg/L	0.57						
K mg/L	0.65						
Org.C %	0.14	Small	2.32	0.02			
		Large	2.21	0.03			
Ca mg/L	0.18						
Mg mg/L	0.01	Small	-2.22	0.03	Small-large	-47.46	0.04
					Medium-medium C	56.8	0.05
pH (KCL)	0.44						
Zn mg/L	0.38						
Mn mg/L	0.34						

Table S6. Results for all foliar analysis showing linear models and Tukey's HSD for each nutrient. Significant combination column has control abbreviated as 'C'.

Focus on *V. karroo*: Effect of *S. myrtina* size							
Linear Model		**Output**			**Tukey's HSD**		
Nutrient	***p***	**Significant size**	***t***	***p***	**Significant combination**	**diff**	***p***
N %	< 0.05	Small	-4.32	< 0.05	Small-Control	-0.75	< 0.05
P %	< 0.05	Small	-4.19	< 0.05	Small-Control	-0.12	< 0.05
K %	< 0.05	Small	-4.13	< 0.05	Small-Control	-0.45	< 0.05
					Small-large	-0.44	0.03
					Small-medium	-0.4	0.05
Ca %	2.87E-05	Small	4.7	3.66E-05	Small-Control	0.5	< 0.05
					Small-large	0.67	< 0.05
					Small-medium	0.51	< 0.05
Mg %	0.94						
Na mg/Kg	0.83						
Zn mg/Kg	0.02	Small	-3.13	< 0.05	Small-Control	-7.13	0.01
Cu mg/Kg	< 0.05	Medium	-2	0.05	Small-large	2.46	0.03
		Small	2.51	0.01	Small-medium	3.24	< 0.05
Mn mg/Kg	4.39E-05	Small	4.89	2.08E-05	Small-Control	74.2	< 0.05
					Small-large	80.6	< 0.05
					Small-medium	75.6	< 0.05
Fe mg/Kg	< 0.05	Large	-2.09	0.04	Small-large	30	< 0.05
		Small	2.18	0.03			
Al mg/Kg	< 0.05	Medium	1.79	0.08	Small-Control	24.4	< 0.05
		Small	4.28	< 0.05	Small-large	31.33	< 0.05
Focus on *V. karroo*: Effect of *V. karroo* size							
Linear Model		**Output**			**Tukey's HSD**		
Nutrient	***p***	**Significant size**	***t***	***p***	**Significant combination**	**diff**	***p***
N %	9.90E-06	Medium	-3.98	< 0.05	Medium-large	-0.81	< 0.05
		Small	-4.14	< 0.05	Small-large	-0.84	< 0.05

P %	1.85E-06	Medium	-4.77	3.38E-05	Medium-large	-0.15	< 0.05
		Small	-4.71	4.07E-05	Small-large	-0.15	< 0.05
K %	6.46E-06	Medium	-4.32	< 0.05	Medium-large	-0.54	< 0.05
		Small	-4.45	8.74E-05	Small-large	-0.56	< 0.05
Ca %	2.40E-06	Medium	4.15	< 0.05	Medium-large	5.56	< 0.05
		Small	5.92	1.08E-06	Small-large	7.92	< 0.05
Mg %	0.6						
Na mg/Kg	0.29						
Zn mg/Kg	7.69E-05	Medium	-3.92	< 0.05	Medium-large	-10.2	< 0.05
		Small	-0.23	< 0.05	Small-large	-10.6	< 0.05
Cu mg/Kg	3.24E-08	Medium	7.16	2.79E-08	Medium-large	4.22	< 0.05
		Small	6.31	3.38E-07	Small-large	3.72	< 0.05
		Small Control	3.9	< 0.05	Small C-large	2.3	0.05
Mn mg/Kg	1.90E-12	Medium	9.36	6.15E-11	Medium-large	114.13	< 0.05
		Small	9.39	5.64E-11	Small-large	114.53	< 0.05
					Small C-small	-98	< 0.05
Fe mg/Kg	0.08	Small	2.52	0.01			
Al mg/Kg	< 0.05	Small	3.55	< 0.05	Small-large	29.26	0.01
					Small C-small	-39.4	< 0.05
Focus on *S. myrtina*: Effect of *S. myrtina* size							
Linear Model		**Output**			**Tukey's HSD**		
Nutrient	***p***	**Significant size**	***t***	***p***	**Significant combination**	**diff**	***p***
N %	1.80E-06	Small	4.68	4.40E-05	Small-large	0.74	< 0.05
P %	7.53E-05	Small	4.44	8.96E-05	Small-large	1.13	< 0.05
K %	2.73E-05	Small	4.52	6.99E-05	Small-large	0.42	< 0.05
Ca %	2.20E-05	Small	-5.1	1.27E-05	Small-large	-1.03	< 0.05
					Small-medium	-0.85	< 0.05
Mg %	0.06	Large C	-2.73	< 0.05			
Na mg/Kg	1.52E-05	Large-C	-3.25	5.13E-15	Large-large C	-521	0.02
		Small	0.18	< 0.05	Small-medium	553	< 0.05

Zn mg/Kg	< 0.05	Small	2.81	< 0.05	Small-medium	6.26	0.05
Cu mg/Kg	< 0.05	Small	-2.64	0.01	Small-medium C	-5.2	< 0.05
Mn mg/Kg	< 0.05	Small	-3.88	< 0.05	Small-large	-106.13	< 0.05
Fe mg/Kg							
Al mg/Kg							
Focus on *S. myrtina*: Effect of *V. karroo* size							
Linear Model		**Output**			**Tukey's HSD**		
Nutrient	***p***	**Significant size**	***t***	***p***	**Significant combination**	**diff**	***p***
N %	4.69E-12	Small	7.51	7.08E-09	Small-large	0.86	< 0.05
		Medium	8.71	2.13E-10	Medium-large	1.01	< 0.05
P %	7.19E-12	Small	6.69	8.41E-08	Small-large	0.13	< 0.05
		Medium	7.98	1.74E-09	Medium-large	0.15	< 0.05
K %	5.71E-08	Small	6.55	1.29E-07	Small-large	0.51	< 0.05
		Medium	4.67	4.05E-05	Medium-large	0.36	< 0.05
Ca %	1.48E-06	Small	-4.64	4.45E-05	Small-large	-1.06	< 0.05
		Medium	-0.79	< 0.05	Medium-large	-0.94	< 0.05
Mg %	0.19	Large	1.98	0.05			
Na mg/Kg	< 0.05	Small	3.9	< 0.05	Small-Control	581.04	< 0.05
		Medium	3.87	< 0.05	Medium-Control	577.14	< 0.05
		Large	3	< 0.05	Large-Control	316.22	0.02
Zn mg/Kg	1.57E-08	Small	6.18	3.97E-07	Small-large	8.4	< 0.05
		Medium	6.43	1.86E-07	Medium-large	8.8	< 0.05
Cu mg/Kg		Small	-4.06	< 0.05	Small-large	-3.6	< 0.05
		Medium	-3.71	< 0.05	Medium-large	-3.24	0.01
Mn mg/Kg	8.13E-07	Small	-4.42	8.52E-05	Small-large	-128.4	< 0.05
		Medium	-4.38	9.64E-05	Medium-large	-127.4	< 0.05
Fe mg/Kg							
Al mg/Kg	< 0.05	Large	2.95	< 0.05	Small-large	-38.33	< 0.05