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Maponga, Tsitsi Sithandiwe

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Mistletoes as drivers of plant community structure and resource heterogeneity in semi-arid savanna ecosystems, Zimbabwe



A thesis submitted to the Faculty of Science, University of the Witwatersrand, in fulfilment of the academic requirements for the degree of Doctor of Philosophy.

Johannesburg, June 2021

By

Tsitsi Sithandiwe Maponga

Academic Supervisors:

Prof E.T. F. Witkowski and Associate Prof H.G.T. Ndagurwa





DECLARATION

I declare that this thesis contains my own original and unaided work. It is being submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg and it has not been submitted before for any degree or examination in any other university. All sources of information in the text are listed in the references.

Signed:  Date: 21 June 2021

Dedication

I dedicate this thesis to my mother Jennifer Khoza. Thank you for believing that I can reach my greatest potential in whatever I set my mind to. You helped me with my revisions from kindergarten up until I got my first degree. Throughout my struggles you continued to be consistent, intentional and selfless. I hope this dissertation brings you the happiness that you have solemnly deserved and it will show you the love and respect that I have for you.

To my niece Ndalwentle Joy Joana you can be whatever you want to be.

There is more that is found in You
What amazing Grace and Love, that You, have continued to show so patiently
The essence is Your Grace and
In You, we boast and we know never to settle for Less.



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Abstract

The role of mistletoes in influencing resource heterogeneity has been shown in many environments. Studies have shown that by providing additional litter and weakening their hosts', mistletoes can change plant community structure by providing sub-ordinate subcanopy species a competitive edge thereby increasing species richness and diversity. These studies have mainly focused on differences between mistletoe-infected and uninfected trees, yet it is possible that different mistletoe-infection degrees' influence species composition within and beyond their canopies. Therefore, this thesis investigated the effects of mistletoe-infection degrees on *Vachellia karroo* (Hayne) Banfi and Glasso on plant community structure in a semi-arid savanna in South-West, Zimbabwe. Firstly, this thesis investigated how high- and low mistletoe-infection degrees on *V. karroo* trees influence abiotic and biotic conditions within and beyond the canopy patches. Further, it examined whether there were variations in species composition, species and functional diversity, and size measurements of understory plants within high- and low mistletoe-infection canopy patches and intercanopy spaces. Thirdly, it explored whether different mistletoe-infection degrees reduced the reproductive and regeneration capacities of their host trees and the recruitment of host juveniles (seedlings and saplings). Lastly, although studies have investigated how uninfected mature trees influence the spatial distribution of other surrounding woody species of different stage classes, little is known on how mistletoe-infected trees influence the spatial patterns of surrounding woody plants. Therefore, the fourth aim investigated how mistletoe-infected *V. karroo* trees influence the spatial patterns of their surrounding conspecific and heterospecific woody plants of different stage classes within three 50m × 50m plots with > 30 mistletoe-infected *V. karroo* trees.

The results show that intercanopy spaces had between 18% to 34%, and 18% higher herbaceous biomass and maximum grass height, respectively, compared to canopy patches. Herbaceous biomass and maximum grass height were 8% to 23%, and 13%, respectively, higher in low- compared to high mistletoe-infection microhabitats. Furthermore, high mistletoe-infection canopy patches had between 29% and 49%, and 30% less herbaceous biomass and maximum grass height, respectively compared to low mistletoe-infection intercanopy spaces, which had the highest measurements. Some of these differences are associated with higher grazing/trampling of between 1.24 and 1.39-fold within high mistletoe-infection canopy patches compared to the other three microhabitats. High animal disturbances contributed to the elevated species richness and species and functional diversity (by reducing understory competition) within high mistletoe-infection canopy patches compared to the other microhabitats, as well as being attracted to the shade from the midday sun in all subcanopy patches. Intercanopy spaces were mainly dominated by high grazing value grasses (e.g., *Setaria incrassata* and *Heteropogon contortus*) and *V. karroo* juveniles, whilst canopy patches particularly of high mistletoe-infection had significantly higher grass (of mixed grazing value), forb, and tree diversity. A ‘mistletophily index’, which calculated the affinity of each species to each of the four different microhabitats, was developed. The index showed that 34% of the recorded species had a strong affinity towards canopy patches whilst intercanopy spaces were significantly associated with 9% of the observed species.

Low mistletoe-infection canopy patches had higher abundance of decreaser grasses which are associated with low-intermediate disturbance, and intermediate disturbance is often linked to higher species diversity. However, grass, forb, and tree diversity were 17% to 43% higher within high mistletoe-infection canopy patches with higher animal disturbances compared to low mistletoe-infected canopy patches. These variations were attributed to higher soil temperature and relative humidity (3% and 0.5%, respectively), measured continuously over 8 months from the wet to the dry season using ibuttons, within high- compared to low mistletoe-infection canopy patches. The raised

soil temperature and relative humidity is interpreted as a result of higher light incidence and decomposition rates from the relatively higher litter turnover, leading to canopy patches with higher soil nutrients and increased rates of nutrient cycling compared to low mistletoe-infection canopy patches. Therefore, high mistletoe-infection canopy patches had a higher occurrence of species that favour semi-shade, high soil moisture and nutrients (*Sporobolus pyramidalis*, *Asparagus africanus*, *Ziziphus mucronata* and *Flueggea virosa*) and those that are prevalent on disturbed sites (*Cynodon dactylon*, *Setaria verticillata*, *Bidens pilosa*, *Sida alba*, and *Lantana camara*). As a result, 15% of the recorded species showed a strong positive affinity to high mistletoe infection canopy patches, whilst only 10% of the species had a high affinity towards low mistletoe-infection canopy patches. Indeed, an increase in mistletoe infection and canopy presence resulted in different species assemblages, higher species diversity (25% to 45%), functional richness (27% to 42%), functional evenness (7% to 30%), functional dispersion (24% to 58%) and RaoQ (26% to 59%) compared to the other microhabitats.

Mistletoe-infection intensity significantly reduced the reproductive and regeneration capacities of *V. karroo* trees. As mistletoe-infection increased there tended to be a general decline in flower buds, flowers, pods, seeds/pod and most importantly seed and germinable seed production/tree, possibly due to changes in the canopy physio-morphological attributes. Flower buds, flowers, and pods were between 40% and 68% higher in low- compared to high mistletoe-infection canopies. Pods were 1.16-fold longer, whilst the number of seeds/pod was 1.15-fold higher in low- than high mistletoe-infection trees. Consequently, seed and germinable seed production/tree were significantly lower in high- (2273 ± 820; 599 ± 216, respectively) than low mistletoe-infection trees (7088 ± 905; 2947 ± 376). The percentage of intact seeds was higher in low- (70%) compared to high mistletoe-infection trees (58%). High mistletoe-infected seeds had higher bruchid beetle seed predation (21%) and aborted seeds (21%) compared to low mistletoe-infection seeds (11% and 19%, respectively). Seed mass per seed was 20% higher (but not significantly different) in low- (0.037 ± 0.003g) compared to high mistletoe-infected trees (0.030 ± 0.003g). The overall percentage germination was 42% and 26% for high- and low mistletoe-infected seeds. However, high mistletoe-infection seeds (12%) had 4-fold higher initial germination rates (pre-scarification) than low infection seeds (3%), whilst after scarification low mistletoe-infection seeds (34%) had 2.83-fold higher germination rates than high mistletoe-infection seeds (12%). This was attributed to variations in seed size and seed coat characteristics. The overall germination rates were very similar at 36.3 ± 2.54 and 36.1 ± 2.57 days for high and low infection seeds, respectively. The soil seed bank was 3.24-fold higher under low- (46.7 ± 10.7 seeds/m^2) than high mistletoe-infection trees (14.4 ± 5.8 seed/m^2).

The number of understory *V. karroo* juveniles was 1.41 to 3.51-fold higher within high mistletoe-infection canopy (n = 172) patches compared to the three microhabitats. There were no significant differences in the juvenile densities across the microhabitats, however, juvenile densities tended to be 2.86-fold higher in high- (1813 ± 528 juveniles/ha) compared to low mistletoe-infection canopy patches (633 ± 218 juveniles/ha) which had the lowest juvenile densities. Seedlings were significantly higher in high mistletoe-infection canopy patches (1275 ± 344 seedlings/ha) followed by adjacent intercanopy spaces (663 ± 121 seedlings/ha), which were much higher than in the low mistletoe-infection canopy patches (294 ± 92 seedlings/ha) and adjacent intercanopy spaces (293 ± 160 seedlings/ha). Although sapling density was higher in low- (706 ± 343 saplings/ha) and high (573 ± 199 saplings/ha) mistletoe-infection intercanopy spaces compared to low- (340 ± 142 saplings/ha) and high mistletoe-infection canopy patches (538 ± 201 saplings/ha), there was no significant difference across microhabitats. This indicates that over time *V. karroo* juveniles are actually persisting better in the intercanopy spaces than under the tree canopies. Therefore, intercanopy spaces were safer for

saplings, compared to the adjacent canopy patches that were apparently safer sites for seedlings, at least prior to the cold dry winter period. Moreover, high mistletoe-infection canopy patches were safer for *V. karroo* juveniles, compared with low mistletoe-infection canopy patches which were safer sites for soil-stored seeds.

Lastly, mistletoe infected *V. karroo* trees had varying relationships with heterospecifics and conspecifics of different stage classes. Mistletoe-infected trees exhibited patterns consistent with a random pattern; this was attributed to bird disperser choices and the already existing random patterns of mature trees. However, due to facilitation, most of the woody conspecific and heterospecifics of seedlings, saplings, and shrubs were clustered around mistletoe-infected trees. Nonetheless, one plot showed significant repulsion of conspecific seedlings and saplings which could be due to both intra- and inter-specific competition. The bivariate relationship between all the mature trees and conspecific mature trees showed a random distribution consistent with the existence of both competition and facilitation. Competition can be attributed to trees with low mistletoe-infection competing with uninfected trees thus inclining the pattern towards regular distribution. In contrast, facilitation could be signifying the weakening of hosts due to high mistletoe-infection intensities coupled with a proliferation in soil nutrients and moisture. This increases the competitiveness of sub-ordinate trees, consequently leaning the pattern towards aggregation. Overall, this part of the study shows that mistletoe infection can increase species and functional diversity and alter the spatial patterns of their nearest neighbours of different growth forms. They can augment plant heterogeneity and functional richness which can positively influence the persistence of semi-arid savanna ecosystems which are nearly always resource-limited.

Key words

Bivariate, canopy patches, competitive exclusion, functional diversity, facilitation, grazing, herbaceous biomass, heterogeneity, intercanopy spaces, litter, Matopos, mistletoes, recruitment, reproduction, regeneration, seedlings, seed banks, semi-arid savannas, spatial patterns, species composition, species diversity, traits, univariate, *Vachellia karroo*, Zimbabwe

List of abbreviations

CA - Canopy area

DPM – Disc pasture meter

FDis - Functional dispersion

Fdiv- Functional divergence

FEve - Functional evenness

FRic - Functional richness

LA - Leaf area

LDMC - Leaf dry matter content

RaoQ - Rao's quadratic entropy

SE – Standard error

SLA - Specific leaf area

SPAD - Single Photon Avalanche Diode (used for estimating chlorophyll content of leaves)

WLT - Whole leaf thickness

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CHAPTER 1

INTRODUCTION

Rationale for the study

African savanna ecosystems are composed of scattered trees in a continous grass layer, driven by soil moisture, nutrients, fire, and herbivory (Walker and Noy-Meir 1982; Witkowski and O'Connor 1996; Scholes and Archer 1997; Mlambo *et al*., 2007). Soil moisture and nutrient availability are amongst the most important drivers of the structure and function of the soil biotic system in savanna ecosystems (Swift *et al*. 1979; Wardle 2002; Moustakas *et al*., 2009). Moustakas *et al*. (2009) also state that the annual rainfall in savanna ecosystems is often patchy, and varies from 100-1300mm. The spatially heterogeneous nature of these and other variables, including topography and herbivory, results in a correspondingly spatially heterogeneous vegetation structure (Venter and Witkowski 2010; Helm and Witkowski, 2012; Fisher *et al*., 2013; 2014; 2015; Mograbi *et al*. 2015).

Recently studies have shown that mistletoes parasitizing on large savanna trees can also increase plant and resource heterogeneity in semi-arid savanna systems (Ndagurwa *et al*., 2013, 2014, 2015, 2016, 2018, 2020; Muvengwi *et al*., 2015) Mistletoes are important resources in forest and woodland canopies as they provide food, shelter, and nesting sites to arthropods, birds, and mammals (Watson 2001, Lira *et al*., 2017; Ndagurwa and Dube 2013a, b). Consequently, there is a strong link between species richness and mistletoe densities (e.g., for birds (Bennetts *et al*. 1996, Watson 2001, Watson and Herring 2012); and arthropods (Burns *et al*. 2011, Ndagurwa *et al*. 2014a)). Recent research has shown that parasitic plants critically influence the structure and function of ecosystems where they are found (Watson 2001, 2009; Fisher *et al*., 2013; Ndagurwa *et al*., 2016), by facilitating changes to competitive dynamics and nutrient inputs (Quested 2008). Some of these impacts can be negative on the host; for instance, mistletoes can induce negative physiological impacts on host tree performance through parasitism by affecting their hosts' size, competitive edge, and reproduction (Lamien *et al*., 2006). A few studies (Gomes and Fernandes, 1994; Silva and Martinez del Rio, 1996; Geil and Hawksworth, 2002; Arruda, 2012; Mourão *et al*., 2009; Cruz Neto *et al*., 2017; Mellado and Zamora, 2020) have also shown that indeed mistletoes weaken their hosts' reproductive performance and survival. However, little or nothing is known about how mistletoes impact the reproduction of their hosts in semi-arid savanna systems of Zimbabwe.

Mistletoe parasitism can potentially shift the competitive balance from host tree species (usually competitive dominants) to non-host plants (competitive subordinates) (March and Watson, 2007;

Ndagurwa *et al*., 2016, 2018; Mellado *et al*., 2016) thereby changing the plant composition and productivity in an area (Press and Phoenix 2005; Fisher *et al*., 2013). These studies have shown that mistletoe presence positively influences the understory productivity of infected tree canopy patches compared to uninfected trees. However, the comparisons were predominantly on infected and uninfected trees, until now, little has been done to understand how the different degrees of mistletoe infection (high and low) can influence the species composition and understory plant productivity within and beyond tree canopy patches.

Mistletoes have been shown to positively alter the physical and chemical environment i.e., nutrient availability and soil moisture in their immediate vicinity through their nutrient-rich litter (Press and Phoenix 2005; Watson 2009; Ndagurwa *et al*., 2013, 2014a, 2015; 2020). They increase nutrient availability to both hosts and non-host species, by having a high leaf turn-over, and leaves with low resorption efficiency at senescence. These characteristics ensure the addition of nutrients that would have otherwise been absent or not returned to the soil system (Mathiasen *et al*., 2008; Spasojevic and Suding, 2011; Demey *et al*., 2013). Conversely, mistletoes have been shown to have contrasting impacts on soil moisture. For example, Ndagurwa *et al.* (2014b) observed lower surface soil moisture content due to high levels of transpiration from mistletoes on infected trees, whilst Ndagurwa *et al.* (2015) report soil moisture variations due to seasonality, mistletoe species, and thicker mistletoe litter layer below the tree canopy which reduced evaporation from the soil. Preservation of soil moisture was attributed to the presence of a thicker mistletoe litter layer below the tree canopy which reduced moisture loss from the soil. As a result, changes in the soil moisture content and nutrient input associated with mistletoe infection, are expected to result in changes in the performance of mistletoe hosts and co-occurring plants (Quested *et al*., 2003) and consequently plant community structure and function (Press and Phoenix, 2005).

Despite recognition of the impacts of litter-fall (nutrient return) as well as parasitism on both hosts and non-host species influenced by mistletoe infection on trees, there has been a handful of studies that have investigated the community-level effects of mistletoes (Watson, 2009; Pennings and Callaway 2002; Press and Phoenix 2005; Fisher *et al*., 2013). This has restricted our understanding of the ecological function of mistletoes in plant communities (Watson 2009). Consequently, although Ndagurwa *et al*. (2013, 2014; 2020) have shown that mistletoes enhance litter-fall and nutrient return in a semi-arid savanna, the effect of different degrees (high and low) of mistletoe infection on the abiotic conditions i.e., temperature and soil moisture have not been fully examined. Furthermore, given the clumped distribution of mistletoes on large savanna trees (Ndagurwa *et al*., 2012), it is inferred that mistletoes would further increase spatial differences, particularly for nutrients and soil moisture, and consequently create even greater patchiness in the quality and quantity of savanna

vegetation. Thus, mistletoes may have a greater impact on the structure and functioning of savanna ecosystems than previously recognised, making them a key priority for further research.

Indeed, mistletoes have numerous effects on overall ecosystems due to their small nutrient-enriched patches under infected hosts in proportion to their surrounding environments (Watson, 2009). Through altering nutrients and soil moisture content, mistletoe-infected trees can result in an understory with different functional traits e.g., plants with traits that respond to environmental factors (i.e., "functional response types"), or those that affect the ecosystem (i.e., "functional effect types") relative to uninfected trees and the surrounding matrix (Joseph *et al*., 2014). Consequently, mistletoe-infected trees may differ in how they influence the overall functional diversity of the savanna ecosystem. Nevertheless, despite the recognition that nutrient-rich patches under mistletoe-infected trees might significantly influence plant functional diversity similar to termite mounds (Joseph *et al*., 2014) no studies have been carried out to ascertain this possibility. Furthermore, the higher levels of nutrients below savanna trees compared to adjacent grassy areas (Belsky, 1992; Belsky, 1994; Davenport *et al*., 1996; Dean *et al*., 1999; Witkowski and Garner, 2000; Munzbergova and Ward, 2002; Pueyo *et al*., 2013; Magandana, 2016) has been well documented. This also has been attributed to bird nests, faecal deposition from birds and mammals, and remains of prey and litter accumulation (Belsky *et al*., 1989; Belsky, 1994; Dean *et al*., 1999; Munzbergova and Ward, 2002; van der Waal *et al*., 2011b). Even so, there has been little mention of the presence and effects of mistletoes on these trees. Hence, this well-known phenomenon of high nutrient and moisture levels may also largely be a contribution of mistletoe infections. Consequently, this study investigated how different levels of mistletoe infection on *Vachellia karroo* (Hayne) Banfi and Glasso influenced the hosts' reproduction, the plant community structure, and abiotic conditions within and outside the host plants canopy. The focus was on *V. karroo* because it is one of the dominant hosts of mistletoes that have been studied extensively at Matopos Research Station (20° 31'S, 28° 31'E) in Zimbabwe, (Ndagurwa *et al*., 2012, 2013, 2014, 2015, 2016; 2020).

Aim of the study

The overall aim of this study is to investigate the role of mistletoes as drivers of plant community structure and resource heterogeneity in a semi-arid Zimbabwean savanna ecosystem.

Specific objectives

1. To determine the effects of high- and low mistletoe-infection on *Vachellia karroo* trees and adjacent intercanopy spaces on the understory biotic and abiotic factors (Chapter 2)
2. To determine the effects of high- and low mistletoe-infection on *Vachellia karroo* trees and adjacent intercanopy spaces on understory species composition, diversity, and functional types (Chapter 3)

3. To determine the effects of mistletoe infection on regeneration of *Vachellia karroo* trees (Chapter 4)
4. To determine the woody plant spatial patterns around mistletoe infected *Vachellia karroo* trees (Chapter 5)

Research questions

1) What are the effects of varying mistletoe infection intensity on understorey plant biomass, litter quantities and animal visitations beneath and beyond *Vachellia karroo* mistletoe-infected trees?
2) Does mistletoe infection degree result in variations in soil temperature and moisture conditions within and beyond infected host subcanopies.
3) Are mistletoe effects on the understorey abiotic and biotic factors restricted to canopy patches or do they also extend to intercanopy spaces
4) What are the effects of mistletoes on the understorey plant species composition?
5) What are the effects of varying mistletoe-infection degrees on understorey plant species and functional diversity?
6) Does variation in mistletoe infection degree result in intra-specific trait plasticity of an understory tree species?
7) Does varying mistletoe-infection degree result in differences in the reproduction of *V. karroo trees*?
8) Does mistletoe infection intensity lead to variations in *V. karroo* regeneration underneath and beyond the subcanopy of infected trees?
9) What is the influence of mistletoes on the spatial patterns of surrounding hetero- and conspecific woody plant species of different stage classes?
10) Does varying mistletoe infection intensity drive plant and resource heterogeneity in semi-arid savannas?

General outline of the thesis

The chapters in this thesis are outlined in Fig 1.1 and they are presented in scientific paper format. Chapter 2 is about to be submitted as a paper to the Journal of Arid Environments. There is a likehood of repetition particularly in the introduction and methods section of each chapter. Although repetition has been minimised as much as possible, certain sections, particular in the introduction and methods, may repeat certain points across some of the chapters.

Chapter 1: Introduction – This chapter comprises a rationale for the study, a broad introduction to the literature on large tree savannas and their effects as fertility islands and it introduces the study host

species, *Vachellia karroo*. The chapter goes on to give an overview of mistletoe biology, dispersal, host preference and their importance in plant communities, and the overall aims and objectives.

Chapter 2: Biotic and abiotic variables - This chapter investigated the effects of high- and low mistletoe-infection on biotic and abiotic components of *V. karroo* trees canopy patches and intercanopy spaces.

Chapter 3: Species and Functional diversity - This chapter explored how varying mistletoe infection degrees influenced species composition, species and functional diversity and size measurements of plants found within and beyond the canopy patches.

Chapter 4: Regeneration - This chapter investigated the effects of mistletoe-infection on reproduction and regeneration of high and low mistletoe-infection *V. karroo* trees.

Chapter 5: Spatial patterns - This chapter examined the spatial patterns of woody plants in relation to mistletoe-infected *V. karroo* trees. No known study has investigated how mistletoe infection influences the spatial patterns of heterospecific and conspecific species of different size classes.

Chapter 6: Synthesis - This chapter provides a synthesis of the findings, regarding how the degree of mistletoe-infection influences the abiotic (soil temperature and moisture) and biotic (herbaceous biomass, grass height, litter and animal activities) variables, species and functional diversity of understory plants, the regeneration of infected *V. karroo* trees and the spatial patterns around mistletoe-infected trees. Furthermore, findings and implications are discussed and recommendations for future research presented.

Appendices: This section presents two published manuscripts that I was involved in, whilst doing my PhD.

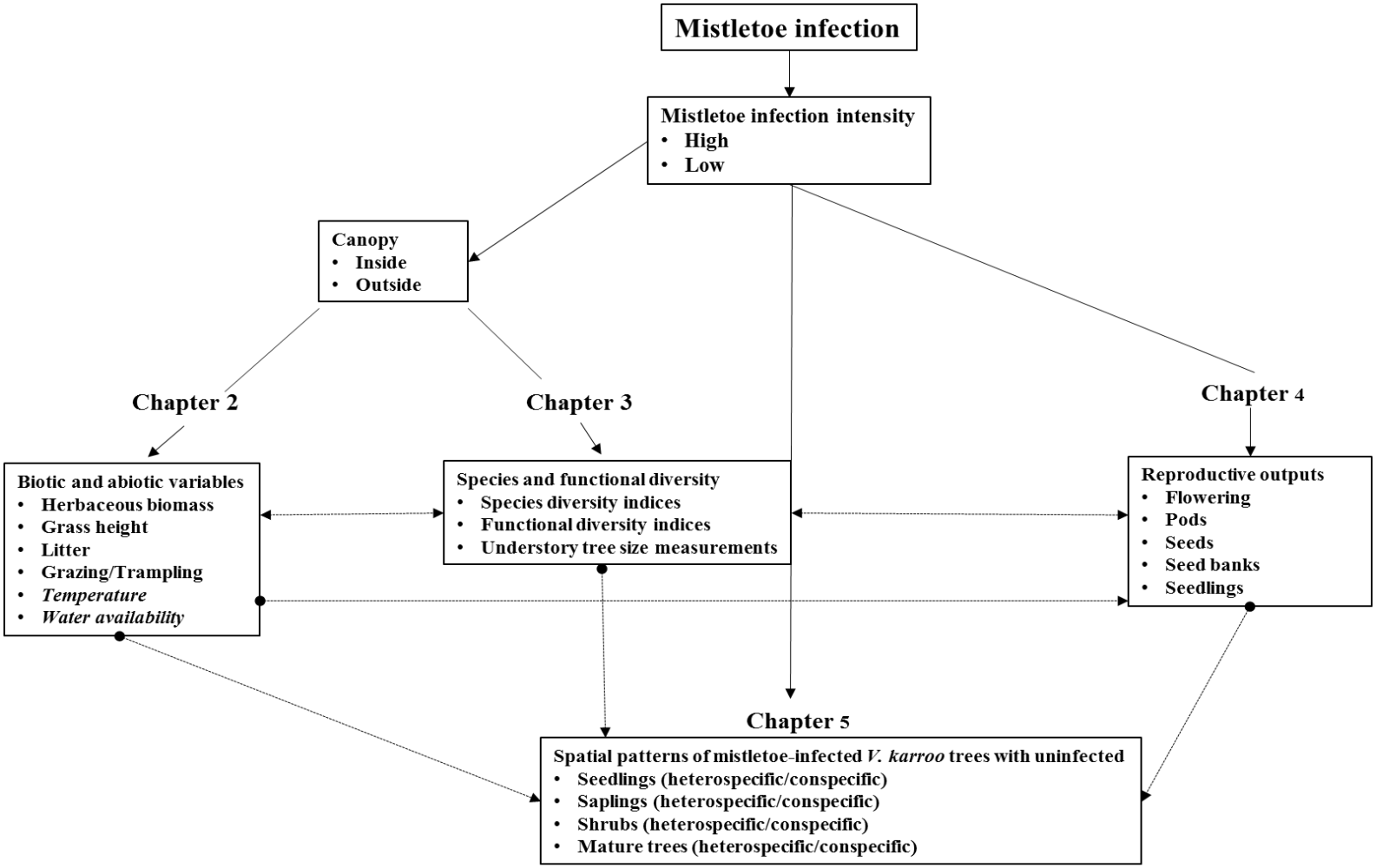


Fig. 1.1 Conceptual framework of the study showing mistletoe infection impacts on different variables that were studied in each chapter. Solid lines with one arrow indicate the chapters that were investigating the direct impacts of mistletoe-infection, whilst the dotted lines with double arrows indicate chapters with outcomes that are directly linked. Dotted one arrow lines are showing chapters that are likely to influence the outcomes of another chapter.

LITERATURE REVIEW

Introduction

The role of mistletoes as drivers of communities and ecosystems remains largely unstudied (Bardgett *et al*., 2006), yet they influence the productivity, functioning, and structure of plant communities through either parasitism or litter inputs or their interaction (Bardgett *et al.*, 2006; Spasojevic and Suding, 2011). Most studies on hemiparasite impacts have focused on their impacts on host plants, and few have investigated how mistletoes influence plant community structure and ecosystem functioning (Spasojevic and Suding, 2011). Nonetheless, understanding the functional roles of mistletoes in ecosystem processes is crucial in enabling further understanding of how plant communities with mistletoes can be conserved and managed (Aukema, 2004; Cullings *et al*., 2005). This chapter aims at contextualising the role of mistletoes as drivers of plant community structures, from the existing literature. However, the chapter begins by providing a general description of the roles of large savanna trees in the ecosystem and then it provides a summary of the biology of the host *V. karroo*, which is the focus of this study. Moreover, a general introduction to mistletoes is given which includes their importance, host preferences and their dispersal, and finally how mistletoes can influence plant community structures.

Large savanna tree impacts on plant community composition

In dry ecosystems, water and nutrient shortages can reduce plant productivity (Barbosa *et al*., 2014). For example, in savanna ecosystems, grasses are often limited by N and P (Zambatis, 2003; van der Waal *et al*., 2011a; Muvengwi *et al*., 2015) whilst woody vegetation seedlings are limited by N (van der Waal *et al*., 2011a, b). Nonetheless, nutrient-poor ecosystems tend to have patches of high-quality resources such as termite mounds, tree canopy patches, and mistletoe host canopy patches that directly and indirectly provide these limiting resources (Treydte *et al.*, 2007; Joseph *et al*. 2013; Seymour *et al*., 2014; Muvengwi *et al*., 2015; Magandana, 2016; Ndagurwa *et al*., 2016, 2020). African savanna trees enrich their understory soils with nutrients and water, hence forming fertility microhabitats. In turn, these fertility microhabitats increase the understory plant productivity, diversity, structure and spatial patterns (Belsky *et al*., 1989, 1993; Weltzin and Coughenour, 1990; Belsky, 1994; Alstad and Veetas, 1994; Kanz, 1996; Dean *et al*., 1999; Sameni and Soleimani, 2007; Treydte *et al.*, 2010; Barbosa *et al*., 2014). Indeed, isolated trees have been observed to change the species composition underneath their canopies (Kanz, 1996). However, this is dependent on the soil nutrient levels underneath each tree, for example, van der Waal *et al*. (2011a, b) reported that dominance of each growth form is resource specific i.e., grasses are better competitors against trees (*Colophospermum mopane* (Kirk ex Benth.) Kirk ex J. Léonard) in fertile soils particularly when soil P is higher, whilst trees can suppress grasses if there is greater soil N. Conversely, Sankaran *et al*. (2008) found a negative association between soil N and tree cover, hence high soil N can also support

more grasses.

The density of trees in a given area can negatively or positively influence the surrounding environment. In general, areas with low tree densities, high soil moisture and nutrient availability have higher productivity beneath tree canopies compared to grasslands (Belsky *et al*., 1989, 1993; Kanz, 1996; Zambatis, 2003; Magandana, 2016). In contrast, areas with high tree densities, low soil resources, high environmental stress, and disturbance, have low productivity and species richness due to only a few species being able to withstand these environments (Belsky *et al*., 1989; Kanz, 1996; Van Coller and Siebert, 2015). High tree densities reduce solar radiation thus they favour shade-tolerant plants and inhibit the presence of shade-intolerant plants (Dantas *et al*., 2013; Charles-Dominique *et al*., 2018). Likewise, grass growth and productivity are significantly reduced by high tree density, due to increased root biomass and below-ground competition for critical resources (Treydte *et al*., 2007, 2010; Randle *et al*., 2018). Consequently, competition especially for water decreases growth or can result in the death of the understory plants (Munzbergova and Ward, 2002; Kröpfl *et al*., 2002; Ludwig *et al*., 2003, 2004; Priyadarshini *et al*., 2016a; Yadeta *et al*., 2018). In addition, trees can release allelopathic substances that can inhibit the growth or establishment of both woody and herbaceous plants within their canopy patches (Kanz, 1996; Kröpfl *et al*., 2002; Sameni and Soleimani, 2007; Sagar, *et al*., 2008). However, competition with understory plants can be surmounted by niche partitioning (Randle *et al*., 2018) and the facilitative effect of trees as conditions underneath their canopies are often better compared to the surrounding matrix (Abdallah and Chaieb, 2010; Tessema and Belay, 2017). Therefore, in savannas, low to medium tree densities (<200 trees ha^{-1}) are ideal to maintain an optimal herbaceous layer, compared to open, and closed trees densities (Treydte *et al*., 2007).

Trees as a source of nutrients

Trees improve soil fertility by directly providing their understories with nutrients through stem flow, bark sloughing of the tree trunk and leaf litter (Belsky *et al*., 1993; Kanz, 1996; Munzbergova and Ward, 2002). Stem flow has been found to increase C, K, Ca, Na, Mg and P concentrations in the canopy patches of *Fagus grandifolia* Ehrh., *Quercus alba* L., and *Acer saccharum* Marshall, (Rhoades, 1996). Consequently, tree bases have the highest quantities of nutrients as they receive more nutrients due to leachates being washed off the stem and leaves and from aerial deposits that reach the soil through stem flow (Belsky *et al*., 1993; Kanz, 1996; Rhoades, 1996). Moreover, trees with smooth barks often have higher stem flow compared to those with rough barks (Rhoades, 1996). However, nutrient quantities decline with an increase in distance from the tree stem (Kanz, 1996; Rhoades, 1996). For example, Belsky *et al*. (1993) reported that soil Mn, extractable P, K, and Ca and soil organic matter declined with distance from the tree bole.

The older the trees, the longer the period of nutrient accumulation underneath the canopy, thus soils near the stem are more enriched throughout the life of the tree (Belsky *et al*., 1993; Kanz, 1996). Still, even though nutrients are concentrated at the tree bole, they are not limited to these areas. Canopies can extend up to 7-12m, and birds prefer to perch on the outer side of the tree canopies at these distances, hence, tree litter and bird droppings increase the distribution of resources beneath the canopy (Belsky *et al*., 1993). Trees also absorb nutrients and moisture horizontally from the intercanopy soils and subsurface, and they deposit them underneath their canopies, through litterfall, thereby increasing canopy patch fertility (Belsky *et al*., 1989; Davenport *et al*., 1996; Kanz, 1996; Breashers *et al*., 1998). However, trees have high nutrient resorption before leaf abscission, and this reduces the quantity of nutrients that are returned to the soil (Seymour *et al*., 2014).

Despite the physical presence of trees, it is their morphological and physiological traits that tend to influence how the trees impact their understory biomass. This is because different tree species and sizes release varying nutrient quantities; moreover, they do this at different temporal scales (Kanz, 1996; Ayres *et al*., 2009; Treydte *et al*., 2009; Muvengwi *et al*., 2015; Tessema and Belay, 2017). Nitrogen fixers usually have greater nutrient (especially N) quantities and higher mineralization compared to non-leguminous trees (Belsky, 1989; Prescott, 2002). Priyadarshini *et al*. (2016a) found that the larger and leguminous *Acacia robusta* (*Vachellia robusta* (Burch.) Kyalangalilwa & Boatwright subsp. *robusta*) had higher total N (1.62-fold), Ca (1.25 to 1.26-fold), available P (1.1 to 2.37-fold), and soil OM (1.96 to 1.36-fold) compared to the two smaller, non-leguminous *Ziziphus spina-christi* (L.) Desf., and *Balanites aegyptiaca* - (L.) Delile. Similarly, Belsky (1992) previously found that in areas with high rainfall, grass shoots within the canopy patches of the leguminous *Acacia tortilis* (*Vachellia tortilis* (Forssk.) Galasso and Banfi subsp. *spirocarpa* (Hochst. ex. A. Rich.) Kyal. & Boatwr.), had higher N concentrations than *Adansonia digitata* A L. due to symbiotic fixation in the former. In contrast, Treydte *et al*. (2007) report that soil N-content, soil OM, grass leaf N, leaf fibre content, and biomass did not vary underneath N-fixing and non-fixing trees. Belsky *et al*. (1989) also state that there were no significant differences in how *V. tortilis* and *A. digitata* influenced their understory total herbaceous layer, herbaceous-layer root biomass, soil microbial biomass-C and soil mineralization rates despite the trees having varying characteristics. Even so, some soil nutrients varied underneath each tree species; for example, *A. digitata* had higher Mg than the *Acacia* sites (Belsky *et al*., 1989). Therefore, similarities and variations of effects by individual trees and different species further increase resource heterogeneity in any landscape (Rhoades, 1996; Treydte *et al*., 2009).

The nutrients that are found within the canopy patches are also influenced by processes that make them available such as rate of decomposition, mineralization and nutrient recycling (Prescott, 2002; Muvengwi *et al*., 2015; Ndagurwa *et al.,* 2016). The physiochemical attributes of the litter which include the amount of lignin, N, hemicellulose, and secondary compounds especially phenolic acids,

coupled with soil abiotic conditions such as temperature and moisture influence these nutrient cycling processes (Facelli, 1991; Prescott, 2002; Ndagurwa *et al.*, 2013, 2015, 2020). Consequently, the rate of decomposition is negatively correlated to the quantities of lignin/nitrogen and the N mineralization in the foliar litter. Older leaves most often have more lignin and secondary compounds, and less protein thus they decompose slower than younger leaves (Facelli, 1991; Seymour *et al*., 2014). However, some studies show that there is no relationship between the rate of decay and the litters' lignin/N ratio or with the N mineralization (Prescott, 2002). Variations in the decomposition of the litter may be due to the dissimilarities in nutrient levels in different tree species and their litter (Prescott, 2002). As a result, the chemical composition and quality of the litter may differ with the species, type of foliage and season, and this has varying impacts on nutrient cycling (Facelli, 1991; Xiong and Nilsson, 1999; Prescott, 2002; Sameni and Soleimani, 2007; Ayres *et al*., 2009; Treydte *et al*., 2009; Spasojevic and Suding, 2011; Ndagurwa *et al.*, 2013; Muvengwi *et al*., 2015). Accordingly, the quantities of nutrients retained in the litter and the amount of carbon both in the litter and soil reflects how each tree canopy stores its nutrients on either the leaves or foliage (Prescott, 2002; Ayres *et al*., 2009). Therefore, although large trees are most likely to increase nutrient levels, due to leaf nutrient resorption, they may have less significant impacts in comparison to mistletoes and termite mounds.

Trees and soil moisture

Soil moisture is the principal variable that limits productivity in arid and semi-arid savannas; as a result, competition for water is usually high in these areas (Belsky, 1994; Kanz, 1996; Breashears *et al*. 1998; Priyadarshini *et al*., 2016b). Usually, tree canopies intercept and redistribute precipitation, and this reduces the quantity and intensity of water reaching the surface. Consequently, raindrop splash and runoff velocity are reduced whilst water infiltration is increased within the canopy patches (Belsky *et al*., 1993; Kanz, 1996; Breshears *et al*., 1997, 1998; Kröpfl *et al*., 2002; Magandana, 2016). Some of the water is stored in the canopy, some is lost through evaporation; the rest of the water slowly reaches the understory biomass where it is further intercepted thus decreasing the quantity of water that reaches the topsoil (Kanz, 1996). For that reason, canopy cover and litter act as a shield that reduces the impact of precipitation on the soil, preventing incidences of soil erosion, leaching, and washing away of nutrients (Kanz, 1996; Prescott, 2002; Davenport *et al*., 1996). Canopy cover can also can also aid in the removal of excess water from the soil through transpiration (Munzbergova and Ward, 2002; Prescott, 2002).

In low rainfall areas, trees and their herbaceous understory have been found to partition water use within the soil using Walters two-layer hypothesis, hence reducing competition and improving soil moisture availability for the herbaceous plants (Belsky, 1994, Breshears and Barnes, 1999; Kröpfl *et al*., 2002; Ludwig *et al*., 2004; February *et al*., 2013; Randle *et al*., 2018). Trees and herbaceous

plants are said to utilise different layers in the soil horizon, and trees either extend their roots deeper into the lower horizons and/or wider to the intercanopy spaces (Kanz, 1996; Breshears and Barnes, 1999). In contrast, herbaceous plants are shallow-rooted, so they utilise the top layers of the soil, and they are thought to have superior mechanisms of uptake for available resources within the soil top layers (Weltzin and Coughenour, 1990; Breshears *et al*., 1997). In agreement, February *et al*. (2013) found that trees are inferior competitors for soil moisture and they switch to deeper soil layers once the topsoil becomes dry whilst grasses keep on exploiting moisture from the upper layers. However, Breashers *et al*. (1997) state that trees can also compete with the herbaceous layer in the topsoil of the intercanopy spaces. They report that *Pinus edulis* Engelm., and *Juniperus monosperma* (Englem.) Sarg. in semi-arid woodlands utilised water in the top 30cm of their adjacent intercanopy spaces, thus competing with the herbaceous plants in these spaces. Nonetheless, the use of water in the intercanopy spaces varies depending on the tree species and its characteristics (e.g., photosynthesis and transpiration rates, root morphology, basal area, plant water potential and water use efficiency) (Breshears *et al*., 1997). Resource partitioning is often used to explain the coexistence of trees and grasses in the low rainfall savannas (Ludwig *et al*., 2004; February and Higgin, 2010). In contrast, in high rainfall areas rigorous competition is expected as tree roots are found throughout the horizons and within the tree canopy matrix thus reducing understory productivity (Belsky, 1994; Kanz, 1996).

Woody species can also make water available in the top layers by bringing it up (vertically) through a process called the hydraulic lift (Kröpfl *et al*., 2002; Sagar, *et al*., 2008; Priyadarshini *et al*., 2016b). This enables shallow-rooted plants and the tree itself to have access to water. Furthermore, the proliferation of soil moisture via hydraulic lift also improves mycorrhizae survival rates. This results in increased nutrient uptake by the tree and its understory plants (Priyadarshini *et al*., 2016b). Consequently, the species composition, growth and productivity of the understory plants are also improved (Ludwig *et al*., 2003, 2004). Nonetheless, hydraulic lift is most effective during the dry season when water is very limiting (Priyadarshini *et al*., 2016b).

Canopy patches *vs*. intercanopy spaces

Trees reduce photosynthetically active radiation (Munzbergova and Ward, 2002) by providing shade to plants beneath their canopies (Prescott, 2002) and this is important where water is limiting as it increases water conservation (Belsky, 1994; Kanz, 1996). The trees intercept solar radiation and thus reduce the understory soil temperature and evapotranspiration and consequently the soil drying rates (Belsky *et al*., 1989, 1993; Belsky, 1994; Kanz, 1996; Breshears *et al*., 1998; Chirara *et al*., 1998; Dean *et al*., 1999; Prescott, 2002). Therefore, water losses are significantly reduced under tree canopies particularly near the tree stem where there is more shade (Kanz, 1996). Abdallah *et al*. (2012) found that canopy patches of *A. raddiana* (*Vachellia tortilis* subsp. *raddiana* (Savi) Kyal. & Boatwr.) had a 1.65-fold greater soil water content (m^3 of water per m^3 of soil) than intercanopy spaces. Similarly, Breashers *et al*. (1997) also reported that soil evaporation was higher (especially in

summer) in the intercanopy compared to the canopy patches. However, in winter, canopy patches lost more water than intercanopy spaces due to higher temperatures and high root densities in the former thus increasing water uptake by plants. These variations give rise to temporal and horizontal heterogeneity in the availability of water within the canopy patches and between the canopy patches and adjacent intercanopy spaces.

Furthermore, Breshears *et al*. (1998) found that intercanopy patches had higher mean ranges of monthly soil temperatures (-1.6°C to 25.9°C) than canopy patches (0.4°C to 22.5°C). The maximum temperature recorded in the intercanopy spaces during the hottest month (July) was 10°C higher than in the canopy patches. Consequently, during the warmer months (April to August) intercanopy spaces were 1°C warmer than the canopy patches. In agreement, Abdallah *et al*. (2012) also reported that light intensity (expressed in Lux) was 2.83-fold lower in the canopy patches than intercanopy spaces. Therefore, lower temperatures within the canopy patches help increase plant nutrient uptake especially N, thus trees improve canopy patch soil fertility by reducing nutrient losses (through leaching) beneath their canopies (Belsky *et al*., 1993; Kanz, 1996). Consequently, understory growth is improved (Belsky *et al*., 1993; Kanz, 1996) and this increases plant spatial heterogeneity in landscapes (Belsky, 1994). However, lower temperatures from shading can decrease soil microbial biomass thereby reducing the rate of organic matter decomposition (Belsky *et al*., 1989; Kanz, 1996).

The variations between canopy and inter-canopy soils with regards to soil nutrients and moisture lead to further differences in plant productivity and biomass in the two spaces (Davenport *et al*., 1996; Rhoades, 1996; Treydte *et al*., 2009; Pueyo *et al*., 2013; Bernadi *et al*., 2016). Abdallah and Chaieb (2010) and Abdallah *et al*. (2012), found that canopy patches were more fertile than intercanopy spaces and they attributed this to *V. tortilis* being nitrogen fixers and also due to the litter produced by these trees. Priyadarshini *et al*. (2016a) also found that pH (1.01 to 1.05-fold), N (1.24 to 2.33-fold), P (1.04 to 1.89-fold), K (1.06 to 1.65-fold) and organic matter contents (1.44 to 1.58-fold) were mostly higher underneath tree canopies compared to outside the canopy. They also attributed this to higher litter deposits, increased litter decomposition (facilitated by higher soil moisture content) and decreased leaching within the canopy patches resulting in fertile microhabitats (Priyadarshini *et al*., 2016a). Similarly, Sameni and Soleimani, (2007), showed that regardless of the tree species they were investigating, topsoil (0-10cm) nutrient (e.g., Na^{+}, K^{+}, Cl^{-1}, SO^{2-}, HCO^{-}_{3}, OM, and total N) contents were 1.13 to 1.93-fold significantly greater in the canopy patches than in the intercanopy spaces. Conversely, as soil depth increased (10-40cm) intercanopy spaces tended to have higher nutrient contents.

Large trees also provide shade, shelter for animals and birds and their canopy patches are highly used as foraging sites (Treydte *et al*., 2010). Granivorous birds' e.g., red-billed buffalo weavers (*Bubalornis niger*) were observed to *V. tortilis* and *Adansonia digitata* (baobab) trees thus providing a major

source of nutrients underneath their canopies (Belsky *et al*., 1989; Kanz, 1996). As a result, trees have high visitations from birds, insects and mammals which leave faecal deposits, fallen nests, and remains of prey (Janzen, 1977; Belsky *et al*., 1989; Belsky, 1994; Dean *et al*., 1999; Munzbergova and Ward, 2002; Treydte *et al*., 2007; Treydte *et al*., 2010; van der Waal *et al*., 2011b; Abdallah *et al*., 2012; Bernardi *et al*., 2016; Tessema and Belay, 2017). In turn, these deposits increase nutrient inputs (N, NO_3-N, Ca, P, and K), organic matter, microbial biomass, and nematode densities beneath tree canopies thereby improving the quality of the undergrowth and species diversity (Kanz, 1996; Belsky, 1994; Belsky, 1992; Treydte *et al.*, 2010). Therefore, understory plants tend to be nutritious, highly palatable and they have high water use efficacy which makes them good sources of food for livestock particularly ruminant grazers (Treydte *et al*., 2007, 2010; Abdallah *et al*., 2008; Bernardi *et al*., 2016). On the other hand, the number of visits, duration of the visit, and the frequency (Kanz, 1996) of the herbivores can reduce the productivity of understory plants within the canopy patches (Weltzin and Coughenour, 1990).

Benefits from trees are often localised under the specific trees particularly in areas with a few trees, low rainfall, and average soil fertility (Belsky, 1992, 1994). They can also vary within the canopy patches, for instance, Weltzin and Coughenour (1990) found that total grass biomass was higher at the tree bole of *V. tortilis* and it declined with increasing distance from the tree. Consequently, herbaceous biomass growth and productivity are often higher within canopy patches where resources are readily available than in the intercanopies (Ludwig *et al*., 2003; Treydte *et al*., 2007, 2009; Abdallah *et al*., 2012; Magandana, 2016). In agreement, Yadeta *et al*. (2018) found that there was significantly more herbaceous biomass and herb cover within *V. tortilis* canopy patches than in the adjacent intercanopies. Likewise, Tessema and Belay (2017) report that canopy patches of three tree species had higher biomass compared to their intercanopy spaces, and Abdallah *et al*. (2008) also showed that total plant cover and the dry matter yield were 17% and 59% (respectively) higher under canopy patches of *Acacia raddiana* in areas with low grazing. This was attributed to the high nutrient levels and the protective effect of the trees. Equally, in areas with high grazing total plant cover and the dry matter yield in the intercanopies tended to be 19% and 20% higher than within the canopy patches. Moreover, Bernadi *et al*. (2016) report that the total biomass in intercanopy spaces tended to be higher than within canopy patches in areas with cattle density of 0.36–1.1 cattle units ha^{-1}.

Canopy patches due to resourceful microhabitats also have higher plant cover, species richness and diversity compared to intercanopy spaces consequently increasing plant heterogeneity (Yadeta *et al*., 2018). For instance, Abdallah *et al*. (2012) report that plant cover and species richness were 33% to 65% and 56% to 70%, (respectively) higher in the canopy patches relative to intercanopy areas depending on whether it was a wet or dry year. Furthermore, shade and higher moisture content provide microclimatic conditions which increase germination and diversity of shade-tolerant species,

broad-leaved trees and C_3 plants (e.g., nitrophilous annuals and flesh-fruited perennials) beneath the canopies (Belsky, 1992, 1994; Kanz, 1996; Dean *et al*., 1999; Munzbergova and Ward, 2002; Barbosa *et al*., 2014; Bernadi *et al*., 2016; Magandana, 2016). Trees growing under low light intensity tend to change their leaf biomass, increase their specific leaf area and photosynthetic characteristics (Barbosa *et al*., 2014). Similarly, shade tolerant grass species (e.g., *Cynodon nlemfuensis, Panicum maximum, Enteropogon macrostachyus, Eragrostis ciliaris and Urochloa panicoides*), can decrease stomata apertures at low to moderate light levels, hence conserving moisture when there is low solar radiation (Belsky *et al*., 1989, 1993; Weltzin and Coughenour, 1990; Belsky, 1994; Magandana, 2016). Conversely, low light incidence can reduce carbohydrate accumulation and this potentially decreases productivity and seedling growth within the canopy spaces (Barbosa *et al*., 2014).

Intercanopies attract shade-intolerant, fine-leaved trees and C_4 species which are highly competitive and photosynthetic (Belsky, 1994; Breashers *et al*., 1997; Kanz, 1996; Dean *et al*., 1999; Bernadi *et al*., 2016; Magandana, 2016). Shade intolerant species are also physiologically incapable of utilising low light conditions, and they have higher conductance, light compensation points, rates of dark respiration, photosynthetic capacities, lower quantum efficiency, and near-saturated photosynthesis (Belsky, 1994; Dantas *et al*., 2013; Barbosa *et al*., 2014). Subsequently, tree canopies reduce productivity of shade-intolerant grass species that have rigid leaves e.g., *Digitaria macroblephara, Eustachys paspaloides, Digitaria velutina*, *Brachiaria leersioides, Aristida adscensionis*, and *Digitaria eriantha,* (Munzbergova and Ward, 2002) and fine-leaved trees (Weltzin and Coughenour, 1990; Barbosa *et al*., 2014; Magandana, 2016). The variations in the micro-climatic conditions of shaded and unshaded areas further cause plant spatial heterogeneity.

Accordingly, in arid and semi-arid areas, large trees are keystone species and nutrient hotspots which foster nurse protégé interactions (Munzbergova and Ward, 2002; Van Coller and Siebert, 2015). Although there are variations in resources beneath the canopy and the surrounding matrix (Davenport *et al*., 1996), the influence of trees extends beyond the canopies to the tree root zones due to litter being deposited in that region (Belsky, 1992). Furthermore, mistletoes have been shown to further augment litter quantities within these canopy patches yet, few studies have looked at how mistletoe-infected trees influence understory species composition and spatial patterns within and beyond canopy patches and intercanopy spaces. Moreover, no known studies have investigated how high- and low mistletoe-infection degrees influence abiotic and biotic factors within and beyond the canopy patches.

Vachellia karroo

Vachellia karroo (Hayne) Banfi & Glasso (sweet thorn, formally *Acacia karroo*) trees are the most widespread *Acacias* and they are indigenous to southern Africa (Botswana, Lesotho, Mozambique, Namibia, South Africa, Swaziland, Zimbabwe and Zambia) (Ross, 1971; Pillay and Ward, 2012). They occur on a wide variety of soils (Chirara, 2002; William, 2015; Csurhers *et al*., 2016) and often

they have a high density of ~400 to 800 trees/ha (O'Connor 1995; Pillay and Ward, 2012). *V. karroo* trees are leguminous and belong to the family Leguminosae, subfamily Mimosoidae, under the subgenus *Acacia Vassal* (Chirara, 2002; Robbertse *et al*., 2014; Magandana, 2016). The tree species nodulates, fixes nitrogen and its root nodules are associated with *Rhizobium* species, thus it is very important in nutrient cycling (Chirara, 2002; William, 2015; Dingaan and du Preez, 2017). Subsequently, canopy patches of *V. karroo* trees are often associated with high understory productivity, particularly of important grasses such as *Panicum maximum*, due to the availability of nutrients and soil moisture within these patches (Dingaan and du Preez, 2017).

V. karroo trees are drought tolerant and have an extensive root system that can obtain water and nutrients from deep soil horizons, and this is probably the reason why *V. karroo* is amongst the most abundant *Acacias* in Southern Africa (Ross, 1971; Chirara, 2002; Robbertse *et al*., 2014; William, 2015; Csurhers *et al*., 2016; Dingaan and du Preez, 2017). *V. karroo* trees are also a pioneer species that invades after many types of disturbances and they are associated with bush encroachment (Ross, 1971; Chirara, 2002; Csurhers *et al*., 2016).

V. karroo physical characteristics vary depending on the areas in which they are found (Ross, 1971). However, *V. karroo* trees tend to have a lifespan of 30-40 years (Magandana, 2016), which can be decreased due to parasitism by mistletoes (Csurhers *et al*., 2016). The trees can grow to a height of up to 25m; though, their heights normally range from 1-15m. Although predominantly they have a rounded or flattened crown, they also have variable canopy shapes (Csurhers *et al*., 2016). The regeneration of *V. karroo* is discussed later in Chapter 4.

What are mistletoes?

Parasitism is common in plant communities, and about 1% of flowering plants are parasitic (Pinto, 2005) with over 5000 species of parasitic plants worldwide (Pennings and Callaway, 1996). Of these parasites, 40% parasitize on the top parts of their host whilst 60% are root parasites, though the genus *Tripodonthus* can attack both stems and roots (Bell and Adams, 2011; Daryaei and Moghadam, 2012). The aerial parasites (i.e., mistletoes, which are the focus of this study) grow within the canopies of the plants (Burns *et al.,* 2011) and they are thought to have originated and evolved from the roots, and their ascent was most likely facilitated by birds and mammals who deposited them on the canopies (Watson, 2017, 2020).

Mistletoes are polyphyletic flowering plants that occur in all regions, except Antarctica (Watson 2001; Mathiasen *et al*., 2008; Arruda *et al*., 2012; Richards *et al*., 2021). They are from five lineages of root parasites (Watson, 2017, 2020; Arruda *et al*., 2012) and they are found in the order Santalales which has 18 families (Mathiasen *et al*., 2008; Bell and Adams, 2011; Watson, 2016, 2020).

Mistletoes are comprised of more than 1400 species from the families, Amphorogynaceae, Loranthaceae, Viscaceae, Eremolepidaceae, and Misodendraceae (Pinto, 2005; Arruda *et al*., 2012; Amico *et al*., 2017). Most mistletoes fall under Loranthaceae and Viscaceae and together they have almost 1300 species (Watson, 2001; Pinto, 2005). However, Loranthaceae has the most genera of mistletoes (76) with ~1000 species that have been described (Manthiasen *et al.,* 2008; Watson, 2020). The savanna biome is the most species-rich area for Loranthaceae and Viscaceae mistletoes, whilst fynbos and forest biomes have low species richness (Dean *et al.*, 1994). Low species richness in forestsis due to low sunlight penetration within forests, which often limits mistletoe establishment and growth (Dean *et al.*, 1994; de Buen *et al.,* 2001; Roxburgh and Nicolson, 2008). Furthermore, mistletoes are richer in southern African savannas compared to other biomes such as fynbos, forests, succulent-karroo, and nama-karroo (Dzerefos *et al*., 2003). The dominant species of mistletoe in semi-arid savannas in the South-West, Zimbabwe fall under Loranthaceae and Viscaceae, and normally have mistletoe species such as *Erianthemum ngamicum* (Sprague) Danser (Loranthaceae), *Viscum verrucosum* Harv (Viscaceae), and *Pliocosepalus kalachariensis* (schinz) Danser (Loranthaceae) (Ndagurwa and Dube, 2013; Ndagurwa *et al.,* 2013, 2016). *E. ngamicum* and *V. verrucosum* are usually attracted to trees with high nutrient contents such as *Acacia* spp. which have high nitrogen levels (Ndagurwa *et al*., 2013).

Mistletoes are also chlorophytic hemiparasites (Preston *et al.,* 2010; Arruda *et al.*, 2012) that obtain their water, minerals, and nutrients through a haustorium, which penetrates and transports solutes from the host plant tissue but also through their photosynthesis (Preston *et al.,* 2010; Arruda *et al.,* 2012; Richards *et al*., 2021). Specifically, they can obtain 60% of their carbohydrates from the host and photosynthesise the other 40% (Watson, 2001; Barbu, 2010; Bishop, 2010). To absorb nutrients and water, the haustorium attaches to the host plant and penetrates the xylem where water transportation occurs (Dzerefos and Wikowski, 1997; Aukema, 2004; Bishop, 2010; Preston *et al.,* 2010; Arruda *et al.,* 2012). Conversely, some hosts through host tissue proliferation at the host-mistletoe interface, prevent the haustorium of the parasite from reaching their xylem (Dzerefos and Witkowski, 1997; Dzerefos *et al*., 1999, 2003).

Mistletoe Uses in the Ecosystem

Mistletoes have a very intimate relationship with their hosts, dispersers and consumers (Bishop, 2010). They are an important food source and shelter for diverse species of birds, mammals, and invertebrates (Burns *et al.*, 2011; Lira *et al.*, 2017; Těšitel *et al*., 2020). Mistletoes bear fruits that are large, sweet, and when ripe they are bright in colour making them very attractive, thus they are highly utilised by frugivores (Watson, 2001; Arruda *et al.,* 2012; Napier *et al.,* 2014). The fruits are also nutritious with high lipids in Loranthaceae species whilst Viscaceae species have more proteins.

However, most of these fruits contain carbohydrates and have high mineral levels for both macro (P and K), and micro (Mn and Fe), nutrients (Watson, 2001).

In Australia, ~30 bird species such as the mistletoe bird and honeyeater rely on mistletoes for their nectar or fruits (Bishop, 2010). Moreover, because mistletoes are succulent and have high nutrient forage they are also a favoured browse for mammals that utilise both fruits and leaves (Watson, 2001; Roxburgh and Nicolson 2008; Bishop, 2010; Ndagurwa and Dube, 2012, 2013). Wild (e.g., Eland and greater Kudu) and domestic animals (goats) in southern Africa also eat mistletoes as they are a reliable food source (Roxburgh and Nicolson 2008; Ndagurwa and Dube, 2012, 2013). Subsequently, their highly nutritious, palatable leaves, and fruits influence the diversity and distribution patterns of both vertebrates and invertebrates (Burns *et al.,* 2011; Napier *et al.,* 2014; Ndagurwa *et al.,* 2014). Nonetheless, some mistletoe may form host-mimicry to avoid predation (Watson, 2001) hence cryptic mistletoes have higher nitrogen concentrations relative to host (Bannister, 1989).

Mistletoes thrive during the dry season or drought due to their chlorophyll-protein complexes which induce low capacities for photosynthesis (Ndagurwa and Dube, 2012). Contrariwise, low capacities to photosynthesise may lead to a failure to produce fibre which is an anti-herbivory compound used by plants (Ndagurwa and Dube, 2012). Accordingly, Ndagurwa and Dube (2013) found that mistletoes had low concentrations of secondary compounds, particularly tannins and lignin, although *Viscum* species were found to have high condensed tannin content. These low concentrations of lignin could potentially be the reason behind the quick breakdown of mistletoe litter for the rapid release of nutrients (Ndagurwa *et al.,* 2013). Subsequently, low anti-herbivory compounds and the presence of mistletoes in the dry season often make them a reliable source of food. However, parasites can also benefit from the host by obtaining host anti-herbivory metabolites which leads to parasites being less affected by herbivory.

Mistletoes are used as structural support for bird nests due to their dense evergreen foliage, and their multi-branched structures, particularly in open canopies. They are also used as nesting sites, roosting or hibernation sites for birds (long-eared owls), mammals (porcupines and pine martens) and raptors (Watson, 2001; Press and Phoenix, 2005; Napier *et al.,* 2014). Mistletoe foliage (e.g., *Viscum* species) also have antibacterial properties and if used in nest linings the foliage can act as an immuno-stimulant for fledglings such as starlings (*Sturnus vulgaris*) (Watson, 2001; Press and Phoenix, 2005). Some vertebrate species also prefer mistletoe clumps for the coolness in extremely hot weather (Ndagurwa *et al.,* 2016b). Arthropods are also known to inhabit mistletoes (Burns *et al.,* 2011) and as a result, mistletoes have species-rich assemblages of insects thus increasing the prevalence of other insectivorous species (Watson, 2001).

Consequently, mistletoes provide nectar, fruit, shelter, and foliage in many ecosystems and they have an impact on ecosystem processes and the abundance and biomass of vertebrate and invertebrates (Watson, 2001; Burns *et al.,* 2011; Napier *et al.,* 2014). Accordingly, mistletoes are a keystone species as their impact on the ecosystem is inordinately large in proportion to their abundance. Studies have shown that higher mistletoe densities are associated with greater flora and fauna richness, and because mistletoes are involved in many interactions, they can have community-level impacts (Watson, 2001).

Mistletoe host preferences

The majority of mistletoes are host generalists and some can also parasitize on other parasites, although, shoot parasites have narrower host ranges in comparison to root parasites (Watson, 2001; Bell and Adams, 2011; Arruda *et al.,* 2012; Lira *et al.,* 2017). Mistletoes can utilise either the most common and abundant host species or the most frequently encountered hosts (Press and Phoenix, 2005). Mistletoes are bound to be generalist in areas with high host species richness, however, in areas with low host species richness they are bound to be host specialists (Kavanagh and Burns, 2012). In agreement, Roxburgh and Nicolson (2005) found that *Plicosepalus kalachariensis* Schinz (Loranthaceae) infection prevalence was significantly correlated (Coefficient = 0.04, $P < 0.01$) with the total number of each tree species. However, few mistletoes are limited to one or two hosts, and the dwarf mistletoe *Arceuthobium minutissimum* (Viscaceae), is specific, only infecting *Pinus griffithii* (syn. *wallichiana*) (Dzerefos *et al*., 2003; Press and Phoenix, 2005; Mathiasen *et al.,* 2008). Parasite host preferences may vary spatially, as the susceptibility of a tree to infection may differ with location (Press and Phoenix, 2005; Roxburgh and Nicolson 2005). In one area a tree species can be heavily infected whilst in another area it is less infected by mistletoes (Hawksworth and Wiens, 1970). This is attributed to the ecotypic variation of the host, mistletoes, environmental factors and/or it can be influenced by habitats (Hawksworth and Wiens, 1970).

Mistletoes aim for hosts that bring the most benefits to their growth, reproduction, and fitness rather than on a variety of hosts that would not increase their productivity (Press and Phoenix, 2005; Daryaei and Moghadam, 2012). Subsequently, they are dominant on larger trees because they have higher access to nutrients such as nitrogen and water. Their deep well-developed roots tap into the lower horizons of the soils even throughout the dry season thus making large trees a more reliable source of these resources (Ndagurwa *et al.,* 2012). However, Lira *et al.* (2017) report that despite high resource availability there was low mistletoe parasitism on deciduous trees and they attributed this to birds preferring not to perch on these trees due to higher chances of predation. Regardless, mistletoes often thrive on hosts with high N content (e.g., leguminous trees, *Acacia*), whose vascular system can easily be tapped into, and those with lower defence capacities (Press and Phoenix, 2005; Hosseini *et al*., 2008; Kavanagh and Burns 2012; Ndagurwa *et al*., 2012). For example, spinescent plants grow on

moist and eutrophic soils hence the occurrence of mistletoes on these plants is for their nutrients and water rather than protection from their spines (Dean *et al.*, 1994). Furthermore, hosts that can access limiting resources are of paramount importance particularly those that can access water during the dry season (Press and Phoenix, 2005). Therefore, mistletoes prefer hosts that offer high resource longevity such as woody perennials to herbaceous plants which are short-lived (Dean *et al.*, 1994; Press and Phoenix, 2005). Consequently, in the savanna biome, the most important genera of hosts for mistletoes are *Acacia* (*Vachellia, Senegalia, Faidherbia*) (24 spp.), *Combretum* (14), *Maytenus* (13), and *Rhus* (*Sersia*) (12) (Dean *et al.*, 1994).

Often long-lived trees have the potential to be more exposed to infections compared to short-lived trees (Roxburgh and Nicolson, 2008). However, larger trees have more chances of recurring mistletoes; hence they have higher mistletoe infection intensities (Dzerefos *et al*., 2003). This is probably because as trees get older the probability of receiving mistletoe seeds rises (Overton, 1994) and the susceptibility to infection increases due to a high likelihood of dispersers visiting that particular tree (Aukema and Del Rio, 2002; Ndagurwa *et al.*, 2012, 2013). In agreement, Barbu (2010) found severe infection in older than younger silver fir (*Abies alba* Mill.) trees. However, factors other than tree size may influence infection of mistletoes. Ndagurwa *et al.* (2012) found a weak positive relationship between tree size (stem diameter, $r^2 = 0.17$, $P < 0.05$; height, $r^2 = 0.08$, $P < 0.05$) and mistletoe infection. However, Roura-Pascual *et al.* (2012) found that trees with big diameters have a positive impact on mistletoe establishment as they have big canopies which increased the chances of visits from birds, as the trees were more visible. Roxburgh and Nicolson (2008) also found that the diameter was 49% bigger for mistletoe-infected- compared to uninfected trees. Consequently, mistletoe infection intensity ($r^2 = 0.15$) and mistletoe infection prevalence increased with stem diameter. Furthermore, mistletoes have also been shown to survive at higher rates on taller hosts (de Buen *et al.*, 2002; Dzerefos *et al*., 2003; Roxburgh and Nicolson, 2008; Ndagurwa *et al.*, 2012). Roxburgh and Nicolson (2005) report that mistletoe-infected trees were 1.59-fold taller than uninfected trees, and there was a positive association (Coefficient = 0.62, $P = 0.02$) between *Phragmanthera dschallensis* (Engl.) (Loranthaceae) infection prevalence and tree height.

Mistletoes are often dominant on the upper than lower parts of the canopy and this can be due to higher light incidence at the top than at the bottom. However, mistletoes can also be dominant at the top of the canopy due to disperser preferences. For instance, birds prefer to perch on the upper parts of the tree canopies thus it is not surprising that a higher number of mistletoe seeds are also deposited there (Roxburgh and Nicolson, 2005, 2008; Amico *et al*., 2017). Conversely, Amico *et al*. (2017) found that the marsupial *Dromiciops gliroides* (Thomas 1894) deposited the seeds in lower parts of the canopy than what has been observed with birds. This is probably due to vegetation preferences and/or the marsupial choosing the lower parts of the canopy as an anti-predatory strategy. Regardless,

the lower canopy has poor light incidence and this can negatively impact mistletoe seed establishment and survival (Dean *et al.*, 1994; Amico *et al*., 2017). Even so, Lira *et al*. (2017) found that light was not an important factor in determining parasitism by mistletoes in a Brazilian rainforest. Therefore, factors such as mistletoe/disperser relationship, and disperser preferences, host resistance and compatibility can influence mistletoe infection patterns (de Buen *et al.,* 2002; Roxburgh and Nicolson, 2005, 2008; Ndagurwa *et al.,* 2012).

Characteristics of the host such as branch architecture, bark thickness, host size, age, height, biochemical composition and compatibility also influence host selection (Aukema, 2004; Roxburgh and Nicolson, 2005; Bell and Adams, 2011; Arruda *et al.,* 2012; Daryaei and Moghadam, 2012; Kavanagh and Burns 2012; Ndagurwa *et al.,* 2012). Seeds can be defecated in clumps on host branches (Aukema, 2004; Roxburgh and Nicolson, 2005, 2008; Arruda *et al.,* 2012) but the diameter of host branches is crucial in the establishment of the mistletoes (Amico *et al*., 2017). Dzerefos *et al*. (1998) reported that the Loranthaceae mistletoe *Erianthemum dregei* (Eckl. & Zeyh.) Tiegh, preferred branches with a cross-sectional area that was between 0.0002 m^2 and 0.025 m^2, whilst the Viscaceae *Phoradendron robustissimum* Eichler, preferred branches between 7.85 x 10^{-5} to 0.0002 m^2. However, mistletoes can establish themselves on thicker branches of softwood species (Lira *et al*., 2017).

Indeed, after processes that get rid of the pericarp that is around the seed (excretion, bill wiping, or regurgitation) the seeds are planted on the branches of host (de Buen *et al.,* 2002; Pinto, 2005; Daryaei and Moghadam, 2012). However, mistletoes need certain chemical conditions to trigger germination and haustorial development. Mistletoes usually have high germination rates, germinating immediately after the epicarp (outer lying layer of the pericarp of the fruit) is removed, even germinating in dry air (Press and Phoenix, 2005; Roxburgh and Nicolson, 2008).

Dispersers of Mistletoes

Mistletoes can resprout (Dzerefos *et al*., 1998) and utilize hydrostatic explosion (Viscaceae) to disperse seeds (Watson, 2001). Equally, mistletoes also rely on dispersers, and they have a symbiotic relationship with their pollinators and dispersers (Mellado *et al*., 2016a). Therefore, pollinator and disperser behaviour is important particularly for mistletoes that rely on dispersers to deposit their seeds on suitable hosts for successful establishment (Amico *et al*., 2017). Dispersers can either be specialist or generalists, who feed on mistletoes and they can be birds (~66 families), mammals (~30 families), and insects (Watson, 2001, 2015; Aukema and Del Rio, 2002; Mellado *et al*., 2016a). Amico *et al*. (2017) found that the marsupial *Dromiciops gliroides* efficiently dispersed the mistletoe *Tristerix corymbosus* (L.) Kuijt on microsites hence increased the chances of their establishment. However, birds are the major transmitters of sticky (substance called viscin) mistletoe seeds and they

are attracted by the mesocarp on the berries which is very nutritious (Dzerefos *et al*., 1998; Watson, 2001; Pinto, 2005; Bishop, 2010; Arruda *et al.*, 2012; Lira *et al.*, 2017).

Birds are rarely interested in trees that have little food resources (Dzerefos *et al*., 1998), but the presence of mistletoes and their high resources attracts a variety of birds to these trees. Therefore, by increasing their species diversity and richness, mistletoes have an effect on the overall community structure. Consequently, mistletoe removal could have adverse impacts on the birds' overall community structure (Lira *et al*., 2017). Even so, most mistletoes are productive during the wet season, but, because of the constant access to nutrients, some mistletoes can have a prolonged flowering, constant and continuous fruiting season. Thus, they have higher chances of being utilised as they are more reliable and available, particularly when food resources are limiting (Watson, 2001; Bishop, 2010; Mellado and Zamora, 2017). Moreover, some mistletoes sporadically ripen their fruits and this ensures continuous fruit availability. Also, individual mistletoes can show variations in the times in which they produce fruits (Watson, 2001). This increases their reliability especially when there is a drought or food shortages (Lira *et al*., 2017).

Birds are normally the natural vectors of mistletoes particularly if their breeding season coincides with mistletoe fruiting. For example, *Phainopepla nitens* breeding success is dependent on the presence and distribution of mistletoe berries (Aukema, 2004; Barbu, 2010). Consequently, *P. nitens* respond to mistletoes. In addition, they distribute mistletoe seeds differently among hosts, favouring other host species over the others, thus they shape mistletoe distribution and create infection patches (Aukema, 2004). Similarly, Roxburgh and Nicolson (2005, 2008) found that the three main dispersers (i.e., *Pogoniulus chrysoconus* (Temminck), *Lybius torquatus* (Dumont), and *Cinnyricinclus leucogaster* (Boddaeert)) of mistletoes (*Phragmanthera dischallensis and Plicosepalus kalachariensis*) in their study nested at the same time as the fruiting season of mistletoes. Consequently, they encountered 2.57-fold more birds on mistletoe-infected than on uninfected trees, and the birds spent 2.65-fold more time on infected than uninfected trees (Roxburgh and Nicolson, 2005). In agreement, Napier *et al.* (2014) also reported that there was greater bird species richness and alteration in the bird community when mistletoes were fruiting, and they observed significantly higher mistletoe birds during the period when mistletoe fruits were ripe. They also observed that even though patterns of fruiting varied, the presence of birds was specifically linked to the mistletoes fruiting (Napier *et al.*, 2014).

These dispersers influence the spatial distribution of mistletoes, and there is a higher chance (70%) that mistletoe seeds are deposited within a range of 100m from the host tree (Bishop, 2010; Amico *et al*., 2017). Usually, mistletoes are aggregated and persist on the same infected trees because seeds are often redeposited on trees that are already infected, eventually resulting in both the host and parasites

dying (Pinto, 2005; Mellado and Zamora, 2017). Contrariwise, Amico *et al*. (2017) found that 55% of seeds were rather deposited on uninfected trees. Nonetheless, when dispersers prefer certain tree species, these species are most likely to be infected by mistletoes as the dispersers' perch, feed, and find shelter on the trees (Roxburgh and Nicolson, 2005). These trees have a higher probability of having more mistletoe seeds deposited on them than others are (Roxburgh and Nicolson, 2005), leading to mistletoe aggregation on the host tree (Aukema, 2004).

Furthermore, trees in low densities have higher parasitism due to birds preferring sparsely located trees (Pinto, 2005). Birds typically prefer taller and larger hosts (Overton, 1994). Roxburgh and Nicolson (2008) found that birds perched on taller and larger diameter trees regardless of whether the trees were infected or not. Therefore, tree architecture and mistletoe intensity positively influence the likelihood of dispersers visiting a particular tree (Aukema and Del Rio, 2002; Ndagurwa *et al.,* 2012). Equally, factors such as distance from the nesting site and spacing of trees are important determinants of disperser behaviour (Roxburgh and Nicolson, 2008).

Mistletoes impacts on plant communities

Plant community assemblage, species diversity, and composition are often influenced by patch dynamics which cause spatial heterogeneity in savanna ecosystems (Joseph *et al*, 2013). Studies done in savanna ecological systems have shown the positive impacts of large tree dominated patches and termitaria patches as nutrient islands on plant community structures (Joseph *et al*., 2013, 2014; Muvengwi *et al.,* 2015). Equally, mistletoes exhibit such patches which on one hand exert significant impacts through extended periods of litter-fall, high leaf turnover, and high resorption efficiency which all results in nutrient-rich litter as compared to surrounding areas (the litter pathway). On the other hand, mistletoes can suppress host plants and influence the competitive interactions that occur between the host and co-occurring species (the parasitism pathway), (Pennings and Callaway, 1996; Cameron *et al*., 2009; Demey *et al*., 2013; Ndagurwa and Dube, 2013; Muvengwi *et al.,* 2015; Ndagurwa *et al*., 2016). Although the effectiveness of the litter pathway is dependent on the quality of the host, the parasitism pathway is reliant on how competitive the co-occurring plants are (Press and Phoenix, 2005). Therefore, this section aims to discuss how productivity and species composition are influenced by the two pathways.

The Mistletoe Parasitism Pathway

Mistletoes draw their resources in a unidirectional manner maintained by higher transpiration rates than their hosts through the connection of haustorium (Pinto, 2005). As a result, mistletoes have higher transpiration rates than their host plants (Bishop, 2010; Scalon and Wright 2017). High transpiration and leaf conductance lead to mistletoes extracting sufficient nutrients such as nitrogen from the xylem of the host (Bannister and Strong, 2001). Indeed, N may be the most important nutrient that can ensure mistletoe growth and when nitrogen is absorbed, the host may not be able to

efficiently absorb more nitrogen to compensate for this loss (Daryaei and Moghadam, 2012, Al-Rowaily *et al*., 2020). However, mistletoes have been found to have a higher concentration of K, which is crucial for osmotic regulation thus increasing water and nutrient uptake in favour of the mistletoes (March and Watson, 2010; Al-Rowaily *et al*., 2020). Ehleringer and Schulze, (1985) also found that concentrations of Cu, Mg, Na and Zn were higher in mistletoes than host trees and this was attributed to high transpiration and negative water potentials. Therefore, mistletoes interfere with water transport mechanisms of their hosts by increasing the leaf area to sapwood cross-sectional area ratios, which increases the demand for water relative to the supply (Sala *et al.,* 2001). Conversely, Richards *et al*. (2021) found no evidence of transpiration-controlled nutrient acquisition in their parasitic plants thus other physiological factors could have been influencing mistletoe nutrient acquisition. They reported that when the host N levels were low, there were smaller variations in N_{mass} of the host and the mistletoes. Nevertheless, if the host plant is starved of water and it suffers from drought stress (Sala *et al.,* 2001), or if other stress factors are affecting it, the host eventually dies (Bishop, 2010).

After an infection, by invading the vascular system of a plant, mistletoes compete with their hosts for water and nutrients. For instance, Al-Rowaily *et al*. (2020) report that the mistletoe *Plicosepalus curviflorus* (Benth. ex Oliv.) Tiegh., significantly reduced nutrients (especially potassium and sodium) in three Acacia species (*Acacia asak* (Forssk.) Willd., *A. ehrenbergiana* (Hayne), *A. gerrardii* Benth., *A. raddiana* Savi, and *A. tortilis* (Forssk.) Hayne). However, the reduction in nutrients varied depending on the host species and degree of mistletoe infection (no infection, low- and high infection) (Al-Rowaily *et al*., 2020). For example, the rank order for nutrient reduction in the *Acacia* hosts varied between high- and low mistletoe-infection, i.e., $K < Na < P < Mg < N < Ca$ and $K < P < Na < N < Mg < Ca$, respectively. Furthermore, different species showed variations in the decline in nutrients compared to uninfected trees i.e., as infection intensity increased *A. gerrardii* had 1.35 and 1.74-fold more N loss for low and high mistletoe-infection trees, (respectively), compared to uninfected trees. Similarly, although uninfected trees had higher N compared to high and low mistletoe-infected *A. asak, A. ehrenbergiana, A. tortilis*, their N losses did not differ with an increase in mistletoe infection.

A decline in nutrients potentially decreases host growth, reproductive output, and physiological attributes, but increases the hosts' susceptibility to other pathogens (Press *et al*., 1999; Press and Phoenix, 2005; Cullings *et al.,* 2005; Hosseini *et al.,* 2008; Preston *et al.,* 2010; Bell and Adams, 2011; Daryaei and Moghadam, 2012; Arruda *et al.,* 2012; Mellado and Zamora, 2017; Silva *et al*., 2021). Changes in the biomass allocation of the host through the loss of leaf area, decline in the number of leaves and reduced biomass decreases the photosynthetic and respiration capacities of the host. Consequently, this can induce abnormal growth and host physiological changes (Press *et al*.,

1999; Sala *et al.*, 2001; Press and Phoenix, 2005; Arruda *et al.*, 2012; Těšitel *et al*., 2020). In agreement, Daryaei and Moghadam (2012) found that the area and weight of hornbeam (*Carpinus betulus* L.) and Alder (*Alnus glutinosa* Gaertn) branches infected by *Viscum album* L. was lower compared to uninfected branches. Similarly, Silva *et al*. (2021) found that mistletoe infections on one branch can also affect neighbouring healthy branches. However, the impacts varied with mistletoe and host species i.e., the mistletoe *Phoradendron crassifolium* increased the specific leaf area (by ~2.6-fold) and reduced the leaf dry matter content (by ~0.8-fold) of neighbouring branches of *Eremanthus erythropappus* compared to parasitism of *Psittacanthus robustus* on *Vochysia thyrsoidea* leading to variations in the hosts' leaf resource use strategies. This was attributed to varying levels of antagonism caused by the different mistletoe species coupled with differences in host susceptibility to parasitism (Silva *et al*., 2021). Similarly, Scalon *et al*. (2017) report that infected branches had lower specific leaf area, thinner and shorter petioles than uninfected branches showing differences in resource use strategies i.e., conservative and resource acquisitive strategies.

The accumulative parasitism impacts result in changes in the overall canopy structure for instance, dwarf mistletoes were observed to decrease Douglas fir (*Pseudotsuga menziesii*) growth by ±65% (Mathiasen *et al*., 1990; Press and Phoenix, 2005). Similarly, Mueller and Gehring (2006) and Mathiasen *et al.* (2008) show that dwarf mistletoe parasitism results in foliage loss and formation of gaps, and alteration of forest stand structure and composition due to mortality (Cullings *et al.*, 2005; Mellado and Zamora, 2017). This increases the amount of light that penetrates the canopy hence benefiting the understory biomass. For instance, Mellado and Zamora (2017) found that *Viscum album austriacum* reduced the dense canopy of *Pinus nigra* which in turn increased the amount of light incidence that penetrated the understory by 19%.

Studies (Gomes and Fernandes, 1994; Silva and del Rio, 1996; Geils and Hawksworth, 2002; Press and Phoenix, 2005; Mourão *et al*., 2009; Arruda *et al.* 2012; Daneshvar *et al*., 2014; Cruz Neto *et al*., 2017) have also shown that mistletoes can also reduce reproductive capacities of their hosts thereby posing a threat to the population of their hosts. Mellado and Zamora (2020) reported that *V. album* lowered cone production (by 1.45 to 11.14-fold), cone length (10 to 18%) and width (5 to 14%), seed weight (14 to 53%), seed germination (0.2-22%) and seedling emergence. However, the extent of mistletoe-parasitism impacts varied depending on the pine species. For instance, the reproductive traits of *Pinus sylvestris* subsp. *Nevadensis* were more affected compared to *Pinus nigra* subsp. *Salzmanii*. Nonetheless, no known study has investigated how reproductive capacities are affected by different mistletoe infection degrees in a semi-arid savanna.

In extreme conditions or high densities, mistletoes have been observed to kill the host (Press and Phoenix, 2005; Preston *et al.*, 2010; Arruda *et al.*, 2012), whilst at low densities, they may not

necessarily kill the host but will reduce its productivity considerably (Press and Phoenix, 2005; Bishop, 2010). Nonetheless, some plants can fight or are resistant and immune to certain mistletoe species. For example, eucalyptus can control the amount of water that is distributed to limbs infected by mistletoes resulting in the loss of the limb (Bishop, 2010; Preston *et al.*, 2010). Some hosts will normally absorb more micronutrients to fight against infections (Hosseini *et al.*, 2008). Daryaei and Moghadam (2012) found that K, Mg, and Zn were higher in the infected branches, though the N content was lower (Daryaei and Moghadam, 2012). High K concentrations in the host plant decrease susceptibility of the host plant to parasitic attacks and harsh environmental conditions (Ehleringer and Schulze, 1985; Hosseini *et al.*, 2008; Daryaei and Moghadam, 2012). Therefore, the high micronutrient levels observed by Daryaei and Moghadam, (2012) could most likely be a mechanism to decrease susceptibility to mistletoe attacks. Some plants can even out-compete the mistletoe for light and hence reduce parasitic growth (Bell and Adams, 2011).

Parasitism impact of mistletoes on the overall community

The impact of mistletoe parasitism is broader than one host-parasite relationship and it may influence entire ecosystems (Cullings *et al.*, 2005). Therefore, if the infection is high, the survival of the host may decrease or the host can become locally extinct, and this may influence plant community succession and understory composition (Press and Phoenix, 2005; Cullings *et al.*, 2005). This can also have negative feedback on the parasite itself and it can lead to its extinction unless the host manages to re-establish itself (Press and Phoenix, 2005).

A reduction in host growth can increase plant diversity as less dominant non-host species are allowed to thrive (Spasojevic and Suding, 2011). Mistletoes similar to other hemiparasites enable inferior co-occurring plants and facilitate the invasion or establishment of new or less dominant species thereby influencing community structures, plant diversity, and richness (Press and Phoenix, 2005; Spasojevic and Suding, 2011; Arruda *et al.*, 2012; Demey *et al*., 2013; Fisher *et al*., 2013; Watson, 2016). Bardgett *et al.* (2006) found that the root hemiparasite *Rhinanthus minor* L. (although not a mistletoe) increased plant diversity although it reduced productivity. The parasite also had an impact on both above ground and underground properties and they increased N cycling. Press and Phoenix (2004) and Pennings and Callaway (1996) in their studies in North America show the same results as that of mistletoes, where *Cuscuta salina* (marsh dodder) which parasitized on *Salicornia virginica*, suppressed *S. virginica* and led to the establishment of co-occurring plants *Limonicum californicum* and *Frankenia salina*, thus increasing plant diversity. Similar to other hemiparasitic herbs and dodder, mistletoes have been shown to have similar impacts on their hosts and surroundings, for instance, Mellado and Zamora (2017) report that mistletoe parasitism increased light incidence which coupled with soil nutrients augmented growth, plant recruitment, species composition and richness of understory woody plants in the Mediterranean pineland. Inevitably, mistletoe parasitism alters the

community structure and dynamics of plant communities by altering the competitive balance of the hosts (Puustinen and Mutikainen, 2001; Těšitel *et al*., 2020).

Consequently, infection of the host by the mistletoes, a decline of the hosts functional attributes and increased light incidence facilitate the parasitism pathway (Spasojevic and Suding, 2011; Demey *et al*., 2013). However, mistletoes can also go on further to negatively affect co-occurring plants by competing with them for the same resources they avail (Press and Phoenix, 2005). Irrespective, the absence of parasites might lead to an increase in above-ground productivity with competitive and fast-growing species thriving at the detriment of other species hence decreasing overall diversity (Bardgett *et al*., 2006). Such a change may influence succession patterns and understory composition (Cullings *et al.,* 2005). Therefore, mistletoes are a keystone species as they facilitate co-existence and diversity through limiting competitive dominants through fitness reduction (Bardgett *et al.,* 2006; Spasojevic and Suding, 2011; Arruda *et al.,* 2012). However, no known study has investigated how understory productivity and species composition is affected by the differences in mistletoe intensity on dominant hosts.

The Mistletoe Litter Pathway

Effects of parasitism can be counteracted by the addition of mistletoe litter into the ecosystem thus resulting in increased diversity and productivity (Těšitel *et al*., 2020). Like termite mounds, mistletoes can directly increase nutrients in their localized patches through their litter foliage or indirectly via litter deposits from animals, birds and insects visiting the mistletoes (Fig 1.2). Mistletoes have high litter-fall and leaf turnover and they add a substantial source of litter which is localized within the host tree canopy patches (Press and Phoenix, 2005; Mathiasen *et al.,* 2008; Mellado *et al*., 2016; Watson, 2016; Lira *et al*., 2017). In semi-arid tropical savannas, mistletoes were found to significantly increase litter by 112 to 156% in the canopy patches of infected trees (Ndagurwa *et al*., 2013, 2014b; Watson, 2016). In Australia, March (2007) also reported an increase in floor litter by a factor of 1.9 attributed to high mistletoe litter-fall. Similarly, Mellado *et al*. (2016) reported that parasitized tree canopy patches received 99% more litter quantities (mistletoe, leaves, flowers, and fruits) than un-parasitized trees. In contrast, under parasitized trees host litter was 24% less compared to that of un-parasitized trees. Consequently, high litter deposits beneath mistletoe-infected hosts can increase nutrient cycling within localised patches, thus increasing understory biomass, productivity and species diversity (Ndagurwa *et al.,* 2016, 2018). In contrast, if the leaf litter effect is weaker it potentially reduces productivity and diversity of the ecosystem (Spasojevic and Suding, 2011; Demey *et al*., 2013).

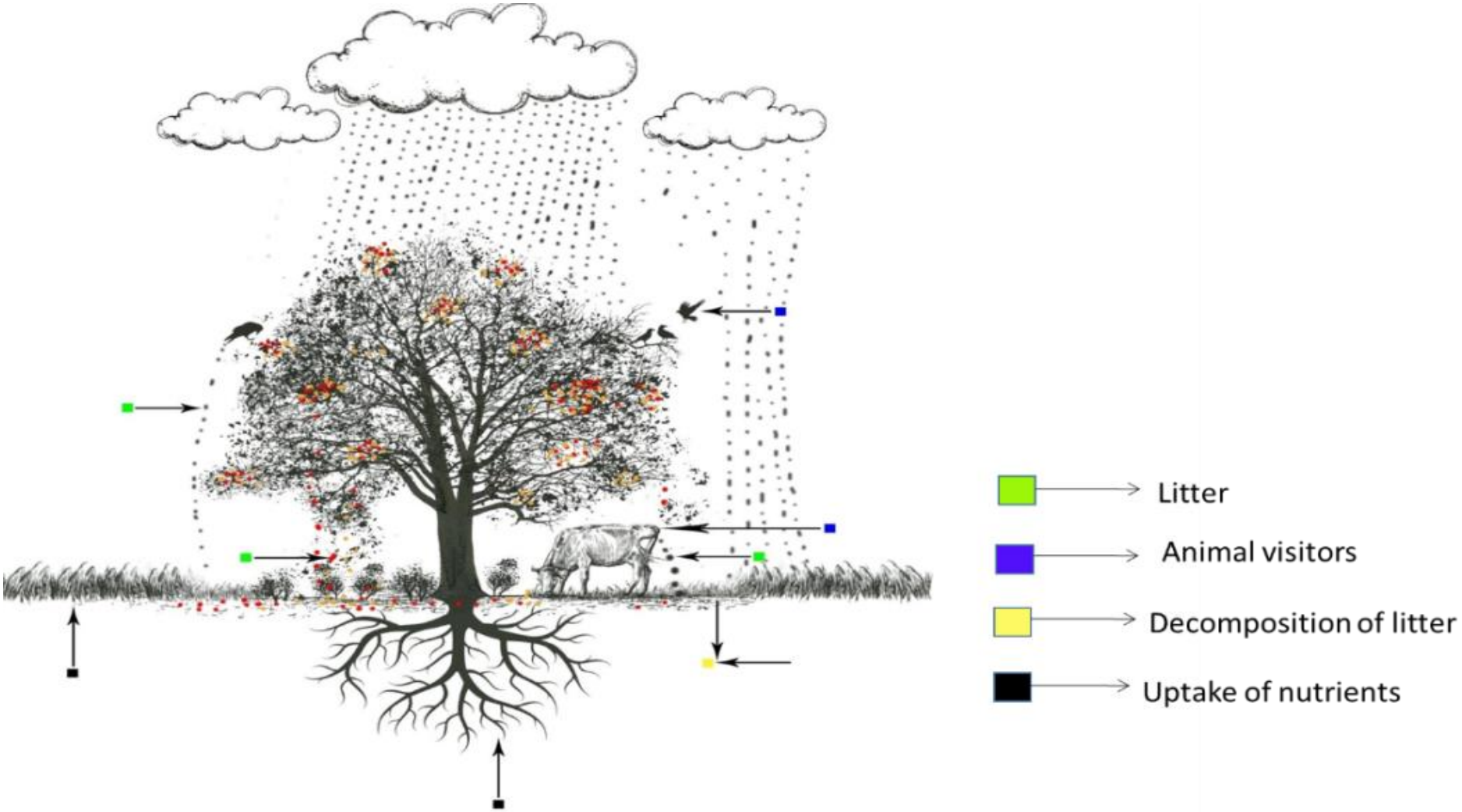


Figure 1.2. Direct and indirect litter deposits under mistletoe-infected tree canopies. Canopy patches receive litter from the host and mistletoe foliage, bird and animal visitors. Nutrients are also obtained from stem flow or/bark sloughing and via litter decompoition.

Mistletoe litter composed of leaves, fruits, and flowers has a high level of nutrients and studies in different locations have shown significant increases in nutrient availability. For instance, Watson (2015), reports that the hemiparasitic herb *Bartsia alpine* L. in a sub-arctic heathland, increased the nitrogen input by 53% and contributed 17% of annual leaf litter, yet the parasitic-plant only comprised 3% to 4% of the total above-ground biomass. Similarly, for aerial mistletoes, Mellado *et al*. (2016) reported that N, P and K in *V. a. austriacum* ranged from 1.5 to 9.8-fold times more than the hosts' litter. Ndagurwa *et al*. (2014) in semi-arid savannas found that N (10-70%), P (10-360%), K (20-270%) and Ca (40%) were relatively higher under infected compared to uninfected trees. Nutrient ranges varied depending on the mistletoe species (Ndagurwa *et al*., 2014). On the contrary, March (2007) found that *Amyema miquelii* (Miq.) Tiegh had 1.36-fold lower N compared to Redgum (*Eucalyptus* species) thus showing that nutrient concentrations can vary depending on the species of host and mistletoe (Dean *et al.*, 1994). Similarly, Al-Rowaily *et al*. (2020) state variations in the nutrient contents of the mistletoes were influenced by their *Acacia* host species and by the degree of mistletoe-infection. They found that mistletoes had significantly higher N, P, and K than their *Acacia* hosts. Additionally, mistletoes had higher N, P, Ca, and Na compared to *Acacias* with low mistletoe infection, whilst high mistletoe-infected *Acacias* had lower K, N, P, Na, and Mg compared to the mistletoes. Generally, as mistletoe infection increased the nutrient concentrations increased in the mistletoes (Al-Rowaily *et al*., 2020).

Mistletoe litter is also highly decomposable, and it can facilitate the decomposition of the recalcitrant litter of the host (Press and Phoenix, 2005; Ndagurwa *et al.,* 2013, 2014; Fisher *et al*., 2013; Muvengwi *et al.*, 2015). Ndagurwa *et al.* (2015, 2020) also reported that the rate of mistletoe litter (*E. ngamicum, P. kalachariensis* and *V. verrucosum*) decomposition was 1.66 to1.9-fold faster than *V. karroo* litter. Consequently, the mixing of mistletoe and the recalcitrant host litter accelerated the overall litter decomposition by 30% to 75% (Ndagurwa *et al.*, 2020). Nonetheless, mixing *V. karroo/V. verrucosum* and *V. karroo/P. kalachariensis/V. verrucosum* did not significantly reduce decomposition rates (Ndagurwa *et al.*, 2013, 2020). Similarly, March (2007) found that mistletoe-litter decomposition rate was slower than red gum litter, and mistletoe litter reduced the decomposition rates of their host litter within 12 months. Differences in the rate of litter decomposition can be explained by the variations in the chemical characteristics (nutrient) of mistletoe and host species and decomposers (arthropods, fungi, bacteria and mycorrhizae) associated with each species (March, 2007; Muvengwi *et al*., 2015; Ndagurwa *et al*., 2020). Therefore, low decomposition rates in mixtures of *V. karroo/V. verrucosum* and *V. karroo/P. kalachariensis/V. verrucosum* can be due to higher secondary compounds in *V. verrucosum* litter and the chemical interactions that arose from mixing the litter (March, 2007; Ndagurwa *et al*., 2020).

Soil nutrient concentrations can also vary depending on the degree of mistletoe-infection and host species (Muvengwi *et al*., 2015; Al-Rowaily *et al*., 2020). Muvengwi *et al*. (2015) found that *Strychnos spinosa* Lam., with the least number of mistletoes had lower soil P (1.13 to 1.28-fold), K (1.6 to 2.6-fold), Ca (3.08 to 4.29-fold) and Mg (1.77 to 2.17-fold) compared to *Diospyros mespiliformis* Hochst. ex A. DC., *Ficus sycomorus* L. subsp. *sycomorus*, *Sclerocarya birrea* (A. Rich.) Hochst., which had relatively a higher number of mistletoe infections. Furthermore, infected trees mostly had higher soil nutrient concentrations compared to their respective uninfected trees. In agreement, Al-Rowaily *et al*. (2020) report that K soil concentrations were 1.67-, 2.34-, 2.79- and 3.26-fold higher under *Acacia tortilis*, *A. ehrenbergiana*, *A. asak* and *A. gerrardii* respectively compared to their respective uninfected trees. Moreover, the rank order of increase in soil elements under high infection trees was K < Ca < N < Na < Mg < P whilst low infection soils had a rank order of Ca < N < K < Na < P < Mg. Additionally, the soil N content varied depending on the *Acacia* host, for instance, *A. asak, A. tortilis, A. ehrenbergiana,* and *A. gerrardii* had 1.65-, 1.81-, 1.99- and 3.22-fold more soil N compared to the respective uninfected trees (Al-Rowaily *et al*., 2020).

There is spatial heterogeneity of resources attributed to the extent of mistletoe infection, the mistletoe species in the canopy, and the soil chemistry (Spasojevic and Suding, 2011; Ndagurwa *et al.*, 2013, 2014; Muvengwi *et al.*, 2015). These variations can be a result of each mistletoe species acquiring different quantities of nutrients e.g., nitrogen, carbon, and water from the host (Sala *et al.*, 2001). Mistletoes differ in their chemical composition and this can also have an impact on nutrient levels. Ndagurwa *et al*. (2014), for example, found that litter contributions varied with species *Plicosepalus kalachariensis* had more litter composed of big and fleshy leaves, whilst *Viscum verrucosum* had small, brittle leaves and produced the least litter. Ndagurwa *et al*. (2013) also show in-depth variations in the impacts of the different mistletoe species, on the litter nutrients, soils and host, for instance, their results showed a difference in both foliar nitrogen and litter with *Erianthemum ngamicum* having the highest concentrations. Therefore, even though mistletoes are known to increase nutrients in the soils through their litter, the nutrient elements may vary depending on the species (Ndagurwa *et al*., 2014). Nutritional profiles can also differ depending on the host species that is being parasitized on, as it influences the host-parasite associations and nutrient transfer characteristics and depending on the host plant, mistletoes may differ in their chemical composition (Ndagurwa and Dube, 2012; Ndagurwa *et al*., 2014; Muvengwi *et al.*, 2015). Therefore, the variations in infection degree and the nutrient elements in litter and soil can further augment spatial heterogeneity of resources within the canopy patches thus altering the understory plants found within the zone of influence of the mistletoes.

The high number of animals visiting mistletoes for their flowers, fruits and leaves can also add ± 90% more litter droppings and other debris underneath parasitized trees (Mellado *et al*., 2016; Mellado and

Zamora, 2017). Through this litter and facilitation (decomposition), mistletoes further proliferate the fertility islands beneath the canopy structures of mistletoe-infected trees. This directly improves the community structure, plant productivity, species composition, and functional diversity of the understory plants (March and Watson, 2007; Mathiasen *et al*., 2008; Spasojevic and Suding, 2011; Ndagurwa *et al.,* 2016, 2018; Watson, 2016; Mellado and Zamora, 2017; Hódar, *et al*., 2018). These studies have made comparisons mostly based on infected or uninfected trees; as a result, there is little known information on whether high and low mistletoe-infected trees vary in their understory herbaceous biomass, species assemblages and their functional traits particularly in a semi-arid savanna.

Having understood all the roles (parasitism and litter pathway) that mistletoes play in suppressing their hosts and eliminating competitive dominance, the role of different mistletoe degrees in influencing plant community structures (productivity, functional diversity, impacts on host reproduction, spatial patterns) has not been fully unpacked (Pennings and Callaway, 1996) particularly in semi-arid savanna ecosystems. Earlier it was also shown that tree densities, canopy presence, mistletoe infection intensity and host species can have different impacts on understory plants. However, no study has investigated how mistletoe-infected trees and their distribution can influence their surrounding woody understory plants within and beyond their zone of influence. High infected trees are often larger and older, and the older the tree the longer the period of nutrient accumulation underneath the canopy with soils near the stem being more enriched (Kanz, 1996). Consequently, the addition of mistletoe litter further augments nutrients underneath infected trees; for that reason, we expect differences in the high and low mistletoe infection understory productivity due to variations in litter quantities and tree size effects. Trees have also been shown to provide moisture through their canopies intercepting water and reducing runoff, through water partitioning and also via hydraulic lift. Yet, it is not clear how soil moisture varies between high and low mistletoe-infection canopy patches. As a result, this study investigated how mistletoe-infection degrees influenced the abiotic and biotic micro-habitats beneath and beyond their canopies.

Based on the literature review it is expected that canopy patches due to higher nutrient quantities and soil moisture will have higher herbaceous biomass and species and functional diversity compared to intercanopy spaces. It is also expected that intercanopy spaces will have fewer *V. karroo* juveniles compared to canopy patches due to seeds falling directly under than further from the tree. Consequently, juvenile stage classes will aggregate around mistletoe infected trees, whilst mature trees will exhibit a regular spatial pattern with mistletoe-infected trees. High- compared to low mistletoe-infected trees would have lower reproductive traits due to changes to the physio-morphological structures of the trees. A representation of these expectations is shown in Figure 1.3.

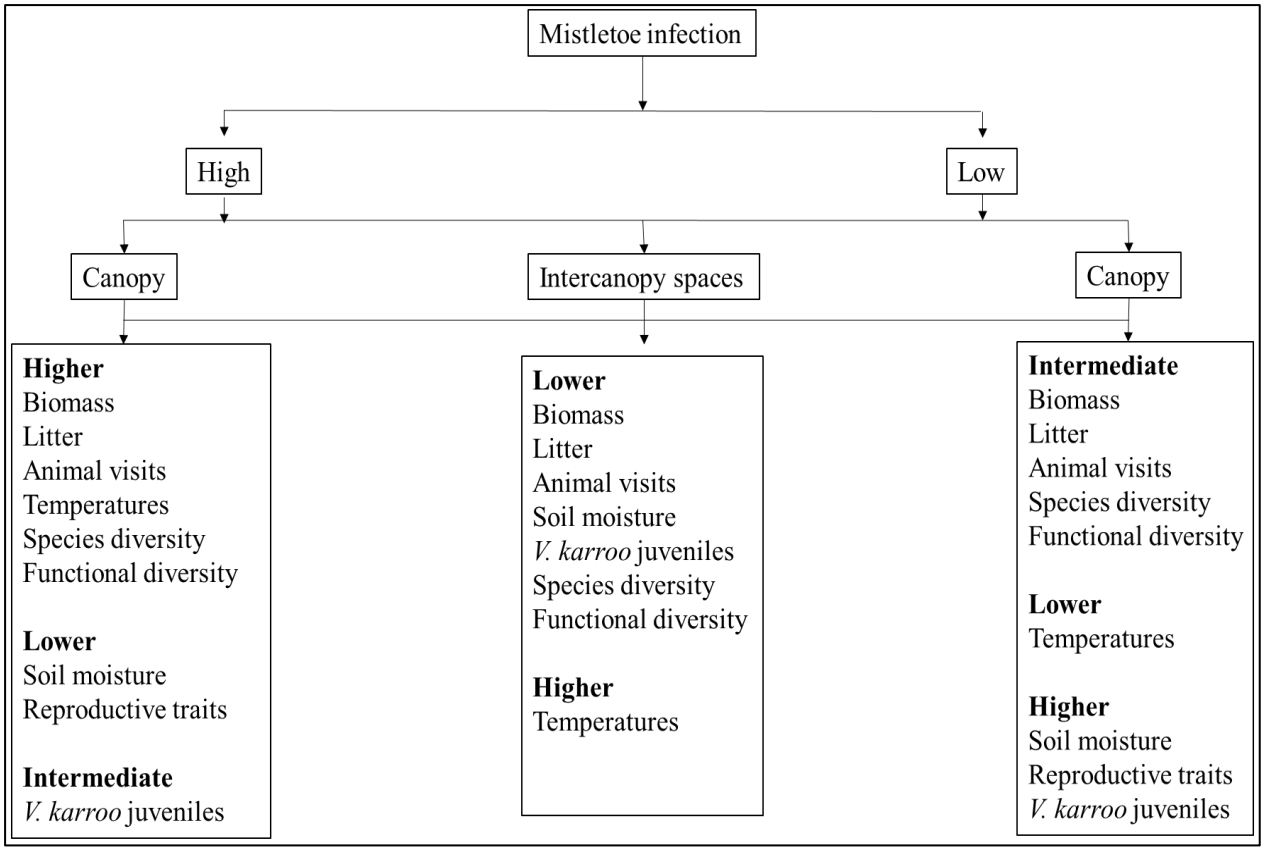


Fig 1.3. Expectations of the effects of mistletoes within and beyond the canopy patches of high- and low mistletoe-infection trees.

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CHAPTER 2

Differences in savanna understory environments with degree of mistletoe infection on *Vachellia karroo* trees

Highlights

- Intercanopies had higher productivity and lower animal visitations than subcanopies.
- Herbaceous biomass was greater beneath low than high mistletoe-infection canopies.
- Litter cover was higher in high- than low mistletoe-infection canopy patches.
- Soil temperature and moisture were higher in high than low infection canopy patches.
- Mistletoe infection degrees increase soil and plant heterogeneity.

Abstract

The role of mistletoes as drivers of plant communities remains largely understudied, yet they influence plant productivity. Over two growing seasons, an investigation of how the degree of mistletoe infection influenced herbaceous biomass, grass height, litter cover, and grazing/trampling, as well as soil relative humidity and soil temperature within *Vachellia karroo* tree canopy patches and their surrounding intercanopy spaces was done. Intercanopy spaces had between 18% and 34% more herbaceous biomass and taller grasses compared to canopy patches. Subsequently, grazing/trampling was 20% lower in the intercanopy compared to the subcanopy, and between 19% and 29%, greater within high mistletoe-infection canopy patches compared to other microhabitats. Litter cover was between 3% and 68% greater in high mistletoe-infection canopy patches compared to the other micro-habitats. Daily mean soil temperatures were 0.5% and relative humidity 3% higher within high- compared to low mistletoe-infection canopy patches. An increase in mistletoe intensity resulted in lower herbaceous biomass, higher grazing/trampling, litter cover, soil temperature, and relative humidity. Therefore, by altering animal activity, litter cover, and soil conditions, mistletoe infection has important implications for plant productivity, decomposition, and nutrient availability. Given the patchy distribution and local abundance of mistletoes, they are a major source of resource and ecosystem spatial heterogeneity.

Keywords: Canopy patch; grazing; herbaceous biomass; intercanopy spaces; litter; mistletoes.

Introduction

Trees through facilitative mechanisms provide resources that are often limiting in semi-arid savannas thereby increasing growth and productivity of understory plants (Belsky *et al*., 1989; Kanz, 1996; Dean *et al*., 1999; Dingaan and du Preez, 2017). Such mechanisms include supplying and redistributing nutrients directly via litter input or indirectly through stem flow, bark sloughing, and animal excreta (Belsky *et al*., 1993; Sameni and Soleimani, 2007; Treydte *et al*., 2007). By providing shade, trees reduce evapotranspiration thereby lowering understory temperatures and evapotranspiration rates of both plants and soils. They also conserve and provide water to their immediate proximity by spatially partitioning water access either through vertical (hydraulic lift) or horizontal access within the soil (or both), hence reducing competition for soil moisture between the tree and understory plants (Kröpfl *et al*., 2002; Ludwig *et al*., 2003; Sagar, *et al*., 2008; Priyadarshini *et al*., 2016). Consequently, the provisioning of nutrients and water underneath tree canopies produces modified, stable, and favourable localized microclimatic patches, often referred to as 'fertility islands', which improve understory plant productivity (Sameni and Soleimani, 2007; Sagar, *et al*., 2008; Treydte *et al*., 2009, 2010). Several studies have shown that canopy patches have significantly higher biomass compared to adjacent intercanopy spaces (Magandana, 2016; Tessema and Belay 2017; Yadeta *et al*., 2018). Abdallah and Chaieb (2010) found that *Acacia* (now *Vachellia*) *tortilis* subsp. *raddiana* canopy patches always had significantly higher biomass (590 to 1530 kg ha^{-1}) compared to intercanopy spaces (86 to 720 kg ha^{-1}). As a result, trees are one of the major influencers of plant and resource heterogeneity within savanna ecosystems.

Trees lodging mistletoes within their canopies also broaden savanna heterogeneity. Mistletoes are chlorophytic hemi-parasites that obtain resources through a haustorium, which penetrates and transports solutes from host plant tissues (Arruda *et al.*, 2012). Generally, mistletoes compete with their host for water and nutrients, thus weakening the hosts' competitive balance, through altering their morphological and physiological attributes (Press and Phoenix, 2004; Mellado and Zamora, 2017). Mistletoe transpiration rates can be up to 5 times more than their host (Bishop, 2010), increasing the demand for water relative to its supply. Consequently, the overall growth of the host can be affected, as observed in dwarf mistletoes that decreased Douglas fir (*Pseudotsuga menziesii*) growth by ±65% (Press and Phoenix, 2004). In a study by Mellado and Zamora (2017), mistletoes increased host foliage loss, formation of canopy gaps, and alteration of tree stand structure and species composition due to tree mortality, thus increasing subcanopy light incidence. As a result, improved light incidence and higher soil nutrients increased understory plant growth by 18% and 31% beneath parasitized compared to unparasitized trees, respectively. Nonetheless, high light incidence can increase soil temperature and evapotranspiration rates thus reducing soil moisture, rate of litter decomposition, and availability of nutrients, leading to a decline in understory productivity (Press and Phoenix, 2004; March and Watson, 2007; Arruda *et al*., 2012; Daryaei and Moghadam, 2012; Melado

et al., 2016; Ndagurwa *et al*., 2016, 2018). In contrast, Chu *et al*. (2021) report that mistletoes increase canopy density and reduce solar radiation, and thus, temperatures were cooler beneath both infected hosts with undisturbed canopies (26.7 ± 0.2°C) and disturbed canopies (26.5 ± 0.2°C) compared to those without mistletoes (28.9 ± 0.8°C).

Despite the negative effects of parasitism, mistletoes can directly increase the diversity, quality, and quantity of litter underneath host canopies (March and Watson, 2007; Ndagurwa *et al.*, 2012, 2013, 2014, 2016; Mellado *et al*., 2016; Watson, 2016). Consequently, higher litter deposition, resulting in greater long-term mistletoe litter accumulation, coupled with mistletoe litter speeding up the decomposition rates of the hosts' litter, has been shown to further increase the rate of nutrient cycling within infected tree canopy patches (Al-Rowaily *et al*., 2020; Ndagurwa *et al*., 2020). This makes resources more readily available, thus facilitating increased plant productivity underneath mistletoe-infected compared to uninfected trees and intercanopy spaces (Spasojevic and Suding, 2011; Muvengwi *et al*., 2015; Ndagurwa *et al*., 2015, 2016). Spasojevic and Suding (2011) found that the hemi-parasite *Castilleja occidentalis* increased biomass production for both dominant and non-dominant species in dry meadow alpine tundra, a nutrient-poor system, by reducing competition for nitrogen. Similarly, March and Watson (2007) found a positive relationship (r^2 = 0.49) between mistletoe biomass in the tree canopy and understory plant biomass. However, no study has assessed how the degree of mistletoe infection (high *vs*. low) influences biotic factors between canopy patches and adjacent intercanopy spaces in semi-arid savannas. Additionally, although abiotic factors have been shown to differ between mistletoe-infected and uninfected tree subcanopies (Ndagurwa *et al*., 2014, 2015), there is seemingly little or no information on how these factors vary between trees with high and low mistletoe-infection.

This study aimed to investigate how the understory a) plant productivity (herbaceous biomass, litter cover, and grass height), b) use by medium-large herbivores (grazing/trampling), and c) soil conditions (soil moisture and temperature) differed in the canopy patches and adjacent intercanopy spaces of high- and low mistletoe-infected *Vachellia karroo* (Hayne) Banfi & Galasso trees in a semi-arid savanna. Specifically, the study investigated how herbaceous biomass, grass height, litter cover, grazing/trampling, soil temperature and soil relative humidity varied between high- and low mistletoe-infected tree canopy patches. It was hypothesised that biomass, grass height, and litter cover would be greater below: a) trees with high relative to low mistletoe-infection and b) under tree canopies than canopy interspaces. Due to higher light incidence and soil respiration, soil moisture, and relative humidity were expected to be lower but soil temperature higher beneath high relative to low mistletoe-infected trees. Immediately after a significant rainfall event, due to absence of tree canopy interception volumetric soil water content (VWC) was expected to be higher in intercanopy spaces compared to canopy patches. However, a few days after it had rained, VWC below high infection canopies was expected to be lower than in low infection canopy patches and canopy interspaces.

Materials and methods

Study area

The study was carried out at the Matopos Research Station (28 000ha, Fig 1), 30km southwest of Bulawayo, Zimbabwe (20°22'60" S and 28°31'0" E, 1340m asl). Annual rainfall and temperature average 586mm and 18°C (Chirara *et al*., 1998). Here, the fine-textured soils are dominated by *Vachellia karroo*, *Vachellia nilotica* (L.) P.J.H. Hurter & Mabb. subsp. *kraussiana* (Benth.) Kyal. & Boatwr., *Vachellia rehmanniana* (Schinz) Kyal. & Boatwr., *Vachellia gerrardii* (Benth.) P.J.H. Hurter, *Senegalia nigrescens* (Oliv.) P.J.H. Hurter, and *Gymnosporia senegalensis* (Lam.) Loes. (Ndagurwa *et al.*, 2012, 2014). Large *V. karroo* trees in this area are typically infected by mistletoes particularly *Erianthemum ngamicum* (Sprague) Danser (Loranthaceae), *Viscum verrucosum* Harv. (Viscaceae), and *Plicosepalus kalachariensis* (Schinz) Danser (Loranthaceae) (Ndagurwa *et al*., 2012, 2014, 2014b, 2016, 2018). The most common grasses are *Themeda triandra* Forssk., *Heteropogon contortus* (L.) Roem. & Schult., *Hyperthelia dissoluta* (Steud.) Clayton, *Hyparrhenia filipendula* (Hochst.) Stapf, with *Cymbopogon plurinodis* (Stapf) Burtt Davy, *Eragrostis* spp., *Loudetia* spp., and *Setaria incrassata* (Hochst.) Hack.. There have not been any recent instances of fire. Livestock such as cows, goats, and sheep graze freely throughout the year. Pictures of mistletoe-infected *V. karroo* trees, understory species diversity and animal vistiations at the study site are shown in Appendix 1.

Selection of trees

A known area, previously used by Ndagurwa *et al*. (2012, 2014a, 2014b), dominated by *V. karroo* trees was traversed using systematic belt transects of 1 km × 10 m with no gaps between transects in search of low- and high-mistletoe infected trees (n = 20 in each category). Height, diameter at breast height (DBH), and the canopy long (D_1) and short (90^o of D_1; = D_2) diameters were measured and canopy area ($\pi(D_1/2)(D_2/2)$ and volume calculated for each tree ($4/3(\pi((D_1/2)(D_2/2)(height/2))$) (Table 1).

The degree of mistletoe infection was determined by the number of adult mistletoes (those with large axial branches with several side branches, Ndagurwa *et al*., 2012) and their canopy cover percentage on each tree (Table 1). The number of mistletoes and tree size measures (canopy area, canopy volume, tree height, and DBH) showed weak positive correlations (Appendix 2).

Herbaceous plant height and biomass, litter cover and grazing/trampling

Within canopy patches and intercanopy spaces of each high and low mistletoe-infected tree (n = 40 trees), 20-disc pasture meter (DPM) measurements were taken systematically at 5 points in each cardinal direction (Fig.2). In the canopy patch, the disc was placed; a) next to the tree bole, b) mid-way between the stem and canopy edge (half the canopy radius), and c) at the edge of the canopy, while in the intercanopy the disc was placed d) at 1.5 and e) at two times the canopy radius.

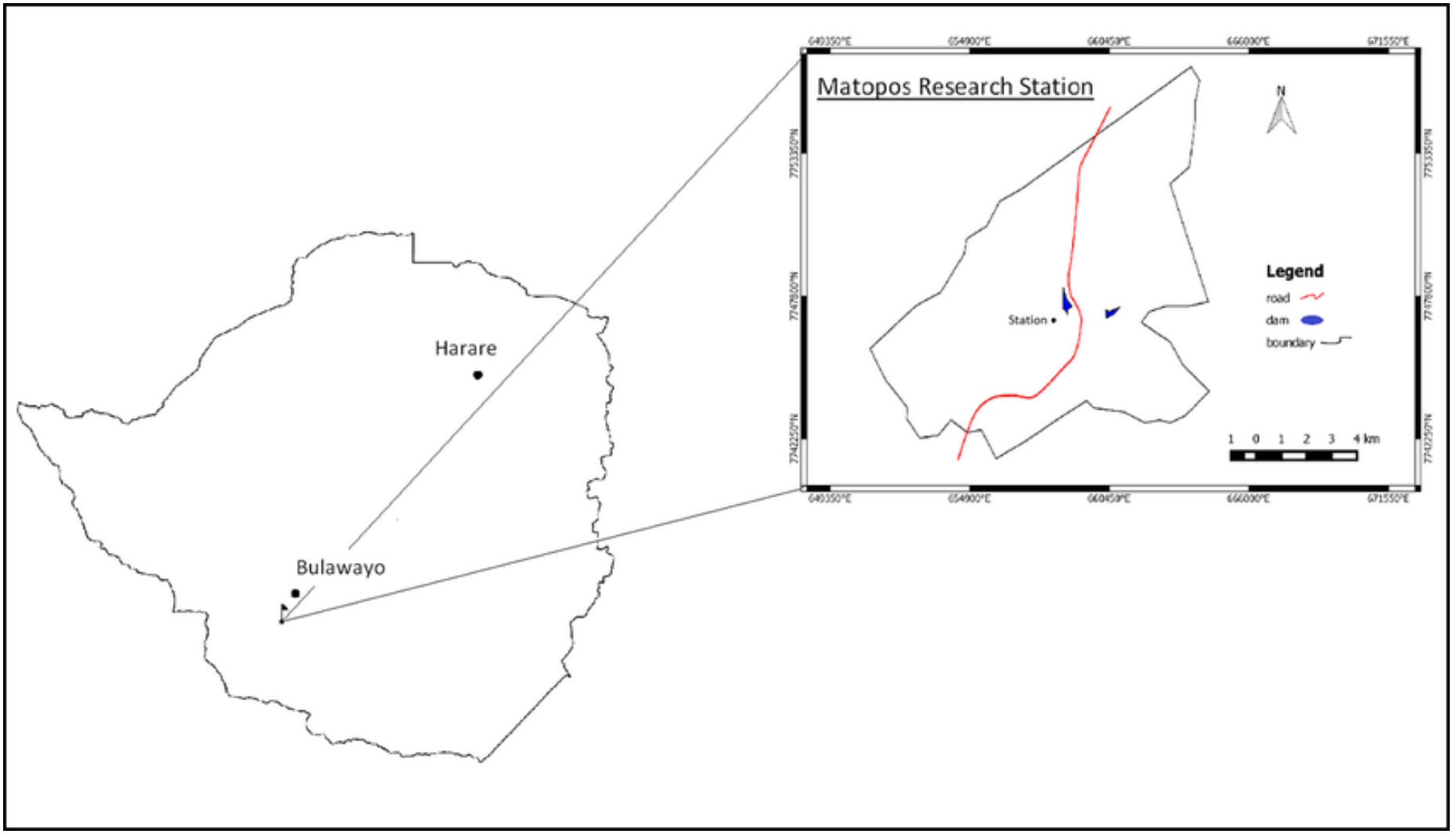


Fig. 1.1 Map showing the location of Matopos research station in Zimbabwe (Ndagurwa, 2015)

Table 1. (a) Criteria used to select high and low mistletoe-infected *Vachellia karroo* trees and (b) the mean (± SE) number of adult mistletoes per tree, their canopy cover, number of each species of mistletoe per tree (numbers in brackets show the presence of each species per individual tree), and tree size dimensions, height, DBH, canopy area and volume and the canopy long (D_1) and short (D_2) diameters. Values in bold indicate significant differences (df = 38, P < 0.05).

Variable	Mistletoe density		*P*-value
	High	Low	
(a) Criteria:			
Canopy cover of adult mistletoes (%)	>70	<10	
Count of adult mistletoes on each tree	>15	<7	
(b) Counts & measurements (mean± SE):			
Canopy cover of adult mistletoes (%)	83.32 ± 2.93	4.67 ± 1.62	**<0.001**
Number of adult mistletoes per tree	23.3 ± 1.64	2.90 ± 0.65	**<0.001**
Viscum verrucosum	19.65 ± 1.59 (20/20)	2.71 ± 0.64 (14/20)	**<0.001**
Plicosepalus kalachariensis	3.4 ± 0.77 (16/20)	0.19 ± 0.11 (3/20)	**<0.001**
Erianthemum ngamicum	0.25 ± 0.12 (4/20)	0 ± 0 (0/20)	0.120
Tree height (m)	8.55 ± 0.42	6.67 ± 0.27	**<0.001**
DBH (cm)	26.33± 1.93	22.95 ± 0.30	0.222
Tree canopy area (m^2)	102.82 ± 9.43	68.80 ± 0.72	**0.010**
Tree canopy volume (m^3)	647.15 ± 91.31	337.67 ± 37.02	**0.001**
D_1 (m)	11.59 ± 0.56	9.59 ± 0.43	**0.005**
D_2 (m)	10.82 ± 0.55	8.82 ± 0.40	**0.003**

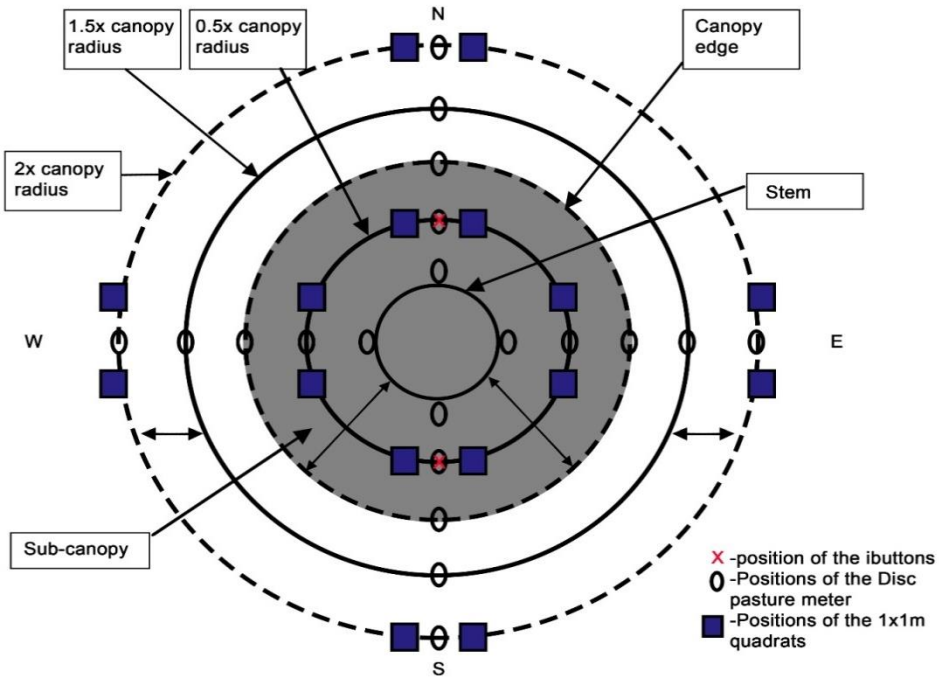


Fig. 2. Top-down view of placement of 1m × 1m quadrats (blue squares) within the canopy patches and intercanopy spaces of *Vachellia karroo* trees. Grass height, litter cover, and grazing/trampling were assessed within the quadrats. Small ellipses show the positions of the DPM at different radii

from the tree bole in the four cardinal positions. The dark grey shaded area shows the canopy patches and the un-shaded area represents the intercanopy spaces. ibutton positions are shown by the red x.

Four distinct microhabitats are represented, high mistletoe-infection canopy patches, high mistletoe-infection intercanopy spaces, low mistletoe-infection canopy patches, and low mistletoe-infection intercanopy spaces. The biomass measurements were carried out four times: early-November 2017 (end of dry season), mid-June 2018 (dry season), late-December 2018 (beginning of wet season), and mid-February 2019 (middle of wet season). There are two equations based on DPM settling height (x), ≤ 26 cm, > 26 cm (Zambatis *et al*., 2006):

Herbaceous biomass (kg ha^{-1}) = [31.7176 (0.3218$^{1/x}$) $x^{0.2834}$]^2 (r^2 = 0.951; P < 0.0005) ($x \leq$ 26cm) (Eq. 1)

Herbaceous biomass (kg ha^{-1}) = [17.3543 (0.9893x) $x^{0.5413}$]^2 (r^2 = 0.882; P < 0.0005) (x > 26cm) (Eq. 2)

Percentage litter cover, grass height (m), and the grazing/trampling impact (%) were assessed in sixteen (1m × 1m) quadrats within canopy patches and intercanopy spaces (Fig. 1) using the 8 point Walker (1976) scale (0: 0%, 1: 1-10%, 2: 10-25%, 3: 25-50%, 4: 50-75%, 5: 75-90%, 6:90-99%, 7: 100%). Data were collected at the end of March 2018 and mid-February 2019, towards the end of the wet season. Maximum grass height with inflorescence and grass table height without inflorescence (maximum height of the foliage) were measured using a calibrated 2m rod. Within the same quadrats, maximum and table height were measured at 10 random points, and mean height within each quadrat calculated.

Soil moisture and temperature

A Hydrosense II Handheld soil moisture sensor with an automatic data acquisition system was used to measure the volumetric soil water content (VWC) between 0–10cm. Soil moisture was measured in the same positions as the DPM (n = 20/tree) (Fig. 1). Measurements were taken thrice consecutively, immediately after what was regarded as a heavy period of rain, and then 3 and 7 days after it had rained in March 2018 (month with the highest and most continuous rainfall).

For a more comprehensive comparison of high *vs*. low mistletoe tree canopy patches, the 10 most and 10 least mistletoe-infected trees were selected for soil temperature and relative humidity measurements. Two ibuttons (temperature and humidity: high precision general-purpose ibuttons) were buried at a depth of 2cm (most biotic activities in semi-arid savannas occur within the top layers of the soil due to low precipitation) at half the canopy radius in the north and south directions, below the selected 20 trees (Fig. 2). The ibuttons were buried on the 8th December 2018 and removed on 10th July 2019, which recorded soil moisture and temperature at 3-hour intervals (8 recordings/24 hours: 7 am, 10 am, 1 pm, 4 pm, 7 pm, 10 pm, 1 am, and 4 am).

Data and statistical analyses

Data were tested for normality using the Shapiro Wilk test. General linear models (GLM) were used to test the effects of canopy presence, cardinal direction, and degree of mistletoe infection on

herbaceous biomass, grass height, litter cover, soil moisture, soil temperature, soil VWC, and grazing /trampling in SPSS 23 for Windows (SPSS Inc., 2012, Chicago, IL U.S.A). Cardinal direction had no significant effect on any of the variables and hence was excluded from the GLM analyses. Differences in the independent variables among the four microhabitats were analysed using Kruskal Wallis tests, followed by a post hoc Kruskalmc for non-parametric data (P = 0.05). To investigate the differences in temperature and relative humidity within the canopy patches and between north and south cardinal directions, t-tests were used when data were normal or Mann-Whitney U tests when not. Data are expressed as mean ± SE, and analyses were conducted in R v. 3.1.5 (R Core Team, 2018).

Results

Effects of canopy and infection on herbaceous biomass

Canopy effects i.e., canopy versus intercanopy generally had more significant effects on the biotic variables than the degree of mistletoe infection (high *vs*. low), but there were numerous but predominantly weaker interactions between them (Table 2). Intercanopy spaces (high and low mistletoe-infected trees pooled) had between 1.22 and 1.53-fold more herbaceous biomass compared to the canopy patches for all the periods of data collection (Table 2, Appendix 3). Overall, herbaceous biomass was 1.09 to 1.30-fold lower in high mistletoe-infection habitats (canopy patches and intercanopy spaces pooled) compared to low mistletoe-infection habitats.

Table 2. General linear model (GLM) analyses of the effects of canopy (canopy *vs*. intercanopy), mistletoe infection (high *vs.* low), and their interactions on aboveground herbaceous biomass (kg/ha) in the dry season and early and middle wet season, grass table and maximum height (cm), litter cover (%) and grazing/trampling (%). Values in bold show significant effects (P < 0.05), error df = 1,636 in every case.

Variable	Effects					
	Canopy Canopy *vs*. Intercanopy		Infection degree High *vs*. Low		Canopy × Infection degree	
	F	Sig.	F	Sig.	F	Sig.
Biomass						
End of dry season	16.87	**<0.001**	9.92	**0.020**	0.30	0.862
Middle of dry season	38.77	**<0.001**	4.07	**0.044**	2.25	0.135
Beginning of the wet season	73.32	**<0.001**	28.57	**<0.001**	4.85	**0.028**
Middle of the wet season	78.90	**<0.001**	8.82	**<0.001**	3.75	**0.053**
Grass height						
Maximum height (cm)	97.77	**<0.001**	56.44	**<0.001**	5.90	**0.015**
Table height (cm)	79.54	**<0.001**	41.77	**<0.001**	4.10	**0.040**
Litter cover (%)	824.62	**<0.001**	15.46	**<0.001**	28.92	**<0.001**
Grazing/Trampling (%)	13.03	**<0.001**	3.29	0.070	4.55	**0.040**

There were significant differences in biomass measured among microhabitats in all measurement periods: end of dry season (Kruskal Wallis χ^2 = 44.30, *df* = 3, *P* < 0.001, Fig.2a), mid- dry season (Kruskal Wallis χ^2 = 44.66, *df* = 3, *P* < 0.001, Fig. 2b), beginning of wet season (Kruskal Wallis χ^2 = 100.32, *df* = 3, *P* < 0.001, Fig. 2c) and mid-wet season (Kruskal Wallis χ^2 = 82.759, *df* = 3, *P* < 0.001, Fig. 2d). In all the periods, intercanopy spaces of low mistletoe-infected trees had between 1.41 and 1.94-fold more biomass compared to canopy patches of high mistletoe-infected trees. Across all periods, herbaceous biomass was 52 to 66% lower at the beginning of the wet season compared to middle of wet season (Fig. 3). Herbaceous biomass declined with an increase in tree size (canopy area, canopy volume), and with number of mistletoes for each period (Appendix 4).

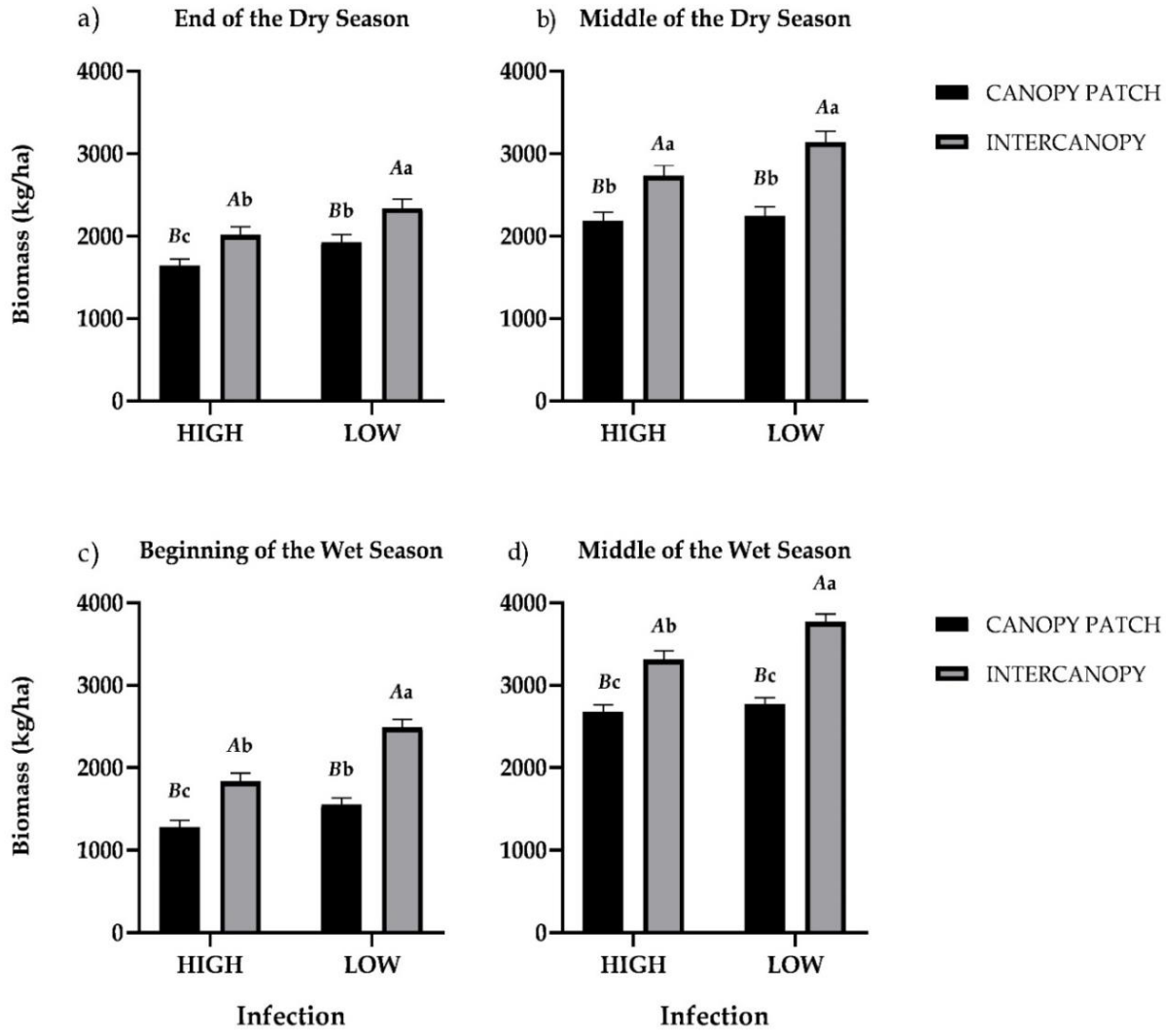


Fig. 3. Comparisons of herbaceous biomass (mean ± SE; kg/ha) among microhabitats at (a) end of the dry season (November 2017), (b) mid-dry season (June 2018), (c) beginning of the wet season (December 2018), and (d) mid-wet season (February 2019). Within each collection period, capital letters show differences within infection microhabitats (canopy patch and intercanopy spaces) whilst lower-case letters show differences among the four microhabitats (Kruskalmc, *P* < 0.05).

Effects of canopy and infection on grass height

The table and maximum grass height within canopy patches were 20% and 18% shorter than in intercanopy spaces, respectively (Table 2, Appendix 3). Grass table and maximum height were 15% and 13% lower within high relative to low mistletoe-infection, respectively (Table 2, Appendix 3).

Between the four microhabitats, grasses were significantly taller (table height: Kruskal Wallis χ^2 = 144.11, *df* = 3, *P* < 0.001, maximum height: Kruskal Wallis χ^2 = 115.71, *df* = 3, *P* < 0.001, Figure 4a, b) in intercanopy spaces of low mistletoe-infected trees and lowest in canopy patches of high mistletoe-infected trees by 33% and 30% for table and maximum height, respectively.

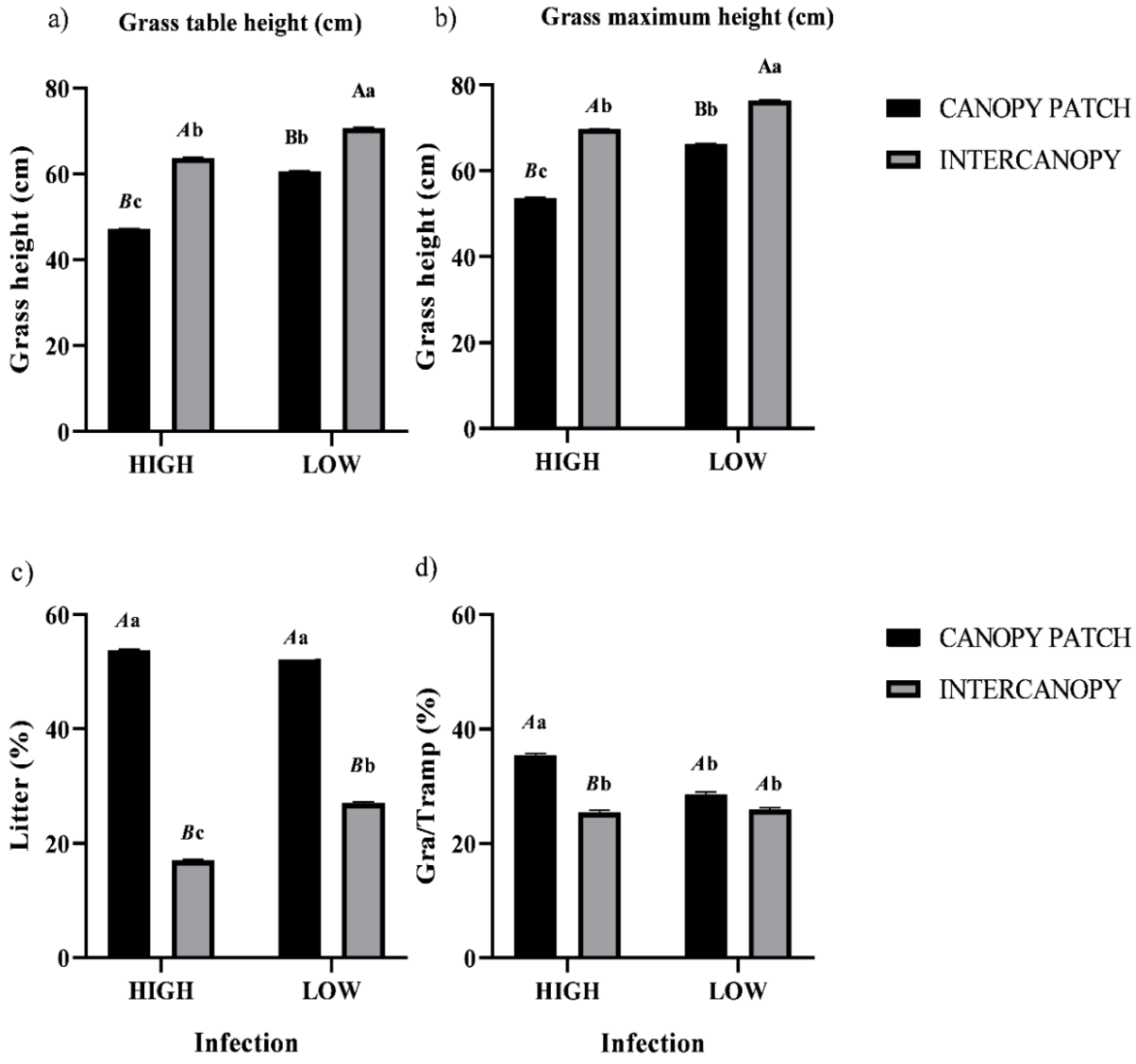


Fig. 4. Comparisons (mean ± SE) of a) grass table height, b) maximum height, c) litter cover, and (d) grazing/trampling among microhabitats. Capital letters show differences within infection microhabitats (canopy patch and intercanopy spaces) whilst lower-case letters show differences among the four microhabitats (Kruskalmc, *P* < 0.05).

Effects of canopy and infection on cover of litter and trampling/grazing

Litter cover was 59% higher within canopy patches compared to intercanopy spaces (Table 2; Appendix 2). High mistletoe-infected trees had 11% less litter compared to low mistletoe-infected trees. However, trampling/grazing was 20% lower in intercanopy spaces compared to canopy patches, whilst low mistletoe-infection had 10% lower trampling/grazing compared to high mistletoe-infection trees.

Litter cover was 3.17-fold higher (Kruskal Wallis $\chi^2 = 533.32$, $df = 3$, $P < 0.001$; Figure 4c) within high mistletoe-infection canopy patches (54 ± 0.15%) than in adjacent intercanopy spaces, which had the lowest litter cover (17 ± 0.21%). Similarly, high mistletoe-infected canopy patches had 1.39-fold more trampling/grazing (Kruskal Wallis $\chi^2 = 26.96$, $df = 3$, $P < 0.001$; Figure 4d) compared with the other three microhabitats, which were not significantly different from each other. Litter cover had weak negative relationships with tree canopy area and volume as well as with mistletoes/tree, but trampling/grazing showed weak positive relationships with these variables (Appendix 5).

Effect of mistletoe infection on soil temperatures and relative humidity

Over the whole period, mean daily soil temperatures were 0.5% higher ($t = 3.78$, $df = 68529$, $P < 0.001$) beneath high- compared to low mistletoe-infection trees. The highest and lowest temperatures for both high and low mistletoe-infection trees were observed in March and June, respectively. Similarly, over the whole period, mean daily soil relative humidity was 3% higher below canopies of high mistletoe-infected trees (91 ± 0.10%) compared to low mistletoe-infected trees (88 ± 0.11%) ($W = 743390078$, $P < 0.001$). Relative humidity was highest in March and May and lowest in July for both high and low mistletoe-infected trees.

The differences in the weekly soil temperatures between high- and low mistletoe-infected trees ranged from 0.01°C to 0.9°C (Figure 5a) whilst differences in relative humidity ranged from 0% to 11.51% (Figure 5b). Weekly mean soil temperature and relative humidity were always equal to or higher in high compared to low mistletoe-infected trees (Figure 6a, b). Mean monthly soil temperatures (Kruskal Wallis $\chi^2 = 34.96$, $df = 13$, $P = 0.001$) and relative humidity (Kruskal Wallis $\chi^2 = 2562$, $df = 13$, $P = 0.001$) were significantly higher under high compared to low mistletoe-infected trees.

Canopy patch and cardinal direction effects on soil temperatures and relative humidity

High mistletoe-infection tree canopy patches had significantly higher temperatures on both the south and north side of the canopies compared to low mistletoe-infection trees (Kruskal Wallis $\chi^2 = 1493.4$, $df = 3$, $P < 0.001$, Figure 7a). Furthermore, temperatures were higher on the north side of high and low-mistletoe-infected trees (both 22 ± 0.03ºC) compared to the south side (both 21 ± 0.03°C), this occurred in February, March, April, May, June, and July (Appendix 6).

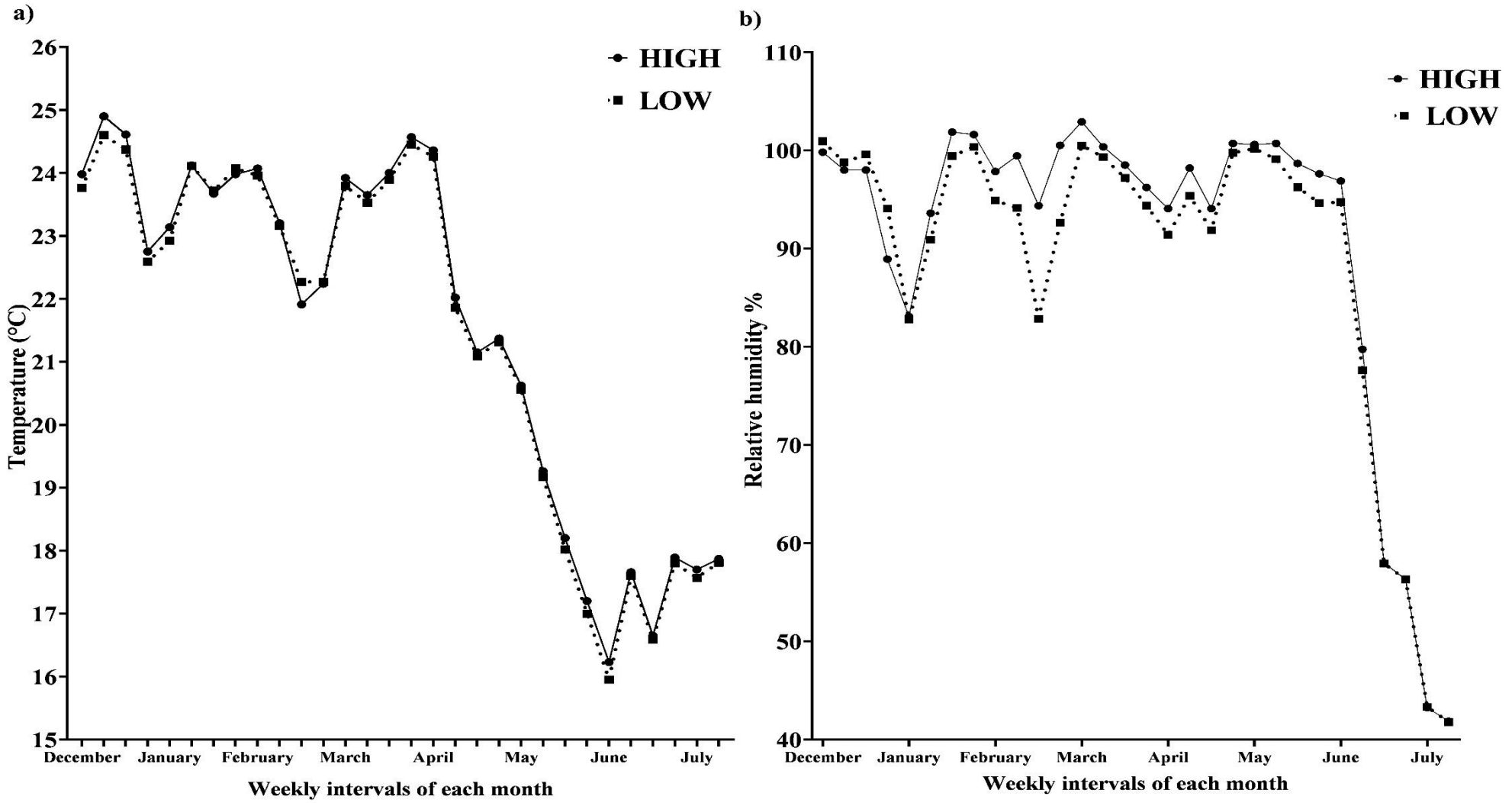


Fig. 5. Average weekly soil (a) temperature (ºC) and (b) relative humidity (%) in the canopy patches of high and low-mistletoe infected *Vachellia karroo* trees.

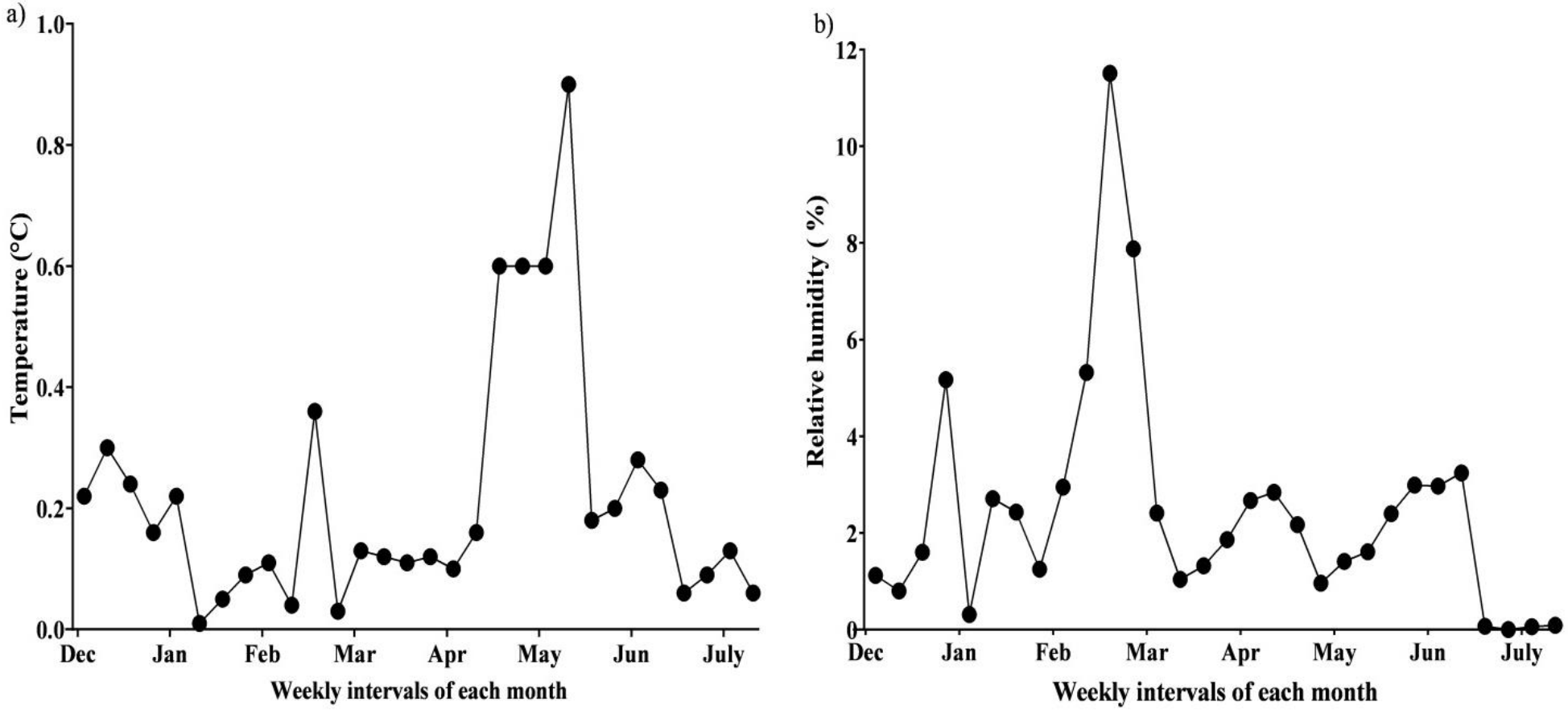


Fig. 6. Differences in the average weekly (a) soil temperatures (ºC) and (b) relative humidity (%) between high and low mistletoe-infection *Vachellia karroo* trees from December 2018 to July 2019.

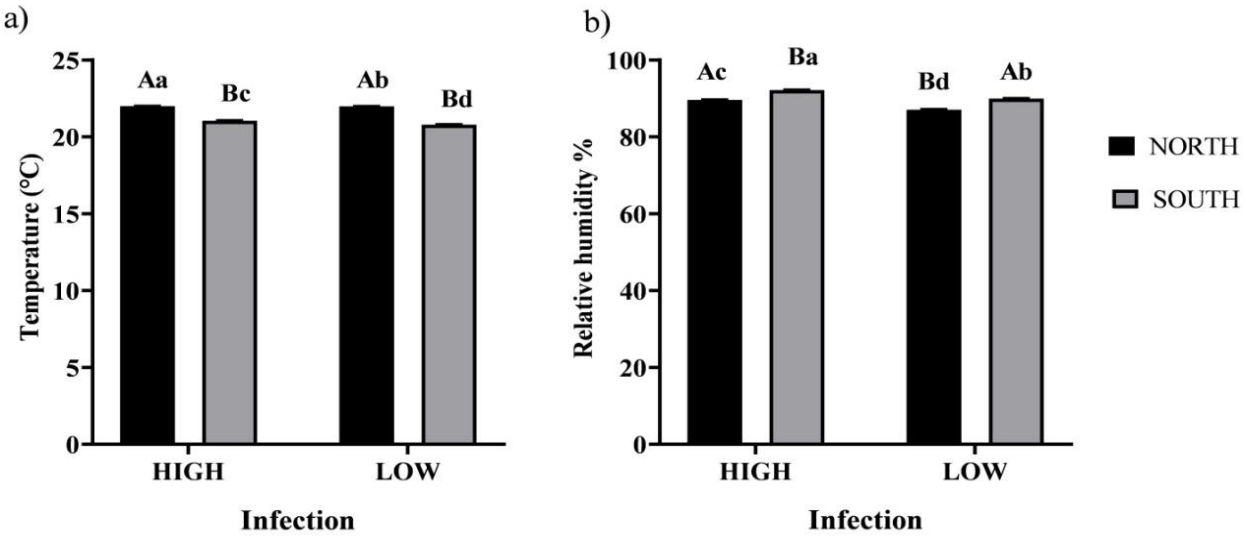


Fig. 7. Comparison of soil (a) temperature and (b) relative humidity in canopy patches of high- and low mistletoe-infection *Vachellia karroo* trees in the two cardinal directions i.e., north and south. Capital letters show differences between cardinal directions (north and south) whilst lower-case letters show differences among these microhabitats (Kruskalmc, $P < 0.05$).

Soil relative humidity was highest in the south side of high mistletoe-infected canopy patches and lowest in the north side of low mistletoe-infected canopy patches (Kruskal Wallis $\chi^2 = 1386.4$, $df = 3$, $P < 0.001$, Figure 7b). The south sides of both high (92 ± 0.14%) and low mistletoe-infected canopy patches (90 ± 0.15%) had higher relative humidity compared to north sides (90 ± 0.17%, 87 ± 0.17%, respectively), which occurred in January, March, May, and June (Appendix 6).

Volumetric soil water content

As expected the soil dried out from day_0 to day_7 by 17% (Table 3, Appendix 6). VWC was 5% and 11% higher immediately after it had rained in the intercanopy spaces of high- and low mistletoe-infection trees compared to adjacent canopy patches, respectively (Table 4). The soil dried out faster in the intercanopy (24%), which is in full sun, than under the canopy of infected trees (11%) (Table 3, Appendix 6). Subsequently, low mistletoe-infection intercanopy spaces had 3.25-fold and 2.89-fold higher rates of VWC losses for day_3 and day_7, respectively, compared to high mistletoe-infection canopy patches which had the lowest losses (Table 4).

There was a strong interaction between canopy effect and level of infection. On day_0 low mistletoe-infection intercanopy spaces had 14% more VWC compared to low mistletoe-infection canopy patches which had the lowest VWC (Table 3, Appendix 7). However, on day_3 and day_7 there was no significant difference in the VWC across the four microhabitats (Table 4). Nonetheless, by day_7, high mistletoe-infection canopy patches tended to have between 1% to 10% higher VWC than the other

microhabitats (Table 4). Furthermore, similar to the relative humidity, VWC within high- compared to low mistletoe-infection canopy patches was always higher by 1% to 1.5%.

Table 3: General linear model (GLM) analyses of the effects of day (day 0, 3, and 7), canopy (canopy *vs*. intercanopy), mistletoe-infection (high *vs.* low), and their interactions on volumetric soil moisture content (%). Values in bold show significant effects ($P < 0.05$) and error $df = 2388$ in every case.

Variables	*df*	F	*P*
Day (0 *vs*. 3 *vs*. 7)	2	49.76	**<0.001**
Infection degree (high *vs*. low)	1	3.21	0.073
Canopy (canopy *vs*. intercanopy)	1	3.79	**0.052**
Day × Infection	2	0.21	0.808
Day × Canopy	2	9.03	**<0.001**
Infection × Canopy	1	6.91	**0.009**
Day × Infection × Canopy	2	0.15	0.865

Table 4. Mean (± SE) volumetric soil water content (%) in high and low mistletoe-infection canopy patches and their respective intercanopy spaces at day_0, day_3, and day_7. Means in columns not sharing a small common letter (subscript) are significantly different, whilst means in rows showing different capital letters (superscripts) are significantly different (Kruskalmc, $P < 0.05$).

Infection	Day_0	Day_3	Day_7	χ^2, *df* (*P*-value)
High				
Inside	$20.30 \pm 0.51^{A}{}_{b}$	19.39 ± 0.49^{AB}	18.17 ± 0.46^{B}	8.76, 2 (**0.01**)
Outside	$21.48 \pm 0.53^{A}{}_{ab}$	19.33 ± 0.48^{B}	16.43 ± 0.41^{C}	50.60, 2 (**< 0.001**)
Low				
Inside	$20.08 \pm 0.56^{A}{}_{b}$	19.08 ± 0.53^{AB}	17.94 ± 0.50^{B}	7.22, 2 (**0.03**)
Outside	$23.31 \pm 0.62^{A}{}_{a}$	20.28 ± 0.54^{B}	17.71 ± 0.47^{C}	46.25, 2 **(< 0.001**)
	$\chi^2 = 21.53$	$\chi^2 = 4.45$	$\chi^2 = 4.62$	
	$df = 3$	$df = 3$	$df = 3$	
	$\boldsymbol{P < 0.001}$	$P = 0.22$	$P = 0.20$	

Discussion

Differences in mistletoe infection intensity enhanced the spatial (and/or temporal) heterogeneity of soil temperature and moisture, animal activity, and litter cover. These factors are associated with soil nutrient availability and they are responsible for the spatial variability of plant productivity reported in this study. However, mistletoe effects are supplementary to canopy effects as the results show that the contrast between inter- and sub-canopy had a greater influence on the herbaceous biomass, grass height, litter cover and trampling/grazing compared to degree of mistletoe-infection. Conversely, soil VWC was more affected by rate of drying compared to canopy and mistletoe-infection effects.

Herbaceous biomass, grass height, grazing and litter cover

Although it was predicted that herbaceous biomass and grass height would be higher under the canopy (Ludwig *et al*., 2003; Treydte *et al*., 2009; Abdallah *et al*., 2012; Magandana, 2016; Tessema and Belay 2017; Yadeta *et al*., 2018), they were actually both higher in the intercanopy. Negative effects

of trees on the understory plants have been observed, and these include competition, shading, and allelopathic influences (Kröpfl *et al*., 2002; Sameni and Soleimani, 2007; Sagar, *et al*., 2008). Therefore, competition between the trees and understory plants could have resulted in lower biomass and shorter grasses within canopy patches than intercanopy spaces (Treydte *et al*., 2007). This competition can be directly from the host tree and/or indirectly from the mistletoes and/or understory subordinates depending on the degree of mistletoe infection. Low mistletoe-infection trees (~3 mistletoes/tree) are unlikely to reduce their competitive edge against subordinate understory species; hence, they directly decrease the productivity of herbaceous plants. Conversely, high mistletoe-infected trees (~22 mistletoes/tree) would have significantly lower competitiveness (Spasojevic and Suding, 2011) thus increasing intra- and interspecific competition due to the establishment of more understory plants (Mellado and Zamora, 2017). Therefore, lower biomass and grass height in high mistletoe-infection canopy patches might be due to either direct competition from the host and/or indirect competition from newly established co-occurring plants and higher number of mistletoes for the limited soil resources.

Below low mistletoe-infection canopies, tree shading could have reduced the understory plant growth rate even if the canopy microsites are predicted to be more resource-rich than intercanopy spaces (Kanz, 1996; Belsky *et al*., 1989). However, high mistletoe infection might have negatively affected the hosts' architecture and canopy structure by increasing foliage loss and created/increased canopy gaps. The resulting increase in light incidence to the understory (Mellado and Zamora, 2017) could have further increased the diversity and number of understory plants, whilst accelerating water loss, thereby increasing competition for the limited soil moisture.

Animal visits measured as grazing/trampling were higher beneath high- than low mistletoe-infected canopy patches (intermediate) and intercanopy microhabitats (low), suggesting that herbivory contributed to differences in understory plant biomass and height between these patches. Higher herbaceous biomass and grass height could be related to differences in animal use since canopy patches, due to their low temperatures, are good grazing and resting sites compared to intercanopy spaces (Belsky *et al*., 1989; Treydte *et al*., 2010; Abdallah *et al*., 2012; Tessema and Belay, 2017). Additionally, higher herbivore visitation could be due to canopy patches being fertility islands, which harbour palatable nutritious plants, fruits (especially pods), seeds (Treydte *et al*., 2007; Abdallah *et al*., 2008; Dingaan and du Preez, 2017) and mistletoe litterfall (March and Watson, 2007; Ndagurwa *et al.,* 2014; Mellado *et al*., 2016). Thus, due to lower grazing and adaptability to low soil moisture and full sunlight, grasses in the intercanopy spaces, may have been able to be productive and recover faster than other growth forms (Muvengwi *et al*., 2017). Furthermore, intercanopy plants may be less preferred as they are often less palatable (Facelli, 1991; Treydte *et al*., 2009; 2010; Seymour *et al*., 2014) due to the high presence of moribund layers and old leaves high in lignin or secondary compounds and less protein. Moreover, grazing in the canopy patches was more prevalent in the dry

season and this was shown by the visibly grazed rings around the trees. In winter, grass production is relatively low due to high grazing and absence of rainfall (Abdallah *et al*., 2008; Treydte *et al*., 2007; Magandana, 2016). Therefore, during the dry season, high mistletoe-infected trees could have provided reliable forage thereby intensifying the frequency and numbers of visitors which explains the low biomass observed within these patches.

Larger trees often have higher soil fertility which translates to their forage quality, and their canopy patches are accessible to animals of different sizes due to high positioned branches compared to small trees (Treydte *et al*., 2007, 2010). Consequently, these characteristics could have increased animal preferences to the larger high mistletoe-infection canopy patches than low infected trees. Regardless, intermediate grazing beneath low mistletoe-infected canopy patches often results in higher soil fertility and facilitation of shoot production (intermediate grazing hypothesis) thus increasing herbaceous biomass (Yadeta *et al*., 2018). In contrast, prolonged overgrazing and trampling, results in soil compaction which leads to a decline in soil water holding capacity, infiltration rates, an increase in soil erodibility and subsequently a decline in nutrient cycling rates within high mistletoe-infection canopy patches (Abdallah *et al*., 2008; Yadeta *et al*., 2018). In turn, these poor soil conditions may indirectly reduce shoot production and growth rate of the herbaceous biomass, below high mistletoe-infected trees. Therefore, the positive feedback (dung and urine) arising from animal visitations (Treydte *et al*., 2007, 2010) could have been limited by the frequency and the number of visitors to the larger high mistletoe-infection canopy patches (Abdallah *et al*., 2008; Yadeta *et al*., 2018).

Canopy patches had significantly higher litter cover compared to intercanopy spaces. The litter was composed of leaves, fruits and flowers, bark and twigs from the *V. karroo* trees, three mistletoe species (*V. verrucosum, P. kalachariensis and E. ngamicum*), grasses, forbs, and trees, similar to other studies (March and Watson, 2007; Ndagurwa *et al.,* 2013, 2014, 2016; Mellado *et al*., 2016). Due to the higher presence of animals especially within canopy patches of high mistletoe-infected trees, there was a high occurrence of animal material which included excreta, feathers, skin, and bones similar to other studies (e.g., Treydte *et al*., 2010). Bird droppings were one of the major components of animal material beneath mistletoe-infected trees consistent with other studies (Ndagurwa *et al*., 2014), which could be due to birds using mistletoe clumps as bird nests or as cooling clumps during hot days (Ndagurwa *et al*., 2016b). Also, birds could have been foraging on the mistletoes for fruits, foliage, or insects (Ndagurwa *et al*., 2016b). Further, thick litter beds and high-quality litter from mistletoes enhance microbial and invertebrate activity, resulting in abundant and distinctive insect assemblages. This increases visitation by insectivorous birds, hence, further increasing the likelihood of bird droppings within canopy patches (Watson, 2015; Ndagurwa *et al*., 2016; Mellado *et al*., 2019).

The diverse and higher litter quantities could potentially increase soil nutrient availability to a greater extent within high- compared to low mistletoe-infected canopy patches (Ndagurwa *et al*., 2014;

Mellado *et al*., 2016; Al-Rowaily *et al*., 2020). Higher nutrient quantities are attributed to higher soil temperature and moisture coupled with mistletoe litter speeding up nutrient cycling and decomposition rates of recalcitrant host litter. Consequently, this will result in higher mineral concentrations, particularly of N, P, Ca, and Mg, within high compared to low mistletoe-infection subcanopies (Ndagurwa *et al*., 2013, 2014, 2020; Muvengwi *et al*., 2015; Al-Rowaily *et al*., 2020). However, nutrient availability even among high mistletoe-infection canopy patches may vary depending on the litter characteristics of the mistletoe species and hosts and the prevailing subcanopy soil chemistry conditions (Al-Rowaily *et al*., 2020; Ndagurwa *et al*., 2020). For example, due to higher quantities of secondary compounds found within *V. verrucosum*/*V. karroo* their decomposition rate is often lower compared to the litter combinations of *E. ngamicum*/*V. karroo* and *P. kalacharensis*/*V. karroo* (Ndagurwa *et al*., 2013, 2014, 2015, 2020). Variations in decomposition and nutrient content further broaden nutrient heterogeneity in semi-arid systems. However, it is anticipated that canopy patches regardless of mistletoe infection intensity have significantly higher soil element concentrations compared to intercanopy spaces, an area open for future research.

Soil conditions

In disagreement with the findings by Chu *et al*. (2021), a higher presence of mistletoes did not moderate understory temperatures. Instead, soil temperature was significantly higher in canopy patches of high- compared to low mistletoe-infected trees, consistent with the previous predictions. Higher temperatures can be explained by the reduction in canopy foliar cover, which increases the penetration of solar radiation underneath high relative to low mistletoe-infection trees (Mellado and Zamora, 2017). Higher soil temperatures are also related to greater rates of soil respiration which is associated with accumulation of litter, microbial activity and litter decomposition rates (Ndagurwa *et al*., 2015, 2016, 2020). This contradicts the assumption that higher litter cover would reduce the impact of solar radiation (Ndagurwa *et al*., 2016). Further, the soil temperatures for high- and low mistletoe-infected canopy patches were also observed to be higher in the north than south, and this is probably due to the slope or orientation of the sun during the day. Consequently, the north sides of all the canopies had lower relative humidity compared to the south due to increased evapotranspiration.

Overall, soil relative humidity was 3% higher within high compared to low mistletoe-infection canopy patches, despite higher temperatures within the former. This could have been a result of higher soil organic matter content which could have decreased evaporation, increased infiltration rates, and improved soil water holding capacity (Kanz, 1996; Dean *et al*., 1999; Treydte *et al*., 2010; Ndagurwa *et al*., 2015, 2016). These results contradict those of Ndagurwa *et al*. (2014b), that mistletoes increase subcanopy moisture losses by ~36% and attributed this to high mistletoe transpiration rates. Similarly, Spasojevic and Suding (2011) found a reduction in soil moisture in areas with hemi-parasites and attributed this to the high transpiration demand of hemi-parasites. Therefore, greater soil moisture observed under high mistletoe-infection trees could mean the transpiration rates associated with high

mistletoe numbers were not sufficiently high to cause a significant decrease in soil moisture. Although not explicitly tested for, another plausible explanation is that high mistletoe-infected trees could have achieved both horizontal uptake and hydraulic lift of water (Kanz, 1996). Hydraulic lift in *Vachellias* has been observed by Ludwig *et al*. (2003), therefore, high mistletoe-infected trees could have used this process to increase water in their subcanopy soils (Priyadarshini *et al*., 2016). Alternatively, high mistletoe-infected trees and their understory subordinates could have spatially (horizontally and vertically in the soil) partitioned their points of water access even during the wet season thus reducing water constraints/competition within their canopy patches (Kröpfl *et al*., 2002; Ludwig *et al*., 2003, 2004). Whilst hydraulic lift could have been present within low mistletoe-infected tree canopy patches, increased competition for water by the trees (with few mistletoes) and their understory plants with higher biomass could have led to lower soil moisture (Ludwig *et al*., 2003, 2004). However, relative humidity was lower in December underneath high than low mistletoe-infected trees. This time usually coincides with leaf flush of both grasses and trees when moisture requirements are high, thus explaining the low moisture readings (Priyadarshini *et al*., 2016). For both infection categories, in December, March, April, and June an increase in sub-canopy temperatures resulted in a decline in relative humidity, possibly through increased evapotranspiration (Breshears *et al*., 1998). In June, canopies were shedding their leaves, therefore higher understory light penetration accelerated soil moisture losses within the canopy patches.

Similar to D'Odorico *et al*. (2007), VWC showed horizontal heterogeneity immediately after it rained, being higher in intercanopy spaces compared to canopy patches. Trees regulate the amount of water reaching the soil during precipitation through leaf interception and stem flow, augmenting soil infiltration capacity (Breshears *et al*., 1998; Kröpfl *et al*., 2002; D'Odorico *et al*., 2007). Higher proportions of litter within tree canopy patches can also intercept rainfall particularly after small rainfall episodes (Kröpfl *et al*., 2002). Therefore, higher VWC in the intercanopy spaces on day_0 could have been due to absence of canopy interception and lower litter quantities. Higher herbaceous biomass within the intercanopy spaces could also have increased water infiltration and soil permeability by reducing rain-splash compaction and runoff (D'Odorico *et al*., 2007). However, high mistletoe-infected trees had higher VWC immediately after the rainfall event compared to low mistletoe-infected trees, possibly due to reduced foliar cover thus reducing interception and allowing more rainfall to reach the soil surface.

The soil dried out progressively from day_0 to day_7, with canopy patches having lower VWC losses compared to intercanopy spaces. Nonetheless day_3 and day_7 showed horizontal homogeneity in the VWC between the canopy patches and the intercanopy spaces. The results contradicted the assumptions of a rapid decline of VWC due to high mistletoe presence compared to low mistletoe-infection canopy patches and the intercanopy spaces. Instead, high mistletoe-infection canopy patches on day_7 tended to have higher VWC. Moreover, high mistletoe-infection intercanopy spaces dried out

at a quicker rate compared to their canopy patches. This could be due to horizontal uptake of water from the intercanopy spaces by the high mistletoe-infected large-rooted trees or because of the higher temperatures in the intercanopy spaces which are associated with high evapotranspiration compared to the shaded canopy patches (Breshears *et al*., 1998; D'Odorico *et al*., 2007).

Lastly, the results contradict some findings from similar studies (Ndagurwa *et al*., 2014, 2016, 2018) in semi-arid savanna systems. These differences are attributed to variations in the experimental design, spatial scale, equipment used, and to this study including animal disturbances. For example, in this study more robust tools of direct measurements (instead of indirect measurements such as oven drying) such as the disc pasture meter and ibuttons/hydrosense soil moisture sensor to measure herbaceous biomass and soil temperature and moisture were used, respectively. Moreover, in contrast to other studies, in addition to using field measurements, the data on soil temperature and soil moisture were recorded over a much longer period, 8 months, rather than once-off core samples that miss dynamics over time.

Conclusion

This study demonstrates that mistletoes modify localized understory plant productivity and microclimatic conditions relative to their surrounding environments, thus increasing the heterogeneity of semi-arid savanna ecosystems. High mistletoe-infected tree canopy patches had higher litter cover, grazing and trampling, soil temperature, and soil moisture content but lower herbaceous biomass and shorter grass height relative to low mistletoe-infected trees. Further, these factors significantly differed between canopy and intercanopy patches of both high and low-mistletoe-infected trees, suggesting that trees are already causing spatial heterogeneity, whilst mistletoes infection intensity is providing a heterogeneous additive effect. Consequently, these results have broader relevance given the widespread distribution of mistletoes in savanna systems and other similar environments. Thus, by modifying resource heterogeneity, mistletoes act as keystone species in this ecosystem as also suggested in a few other studies.

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Author Contributions

TSM, HGTN, ETFW designed the study. TSM collected and analysed the data. TSM, HGTN, ETFW discussed the results and wrote the paper.

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Appendices

Appendix 1a. Mistletoe infected *Vachellia karroo* tree during the wet season (showing some yellow flowers)



Appendix 1b. *Vachellia karroo* tree infected by *Viscum verrucosum*



Appendix 1c. Grazing rings around mistletoe-infected *Vachellia karroo* trees



Appendix 1d. Species diversity underneath mistletoe-infected *Vachellia karroo* trees



Appendix 1e. Goats feeding on pods and the remaining herbaceous biomass at peak of the dry season



Appendix 1f. Canopy patches during at the peak of the dry season



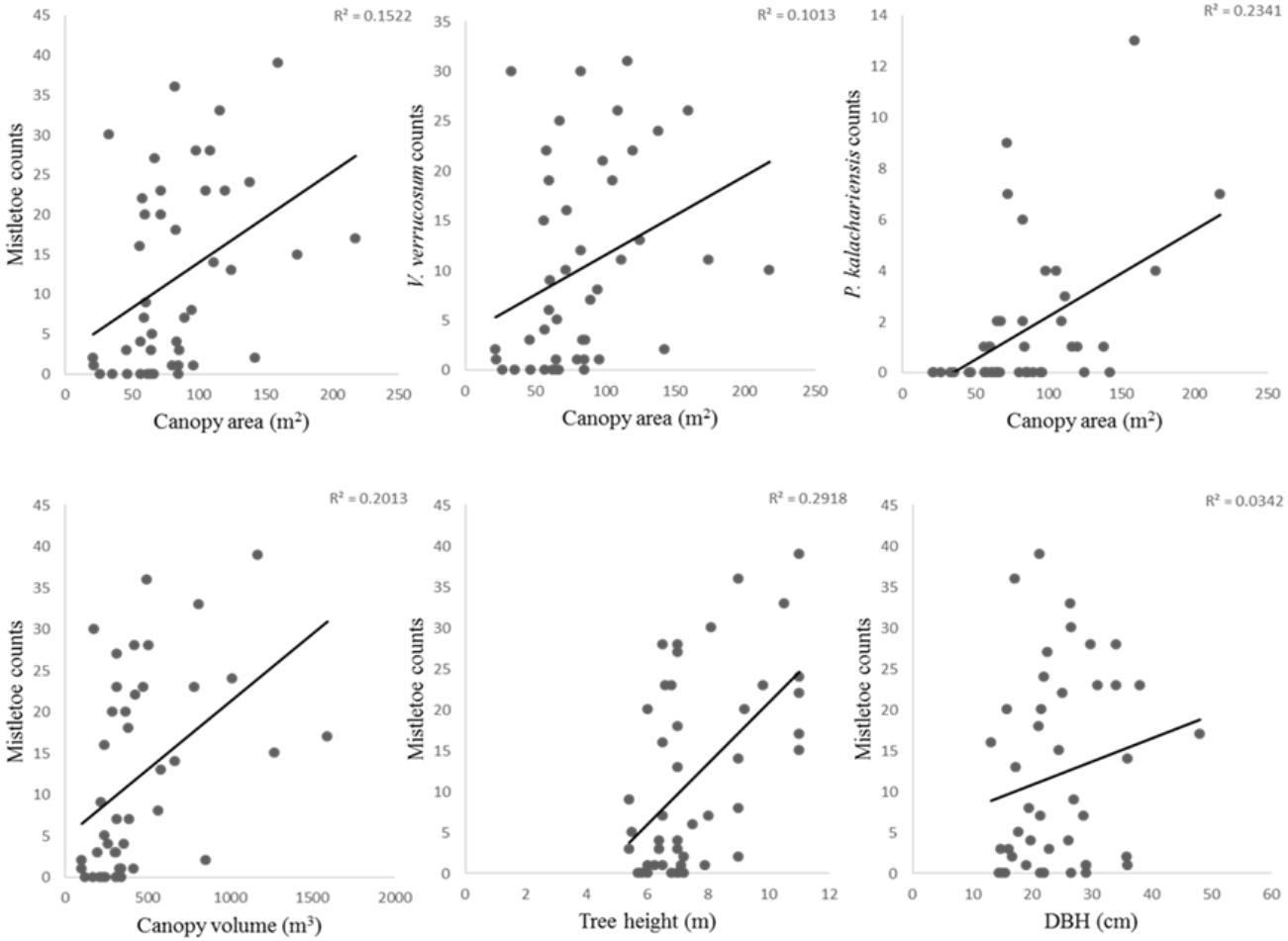
Appendix 1g. Example of a *Vachellia karroo* dominated stand at Matopos Research Station.



Appendix 1h. Evidence of tree cutting in the area



Appendix 2: Mistletoe infection in relation to tree size measures.

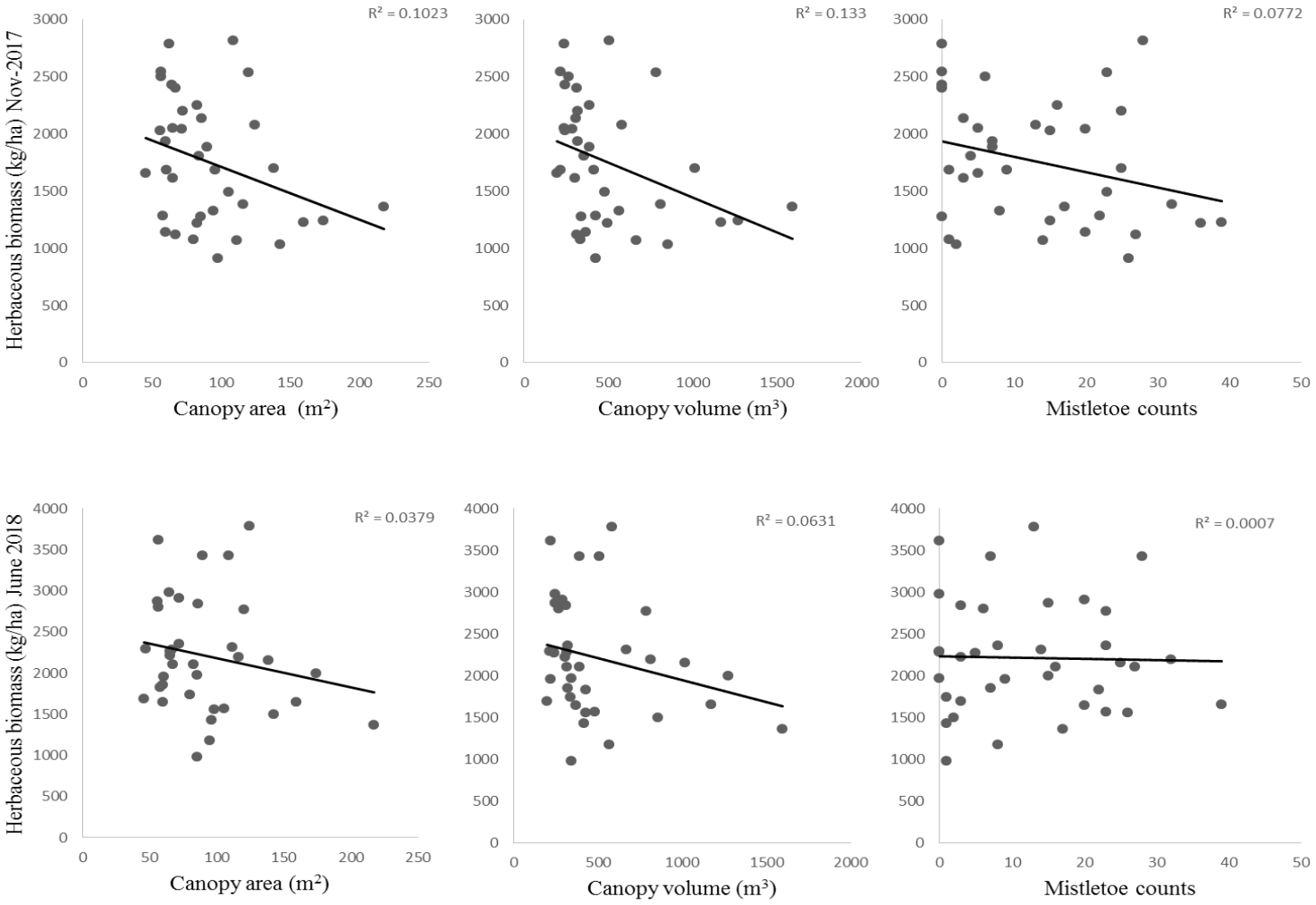


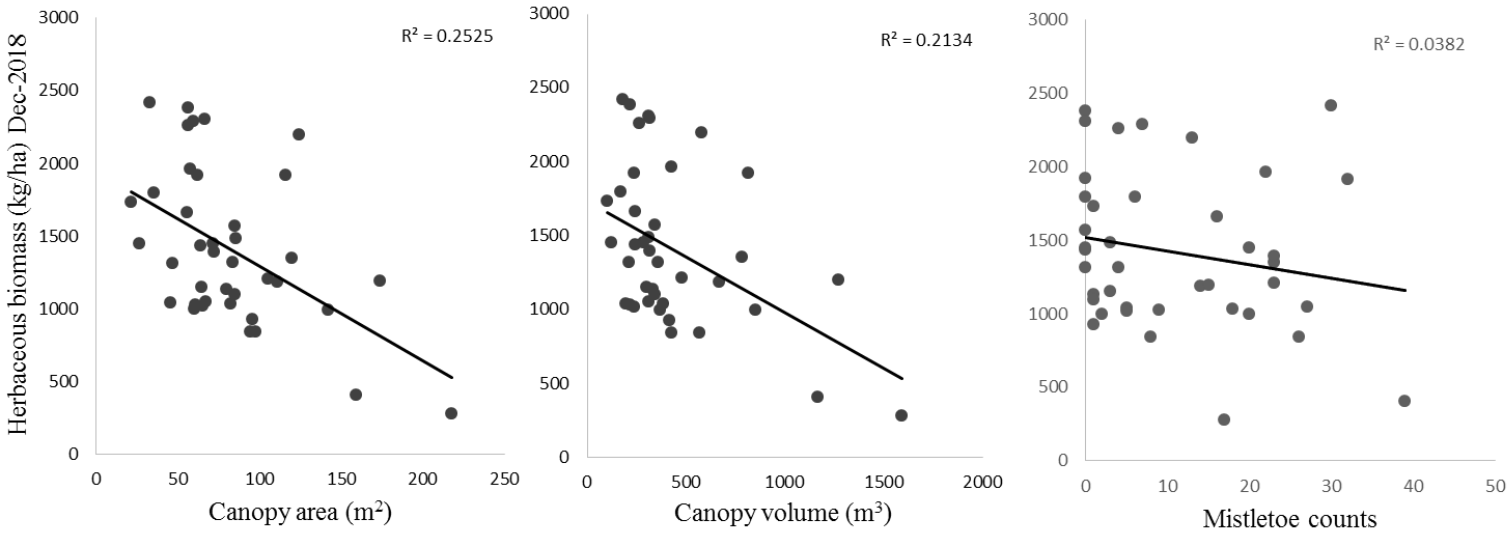
Appendix 3

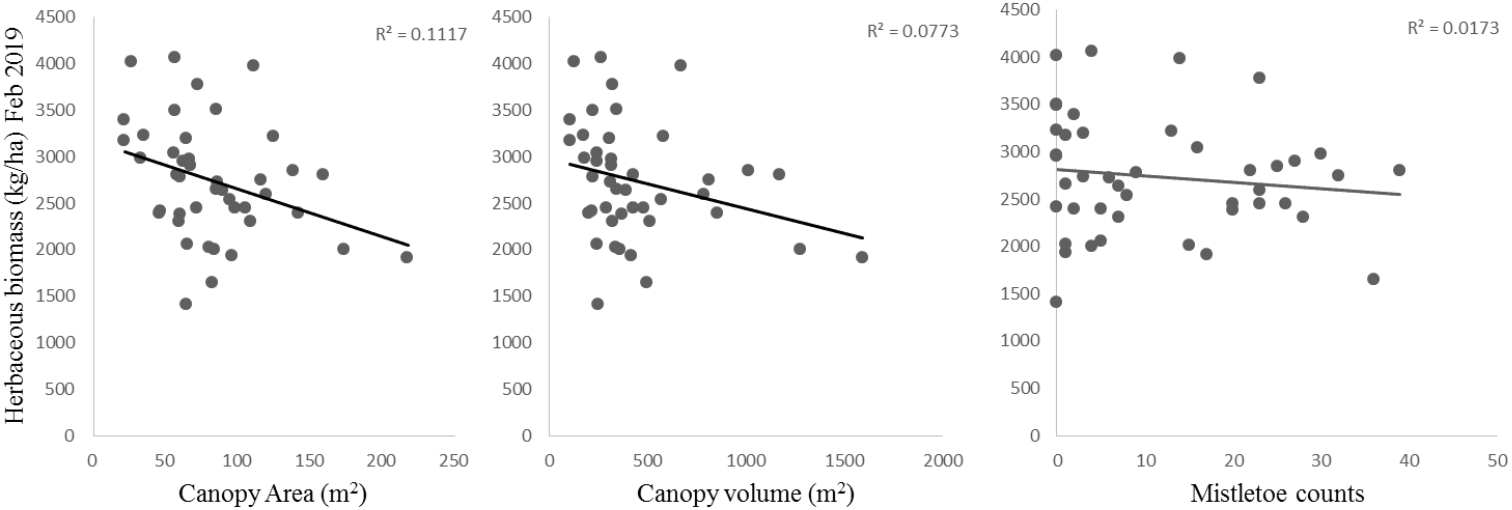
General linear model (GLM) analyses results showing mean (±SE) of the effects of canopy (canopy *vs*. intercanopy spaces) and mistletoe infection degree (high *vs*. low) on aboveground herbaceous biomass (kg/ha) collected at the end of the dry season (November 2017), mid-dry season (June 2018), beginning of the wet season (December 2018) and mid-wet season (February 2019), grass table height (cm) and maximum grass height (cm), litter (%) and grazing/trampling (%). Different letters indicate significant differences between the four patch types (Tukey HSD, $P < 0.05$).

Variable	**Infection**		**Canopy**	
	High	**Low**	**Inside**	**Outside**
Biomass (kg/ha)				
End of dry season (2017)	1831 ± 62.74^{b}	2132 ± 71.88^{a}	1785 ± 60.34^{b}	2178 ± 73.90^{a}
Middle of dry season (2018)	2463 ± 78.40^{b}	2695 ± 84.48^{a}	2219 ± 72.75^{b}	2939 ± 89.40^{a}
Beginning of the wet season (2018)	1559 ± 63.04^{b}	2024 ± 59.84^{a}	1419 ± 54.92^{b}	2164 ± 67.38^{a}
Middle of the wet season (2019)	2997 ± 66.66^{b}	3270 ± 63.28^{a}	2725 ± 58.42^{b}	3541 ± 70.95^{a}
Grass height (cm) 2018-2019				
Maximum height (cm)	61.76 ± 1.06^{b}	71.30 ± 1.03^{a}	59.94 ± 1.04^{b}	73.12 ± 1.05^{a}
Table height (cm)	55.46 ± 0.97^{b}	65.61 ± 0.94^{a}	53.86 ± 0.96^{b}	67.21 ± 0.96^{a}
Litter cover (%) 2018-2019	35.42 ± 0.77^{b}	39.64 ± 0.76^{a}	53.04 ± 0.77^{b}	22.02 ± 0.77^{a}
Grazing/Trampling (%) 2018-2019	30.48 ± 1.23^{a}	27.28 ± 1.23^{a}	32.04 ± 1.24^{b}	25.70 ± 1.24^{a}

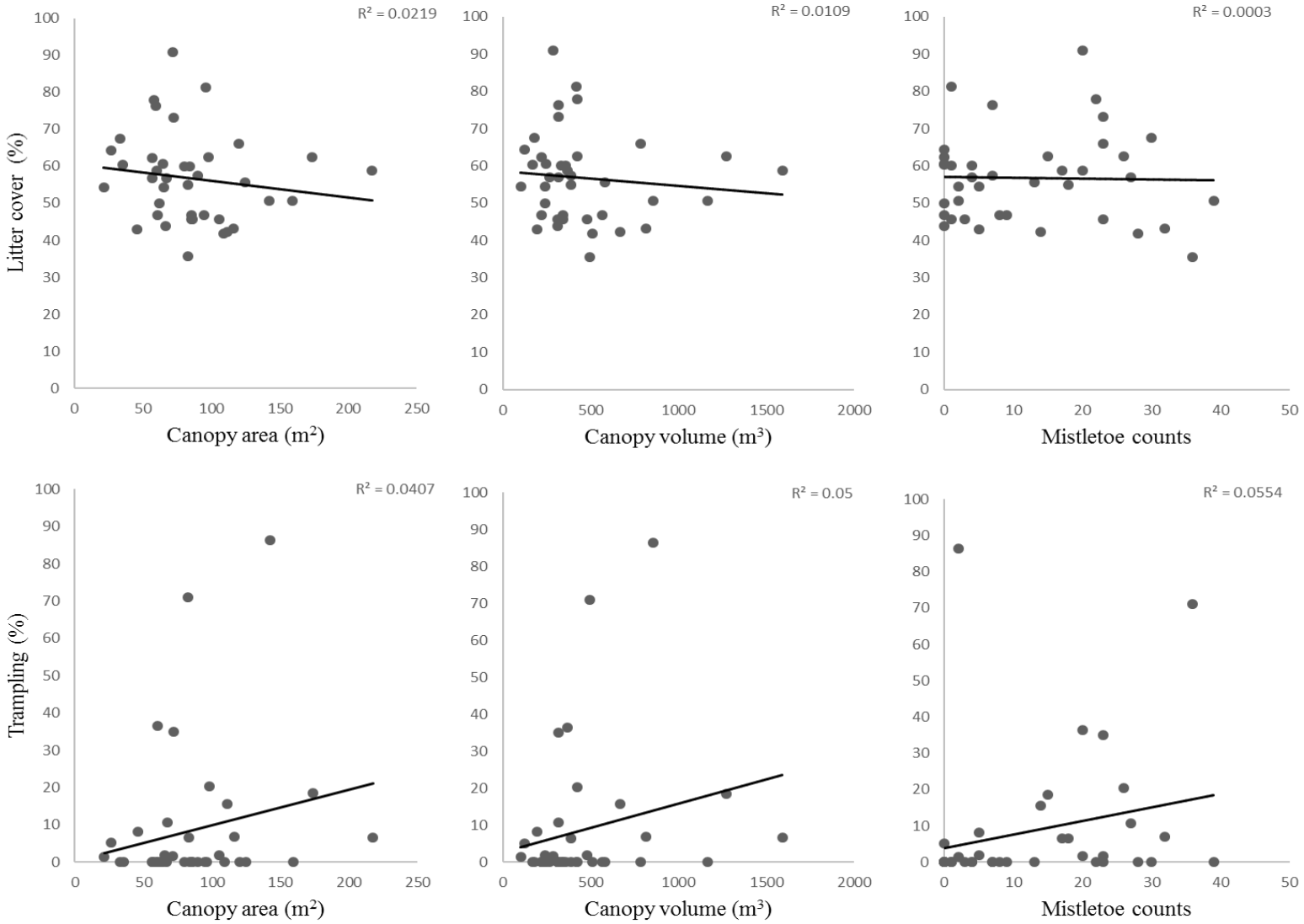
Appendix 4: Relationship between herbaceous biomass (kg/ha) and tree size (canopy area and volume) and mistletoe count for November 2017, June 2018, December 2018, and February 2019.



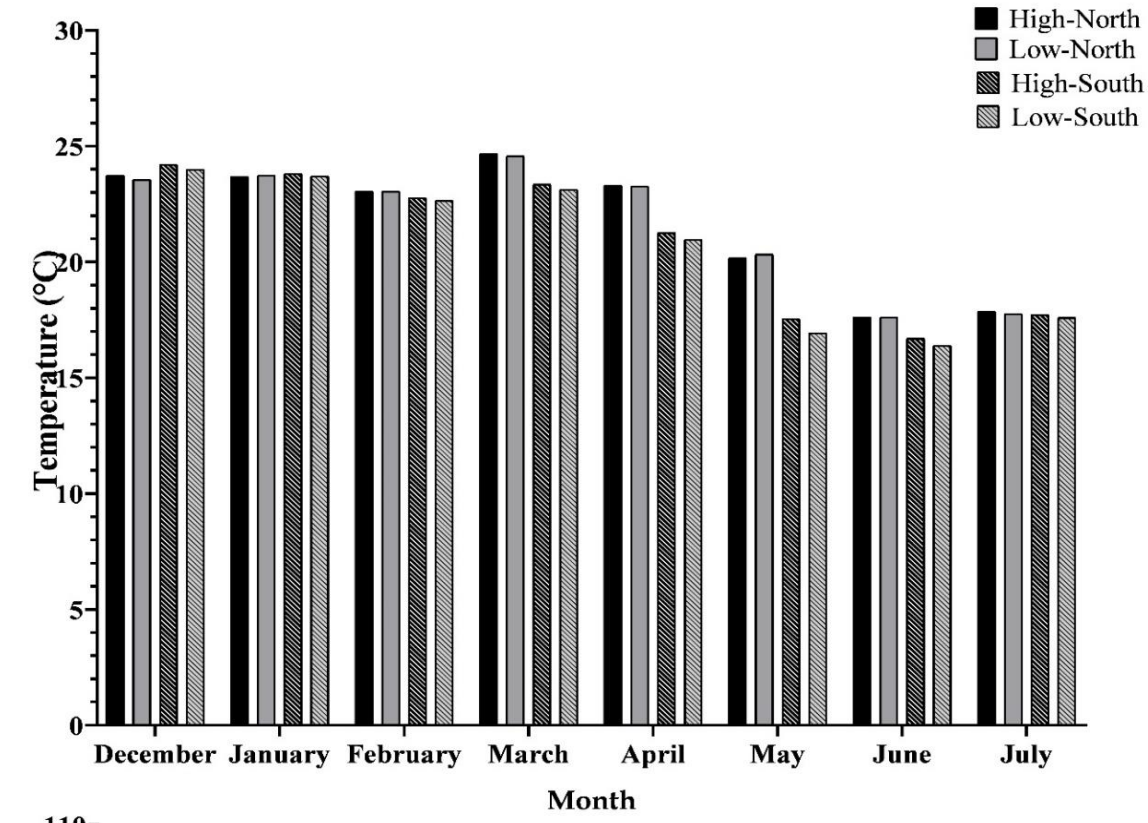

Herbaceous biomass (kg/ha) Dec-2018
R² = 0.2525
Canopy area (m²)
R² = 0.2134
Canopy volume (m³)
R² = 0.0382
Mistletoe counts


Herbaceous biomass (kg/ha) Feb 2019
R² = 0.1117
Canopy Area (m²)
R² = 0.0773
Canopy volume (m²)
R² = 0.0173
Mistletoe counts

Appendix 5: Relationships of litter cover (%) and tramping (grazing %) with tree size (canopy area and volume) and mistletoe numbers.



Appendix 6: Mean monthly temperature (ºC) and relative humidity (%) recorded in the north and south direction of high and low mistletoe infected trees from December 2018 to July 2019.





Appendix 7: Means (±SE) from the general linear model (GLM) analyses of the effects of day (day 0, day 3, day 7), canopy (canopy *vs*. intercanopy spaces), mistletoe infection (high *vs*. low), and their interactions on volumetric soil moisture content (VWC) (%).

Variable				**Mean ± SE**
Day	0			21.29 ± 0.27
	3			19.52 ± 0.27
	7			17.56 ± 0.27
Infection	High			19.18 ± 0.22
	Low			19.32 ± 0.22
Canopy	Canopy patch			19.16 ± 0.19
	Intercanopy spaces			19.76 ± 0.24
Day × Infection	Day 0	High		20.89 ± 0.37
		Low		21.70 ± 0.37
	Day 3	High		19.36 ± 0.37
		Low		19.68 ± 0.37
	Day 7	High		17.30 ± 0.37
		Low		17.82 ± 0.37
Day × Canopy	Day 0	Canopy patch		20.19 ± 0.34
		Intercanopy spaces		22.39 ± 0.41
	Day 3	Canopy patch		19.24 ± 0.34
		Intercanopy spaces		19.80 ± 0.41
	Day 7	Canopy patch		18.05 ± 0.34
		Intercanopy spaces		17.07 ± 0.41
Infection × Canopy	High	Canopy patch		19.29 ± 0.27
		Intercanopy spaces		19.08 ± 0.34
	Low	Canopy patch		19.03 ± 0.27
		Intercanopy spaces		20.43 ± 0.34
Day × Infection × Canopy	Day 0	High	Canopy patch	20.30 ± 0.47
			Intercanopy spaces	21.48 ± 0.58
		Low	Canopy patch	20.08 ± 0.47
			Intercanopy spaces	23.31 ± 0.58
	Day 3	High	Canopy patch	19.39 ± 0.47
			Intercanopy spaces	19.33 ± 0.58
		Low	Canopy patch	19.08 ± 0.47
			Intercanopy spaces	20.28 ± 0.58
	Day 7	High	Canopy patch	18.17 ± 0.47
			Intercanopy spaces	16.43 ± 0.58
		Low	Canopy patch	17.94 ± 0.47
			Intercanopy spaces	17.71 ± 0.58

CHAPTER 3

Functional and species composition of understory plants varies with mistletoe-infection on *Vachellia karroo* trees in a semi-arid African savanna

Abstract

In savanna ecosystems, tree canopy patches differ in plant species composition compared to adjacent intercanopy spaces due to different levels of resource availabilities. Mistletoes further augment nutrients underneath tree canopies whilst reducing their hosts' competitive edge, thus providing more resources and creating patches that support higher understory species richness. However, little is known on how understory species and functional trait assemblages, in both canopy and intercanopy spaces, are affected by varying overstory mistletoe intensities. This study investigated how functional and species composition varied within and between canopy patches and intercanopy spaces of high- and low mistletoe-infected *Vachellia karroo* trees. The affinity of individual species to the different canopy patches and intercanopy spaces was also assessed. Furthermore, an investigation of how plant trait measurements of *Ziziphus mucronata*, a dominant subcanopy woody species, varied between the two canopy patches was done. Microhabitats had significantly different species compositions. A higher proportion of species (34%) showed a strong positive affinity towards canopy patches whilst intercanopy spaces were strongly associated with only 9% of the recorded species, which also indicates greater dominance of some species here. Generally, subcanopy patches had significantly higher species richness and diversity, and functional diversity, compared to their adjacent intercanopy spaces. These variables increased with increasing mistletoe infection, thus grass, forb and tree species diversity were 17% to 43% higher and functional diversity indices were 0.5% to 28% greater in high- compared to low mistletoe-infection canopy patches. Chlorophyll content and height and of *Z. mucronata* were 6% and 27%, (respectively) higher within high- than low mistletoe-infection canopy patches, but SLA, LA, WLT and CA were not different. By enhancing spatial heterogeneity, variations in mistletoe infection facilitate biodiversity and to a lesser extent vegetation structural diversity in these semi-arid savannas.

Keywords: Functional diversity; intercanopy spaces; mistletoe-infection; plant traits; spatial heterogeneity; species diversity.

Abbreviations: CA - canopy area; FDis - Functional dispersion, Fdiv- Functional divergence, FEve - Functional evenness, FRic - Functional richness, LA - leaf area, LDMC - leaf dry matter content, RaoQ - Rao's quadratic entropy, SLA - specific leaf area, WLT - whole leaf thickness.

Introduction

The influence of large trees on understory species composition has been widely documented. Studies have shown that tree canopy patches are fertility islands that have lower soil temperatures and higher soil nutrients and moisture compared to adjacent intercanopy spaces (Dean, *et al*., 1999; Ludwig *et al*., 2004; Munzbergova and Ward, 2002; Treydte *et al*., 2008, 2009; Linstädter *et al*., 2016). Consequently, canopy patches often have distinct plant assemblages that vary in function and structure compared to surrounding intercanopy spaces. For instance, canopy patches primarily support native, shade tolerant (C_3), and moisture-loving plant species through facilitation (Dean *et al*., 1999; Linstädter *et al*., 2016). In contrast, intercanopy spaces are dominated by shade-intolerant C_4 plants which are adapted to high temperatures and low soil moisture levels (Linstädter *et al*., 2016).

Mistletoes on tree canopies have been shown to increase the availability of nutrients through augmented litterfall, thus further enhancing heterogeneity and increasing species richness of understory assemblages (March and Watson 2007; Ndagurwa *et al*., 2016, 2018; Muvengwi *et al*., 2015; Mellado *et al.*, 2016; Hódar, *et al*., 2018; Al-Rowaily *et al*., 2020). For example, Ndagurwa *et al.* (2013) showed that mistletoes produced significantly more litter than their host *Vachellia karroo* (Hayne) Banfi & Galasso trees, thus increasing nutrients within the canopy patches of mistletoe-infected trees. Furthermore, Ndagurwa *et al.* (2015, 2020) reported that mistletoe leaf litter decayed faster than that of *V. karroo* and potentially catalysed the rapid decomposition of *V. karroo* litter. This facilitation makes the nutrients more readily available to the surrounding plant community, thereby increasing understory primary productivity, and potentially counteracting the negative impacts of parasitism (Spasojevic and Suding, 2011; Ndagurwa *et al.*, 2013, 2016; Muvengwi *et al*., 2015). Consequently, high animal visitations to mistletoe flowers and fruits were observed to increase seed dispersal via excreta, resulting in the accumulation of seed-filled excreta leading to aggregation of a variety of seedlings and saplings within the parasitized tree canopy patches (Mellado *et al*., 2016; Mellado and Zamora, 2016, 2017). This contributes to further variation in species assemblages and most likely plant sizes and traits underneath mistletoe-infected compared to uninfected trees and adjacent intercanopy spaces (March and Watson 2007; Ndagurwa *et al*., 2016, 2018; Mellado and Zamora, 2017; Hódar, *et al*., 2018; Ramsauer, 2019).

Mistletoes through parasitism can also alter the understory species assemblages by suppressing the competitive edge of their hosts, and alternately increase the competitive edge of non-host or co-occurring understory species (Spasojevic and Suding, 2011). High mistletoe infection weakens the health and defence mechanisms of the host by invading their vascular system and increasing the demand for water and nutrients relative to supply (Sala *et al.*, 2001). Consequently, high mistletoe infection decreases host growth (biomass), physiological attributes (i.e., leaf and branch area, and the number of leaves) and can lead to death of the host (Press and Phoenix, 2004; Mellado and Zamora,

2017; Monteiro *et al*., 2020). For example, dwarf mistletoes decreased the growth of Douglas fir (*Pseudotsuga menziesii*) by ~65% (Mathiasen *et al*., 1990; Press and Phoenix, 2004). Subsequently, there is loss of photosynthetic and respiration area leading to formation of canopy- and within canopy gaps thus changing both the biotic and abiotic conditions underneath the hosts (Sala *et al.*, 2001; Manthiasen *et al*., 2008; Mellado and Zamora, 2017). Mellado and Zamora (2017) also showed that *Viscum album subsp. austiacum* parasitism on pines resulted in ~18% more light incidence compared to un-parasitized trees. Similarly, in semi-arid savannas, high mistletoe-infected *V. karroo* canopy patches had higher soil temperatures and relative humidity compared to low mistletoe-infected trees (Chapter 2). These conditions open up space for growth and productivity of sub-dominant plant species that prefer intermediate light conditions thus, potentially increasing understory species diversity as shown in other studies (Sala *et al.*, 2001; Spasojevic and Suding, 2011; Monteiro *et al*., 2020).

A reduction in host growth (parasitism), intermediate light incidence, coupled with the high mistletoe and host litter are likely to result in greater variation in species composition between high and low mistletoe-infected trees and their surrounding intercanopy spaces. No known study has investigated how the extent of mistletoe infection influences species composition, structure and function within and beyond canopy patches. Therefore, there is a need to determine if there are spatial differences in the plant assemblages, plant sizes and qualitative functional traits (e.g., morphological and physiological traits). Furthermore, it is pertinent to investigate the different functional responses that can arise from each set of environmental conditions. Most biodiversity studies use traditional species diversity indices, here both the taxonomic and functional traits were used in-order to understand how community assemblages respond to varying degrees of mistletoe infection (Schirmel *et al*., 2012; Török *et al*, 2016; Schellenberger, 2017; Solbrig *et al*., 2017).

This study investigated how understory plants respond to different levels of mistletoe infection (high *vs*. low) between canopy and adjacent intercanopy patches. It was expected that (i) canopy patches and intercanopy spaces would have different species compositions; (ii) that abundance of grass species would be highest in the intercanopy spaces, whilst forbs and trees would dominate the tree canopy patches. Furthermore, it was expected (iii) that the affinity of species would be highest within the canopy patches and particularly high mistletoe-infection canopy patches, in a similar way that many tree species have affinities for termite mounds (Cuma Mushagalusa *et al*., 2019); (iv) species richness, evenness, and diversity would be highest below canopies of high mistletoe-infected trees; and (v) that functional diversity indices would be higher in canopy patches, particularly those with high mistletoe-infection. Lastly, it was expected that due to higher light incidence (vi) high mistletoe-infection canopy patches would have saplings with smaller, leaf area (LA), specific leaf area (SLA), and higher leaf dry matter content (LDMC) compared to those found in low mistletoe-infection

canopy patches.

Methods

Study area

The study was carried out at the Matopos Research Station (MRS) (20° 31'S, 28° 31'E), south-west Zimbabwe. The average rainfall and temperature are 586mm and 18°C, respectively. This study focused on the area with fine-textured soils which are mainly dominated by *Vachellia* spp. (formerly *Acacia*) (Ndagurwa *et al.,* 2012). The study tree *V. karroo* is one of the dominant species in the site (see Chapter 2 for further details).

Species composition

Herbaceous species composition and species diversity were assessed under 'canopy patches' and adjacent 'intercanopy spaces' of 20 high- and 20 low- mistletoe-infected *V. karroo* trees, towards the end of the growing season (end of February to early March of both 2018 and 2019, when plants have largely flowered/fruited, facilitating easy species identification). However, only the 2019 results are presented, when species identifications were more straightforward, although the 2018 results follow the same overall patterns. Beneath each study tree, two 1m × 1m quadrats were placed in each of the four cardinal directions at different distances (half the canopy diameter (8 quadrats) and at 1½ times the canopy radius (8 quadrats)), see Fig. 2 in Chapter 2. Therefore, there were four microhabitats i.e., high mistletoe-infection canopy patches, high mistletoe- infection intercanopy spaces, low mistletoe-infection canopy patches, and low mistletoe-infection intercanopy spaces. All the understory plants within each quadrat were identified up to species level (where possible) and recorded. Voucher specimens of unidentified species were pressed, taken to MRS or to the Department of Forest Resources and Wildlife Management laboratory at the National University of Science and Technology, Zimbabwe, where the plants were either cross-referenced with plant identification guidebooks or identified by an expert. Percentage cover of each species, the proportion of grasses, forbs, and all woody species within each quadrat were determined using the 8 point Walker (1976) scale.

Species affinity to different microhabitats

The affinity of each species to each of the four different microhabitats ('mistletoephily index') was calculated using the equivalent of the "termitophily index" (Cuma Mushagalusa *et al*., 2019). In this case, the following formula was used :

$$\text{IndexMicrohabitat} = \frac{\text{SpeciesX}_{\text{microhabitat}}}{\text{SpeciesX}_{\text{microhabitat}} + \text{SpeciesX}_{\text{m}}} \quad ,$$

where $\text{SpeciesX}_{\text{microhabitat}}$ and $\text{SpeciesX}_{\text{m}}$ are the number of occurrences of each species on and off the

two different microhabitats and IndexMicrohabitat is the index for each microhabitat.

Functional traits

Functional trait data were obtained from existing literature, handbooks, and field guides (Cornelissen *et al*. 2003; Van Wyk, 2013; Van Oudtshoorn, 2014; Palgrave, 2015; Perez-Harguindev *et al*., 2016). Online literature databases/data sources i.e., TRY global database (Kattge *et al*., 2011), Catalogue of Life, Encyclopaedia of life, Flora of Zimbabwe, and PlantZAfrica.com were also used to obtain information on traits for each species. The focus was on traits that show the response of the understory plants to soil resources, which included whole-plant traits (i.e., life history, life form, photosynthetic pathway, and nitrogen fixation) and whether the plants were native or exotic. Furthermore, grasses were grouped according to plant succession, grazing status (increaser or decreaser), and grazing value (palatability) (Van Oudtshoorn, 2014). Therefore, most traits were categorical, and hence had to be converted to binary or ordinal formats to calculate the FD indices. Most continuous and some categorical traits, including presence of mycorrhizae, were not included because of insufficient data in the traits databases.

Specific trait measurements

To investigate intraspecific trait variability, the characteristics of one of the most dominant subcanopy woody plants i.e., *Ziziphus mucronata* Willd., were compared within 10 highest- and 10 lowest mistletoe-infection canopy patches. Within each of the canopy patches, ten *Z. mucronata* individuals (200) found in the least shaded areas were selected and their height, stem diameter, and the canopy long (D_1) and short (90^o of D_1; = D_2) diameters measured. Ten fully expanded photosynthetically active leaves were identified per tree, and chlorophyll content measured non-destructively using a SPAD-502-Plus (Spectrum Technologies, Inc, Plainfield IL, USA). The leaves were then cut off, individually marked, and photographed using a digital camera, and then leaf area was calculated using ImageJ. Leaf fresh and dry weight was determined before and after oven drying at 70°C for 72 hours using a 0.0001 g precision scale, respectively.

Data Analysis

Rarefaction curves were produced using the function *specaccum* and the method rarefaction in the *vegan* package in *R*. The method rarefaction calculates the exact species richness and standard deviation by selecting and using abundances of individual species instead of sites. Species composition was analysed by correspondence analysis (CA), using the packages *FactoMineR* (functions CA ()) and *factoextra* (graphical presentation) in R (Kassambara, 2017).

Species diversity

Biodiversity R was used to calculate species richness, abundance, evenness, and Shannon–Wiener (*H*') diversity index) of each $1m^2$ quadrat (n = 160/microhabitat) within each of the four microhabitats. Data were tested for normality using the Shapiro Wilk test and data was not normal even after

transformations. General linear models (GLM) were used to test the effects of canopy and infection on species diversity indices in SPSS 23 for Windows (SPSS Inc., 2012, Chicago, IL U.S.A). Kruskal-Wallis tests were used to compare the species diversity indices (richness, evenness, diversity, and abundance) across the four microhabitats, followed by a post hoc Kruskalmc for non-parametric data (P = 0.05). ANOSIM was used to determine differences in species composition across the four microhabitats using the *Vegan* package in R (Vegan *et al*., 2013).

Species affinity to different microhabitats

Fishers' exact test was used to calculate the affinity of each species ('mistletoephily index') to the different microhabitats by comparing the ratio of each species (Species X) on and off each microhabitat, and calculated as follows:

$$\frac{\text{SpeciesX}_{\text{microhabitat}}}{\text{SpeciesX}_{\text{m}}} \qquad \text{versus,}$$

where $\text{SpeciesX}_{\text{microhabitat}}$ and $\text{SpeciesX}_{\text{m}}$ are the number of occurrences of each species on and off the two different microhabitats.

Total count ratio of all the species without the Species X

$$\frac{\text{AllSp}_{\text{microhabitat}} - \text{SpeciesX}_{\text{microhabitat}}}{\text{AllSp}_{\text{m}} - \text{SpeciesX}_{\text{m}}}$$

where, $\text{AllSp}_{\text{microhabitat}}$ and AllSp_{m} is the abundance of all the species found in the microhabitat of interest and the abundance of all the species in the contrasting microhabitat, respectively (Cuma Mushagalusa *et al*., 2019). A significantly low ratio of each species compared to the rest of the community inferred a strong negative association (<10%) of the species to that microhabitat, whilst a significantly high ratio means a positive association (> 90%). Species that were associated with both microhabitats were referred to as neutral species, whilst species that had less than five individuals for both microhabitats were not assessed (Cuma Mushagalusa *et al*., 2019).

Functional diversity

FD was unpacked by investigating five indices: functional richness (FRic), functional evenness (FEve), functional divergence (FDiv), functional dispersion (FDis), and the Rao's quadratic entropy (Rao Q). The functional diversity indices were calculated using the FD (Laliberté *et al*., 2014) and vegan packages in R (version 3.6.3, R Core Team, 2020). The species abundance and composition data coupled with the trait matrix were used for this analysis. Due to the data being mostly qualitative (non-continuous) the Gower Distance (gowdis) which can incorporate different variables (i.e., continuous, nominal, ordinal and binary), and converts the species by trait matrix into a trait distance matrix was used (Katovai *et al*., 2011; Villéger *et al*., 2008; Laliberté and Legendre, 2010; Joseph *et al*., 2014). Functional diversity was calculated using the dbFD function, coupled with the correction method "lingoes". FRic, FEve, FDiv, FDis, and the Rao's Q for each quadrat (160 quadrates/

microhabitat = 640 quadrates) were then calculated. GLMs were used to test the effects of canopy and infection on functional diversity indices in SPSS 23. Values for the indices for each quadrate were used to calculate the differences between the four microhabitats using Kruskal-Wallis, followed by post hoc Kruskalmc for non-parametric data ($P < 0.05$).

Specific trait measurements

The mean SLA, LDMC, WLT, and CA of *Z. mucronata* were calculated using the following equations:

Canopy area (m^2) = $\pi(\frac{D1}{2} \times \frac{D2}{2})$

Specific leaf area ($m^2\ kg^{-1}$) = $\frac{Fresh\ leaf\ area}{Oven\ dry\ mass}$

Leaf dry matter content ($mg\ g^{-1}$) = $\frac{Oven\ dry\ mass\ (mg)}{Water\ saturated\ fresh\ mass\ (g)}$

Whole leaf thickness (µm) = $1/SLA \times LDMC$

Mann-Whitney tests were used to compare the measured *Z. mucronata* traits between high- and low mistletoe-infection tree canopy patches.

Results

Species composition

A total of 82 species (including morphospecies) of plants from 25 families were observed. These comprised 29 (including 1 unknown) grasses, 31 forbs (including 9 unknown), and 22 tree (including 4 unknown) species (Table 1). Grasses (Poaceae) were dominated by Increaser II grass species (n = 22, Table 1) and a few (n = 5) decreaser species, i.e., *Digitaria eriantha, Setaria incrassata*, *Themeda triandra, Panicum maximum* and *Cenchrus ciliaris*, and one Increaser I species i.e., *Bothriochloa insculpta* (Van Oudtshoorn, 2014). Overall, intercanopy spaces were dominated by grasses (decreasers), whilst canopy patches were dominated by all three growth forms. *V. karroo* trees seemed to favour intercanopy spaces. This supported our initial hypotheiss that canopy patches and intercanopy spaces would have different species compositions and that grass species would be highest in the intercanopy spaces, whilst forbs and trees would dominate the tree canopy patches. The most dominant grass species was *S. incrassata*, with 24 to 69% higher cover within low compared to high mistletoe-infection microhabitats (Table 1). Similarly, *S. incrassata* cover was 27 to 60% more in the intercanopy spaces than canopy patches. *Setaria pumila* was 13% to 71% greater in low mistletoe-infection intercanopy spaces than within other microhabitats. Three increaser II grasses i.e., *S. verticillata*, *Sporobolus pyramidalis, Eragrostis aspera* (Van Oudtshoorn, 2014) were 38 to 100% more abundant within high mistletoe-infection canopy patches compared to other microhabitats (Table1).

Table 1. Mean **(**± SE) percentage cover of grass, forb and woody species at the 1 m^2 scale in high mistletoe-infection canopy patches and intercanopy spaces and low mistletoe-infection canopy patches and intercanopy spaces and their taxa codes for Fig. 2. *, vernacular names of the species, **non-native species. Unknowns (morphospecies) were excluded from this table.

Plant species	**Taxa code**	**High infection**		**Low infection**	
		Canopy	**Intercanopy**	**Canopy**	**Intercanopy**
Grasses					
Aristida congesta Roem. & Schult. subsp. *barbicollis* (Trin. & Rupr.) De Winter[IC2]	ACB	0.04±0.03	1.02±0.34	0±0	0.12±0.06
Aristida congesta Roem. & Schult. subsp. *congesta* [IC2]	AC	0.02±0.02	0.01±0.01	0±0	0±0
Aristida scabrivalvis Hack. subsp. *scabrivalvis* [IC2]	AS	0±0	0.79±0.40	0±0	0±0
Bothriochloa insculpta (Hochst. ex A. Rich.) A.Camus [IC1]	BI	0.06±0.04	1.29±0.25	1.1±0.25	1.23±0.20
Cenchrus ciliaris L. [D]	CC	0.16±0.08	0.01±0.01	0.13±0.08	0.28±0.13
Chloris pycnothrix Trin. [IC2]	CP	0.21± 0.06	0.1±0.04	0.01±0.01	0.01±0.01
Chloris virgata Sw. [IC2]	CV	0.85±0.21	1.25±0.37	0.09±0.04	0.15±0.07
Cynodon dactylon (L.) Pers. [IC2]	CD	2.01±0.35	1.78±0.40	1.4±0.30	0.67±0.28
Dactyloctenium aegyptium (L.) Willd. [IC2]	DG	0.01±0.01	0±0	0±0	0±0
Digitaria eriantha Steud. [D]	DE	0.23±0.06	0.33±0.12	0.24±0.08	0.28±0.14
Eragrostis aspera (Jacq.) Nees [IC2]	EA	4.76±0.97	0.29±0.18	0.07±0.03	0.16±0.07
Eragrostis biflora Hack. ex Schinz [IC2]	EB	0±0	0±0	1.33±0.50	0±0
Eragrostis curvula (Schrad.) Nees [IC2]	EC	3.28±0.82	3.65±0.60	0.65±0.14	1.21±0.26
Eragrostis lehmanniana Nees [IC2]	EL	0.29±0.18	0±0	0.04±0.04	0±0
Eragrostis superba Peyr. [IC2]	ES	0±0	0.13±0.10	0±0	0±0
Heteropogon contortus (L.) Roem. & Schult. [IC2]	HC	1.43±0.29	8.43±1.40	0.81±0.25	0.85±0.31
Heteropogon melanocarpus (Elliott) Benth. [IC2]	HM	0.71±0.28	0.59±0.21	0.08±0.04	0.06±0.03
Melinis repens (Willd.) Zizka *subsp. repens* [IC2]	MR	0±0	0±0	0.02±0.01	0.02±0.01
Panicum maximum Jacq. [D]	PM	0±0	0.08±0.06	0±0	0±0
Pogonarthria squarrosa (Roem. & Schult.)Pilg. [IC2]	PS	0.01±0.01	0.11±0.07	0±0	0.16±0.13
Setaria incrassata (Hochst.) Hack. [D]	SI	18.26±1.75	45.1±2.37	43.01±1.82	59.06±1.64
Setaria pumila (Poir.) Roem. & Schult. [IC2]	SP	0.41±0.14	1.14±0.31	0.38±0.10	1.31±0.63

Setaria verticillata (L.) P. Beauv. [IC2]	SVT	3.12±0.65	0.01±0.01	0.23±0.12	0±0
Sorghum versicolor Andersson [IC2]	SV	0.07±0.04	0.44±0.25	0.01±0.01	0.12±0.07
Sporobolus ioclados (Trin.) Nees [IC2]	SIC	0.02±0.02	0±0	0±0	0±0
Sporobolus pyramidalis P. Beauv. [IC2]	SPY	3.21±0.63	0.78±0.34	1.98±0.44	0.63±0.24
Themeda triandra Forssk. [D]	TT	0.02±0.02	0±0	0±0	0±0
Urochloa mosambicensis (Hack.) Dandy [IC2]	UM	1.03±0.20	1.65±0.32	0.04±0.02	0.54±0.15
Total grass cover		**40.21**	**68.98**	**51.62**	**66.86**
Forbs					
Achyranthes aspera L. var. *pubescens* (Moq.) C.C. Towns.**	AAL	0.56±0.15	0±0	0.88±0.34	0±0
Asparagus africanus Lam. var. *africanus*	AA	8.66±0.78	0.01±0.01	2.58±0.48	0±0
Berkheya radula (Harv.) De Wild.	BR	0±0	0±0	1.74±0.47	0.13±0.07
Bidens pilosa L.**	BP	1.71±0.29	0.05±0.03	1.68±0.34	0.04±0.03
Commelina benghalensis L.	CB	0.23±0.07	0.08±0.04	0.19±0.05	0.11±0.05
Corchorus olitorius L.	CO	0.01±0.01	0±0	0.13±0.06	0.29±0.11
Hibiscus sidiformis Baill.	HS	3.31±0.57	0.05±0.03	0.39±0.13	0±0
Igambule*	MP8	0.01±0.01	0.01±0.01	0.01±0.01	0±0
Indigofera schimperi Jaub. & Spach var. *schimperi*	IS	0.04±0.04	0±0	0±0	0±0
Ipomoea plebeia R. Br. subsp. *africana* A. Meeuse	IP	0.23±0.09	0.09±0.06	0.26±0.06	0.01±0.01
Isagenama (Drimia sanguinea (Schinz) Jessop)*	MP10	0±0	0±0	0.03±0.03	0.02±0.02
Lantana camara L.**	LC	1.34±0.35	0±0	0.24±0.16	0±0
Leucas martinicensis R. Br.	LM	0.03±0.03	0.04±0.03	0±0	0±0
Lippia javanica (Burm.f.) Spreng.	LJ	0.41±0.19	0.13±0.11	0.65±0.29	0.19±0.11
Lippia oatesii Rolfe	LO	0.14±0.06	0.12±0.07	0.05±0.05	0±0
Oxalis corniculata L.**	OC	0.02±0.02	0±0	0.01±0.01	0±0
Senna italica Mill. subsp. *arachoides* (Burch.) Lock	SIT	0.16 ±0.07	1.09±0.16	0.03±0.01	0.63±0.11
Sida alba L.	SA	2.37±0.28	0.04±0.03	2.01±0.41	0±0
Solanum aculeastrum Dunal var. *aculeastrum*	SAA	2.66±0.39	1.75±0.33	0.32±0.10	0.18±0.08
Solanum catombelense Peyr.	SC	0±0	0±0	0.04±0.03	0±0
Tagetes minuta L.**	TM	0.53±0.11	1.13±0.25	0.34±0.08	0.49±0.15
Tagetes patula L.**	TP	0.08±0.08	0.05±0.03	0.02±0.02	0.06±0.03

Taraxacum officinale Weber**	TO	0.13±0.07	0±0	0±0	0±0
Total forb cover		**22.63**	**4.64**	**11.60**	**2.15**
Trees					
Amehlo enkomo *	MP12	1.39±0.20	0.03±0.03	1.69±0.36	0.39±0.16
Berchemia discolor (Klotzsch) Hemsl.	BD	0±0	0±0	0.01±0.01	0±0
Combretum hereroense Schinz subsp. *hereroense*	CH	0±0	0±0	0.33±0.25	0±0
Combretum imberbe Wawra	CI	0±0	0±0	0±0	0.01±0.01
Combretum molle R.Br ex G. Don	CM	0±0	0±0	0.03±0.03	0±0
Dichrostachys cinerea (L.) Wight & Arn.	DC	0±0	0±0	0.04±0.04	0.02±0.02
Elaeodendron transvaalense (Burtt Davy) R.H. Archer	ET	0.24±0.07	0±0	0.01± 0.01	0±0
Euclea divinorum Hiern	ED	0.44±0.10	0±0	0.18±0.06	0.08±0.08
Flueggea virosa (Roxb. ex Willd.) Voigt subsp. *virosa*	FV	3.56±0.49	0±0	1.18±0.30	0±0
Grewia flavescens Juss. var. *flavescens*	GF	0.94±0.17	0±0	0.31±0.10	0±0
Pseudolachnostylis maprouneifolia Pax var. *maprouneifolia*	Pseudo	0±0	0±0	0.08±0.08	0±0
Rhus lancea L. f.	RL	0.43±0.13	0±0	0.09±0.05	0±0
Sclerocarya birrea (A. Rich.) Hochst. *subsp. caffra* (Sond.) Kokwaro	SB	0±0	0±0	0.01±0.01	0±0
Senegalia nigrescens (Oliv.) P.J.H. Hurter	SN	0±0	0±0	0±0	0.28±0.20
Vachellia karroo (Hayne) Banfi & Galasso	VK	0.51±0.09	3.32±0.39	0.67	2.36±0.29
Vachellia sieberiana (DC.) Kyal. & Boatwr. var. *woodii* (Burtt Davy) Kyal. & Boatwr.	A.SIEB	0±0	0±0	0±0	0.07±0.05
Vachellia tortilis (Forssk.) Galasso & Banfi	VT	0±0	0±0	0±0	0.02±0.02
Ximenia americana L. var. *microphylla* Welw. ex Oliv.	XA	0±0	0.03±0.03	0±0	0±0
Ziziphus mucronata Willd.	ZM	4.36±0.36	0.03±0.03	2.54±0.28	0.11±0.07
Total tree cover		**11.87**	**3.41**	**7.17**	**3.34**

Superscripts (1) D represents decreaser grasses which are highly desired grasses, prevalent in areas with good range condition, but decrease with overgrazing (over-utilized), (2) IC1 represents increaser I grasses that increase when they are under grazed (under-utilized), (3) IC2 represents increaser II grasses that increase when there is overgrazing (over-utilization) (Van Oudtshoorn, 2014).

Occurrences of *Heteropogon contortus*, *Urochloa mosambicensis*, and *Eragrostis curvula* were 10% to 98% greater within high mistletoe-infection intercanopy spaces compared to other microhabitats. Canopy patch microhabitats had a higher occurrence of *Cynodon dactylon* and *S. pyramidalis* 11% to 52% and 68% to 76%, (respectively) compared to their adjacent intercanopy spaces.

High mistletoe-infection intercanopy spaces had three unique grass species, *Panicum maximum, Eragrostis superba,* and *Aristida scabrivalis* and similarly, high mistletoe-infection canopy patches had another three unique grass species *Themeda triandra*, *Sporobolus ioclados*, and *Dactyloctenium aegyptium*. Low infection canopy patches had one unique grass species, *Eragrostis biflora*, whilst low mistletoe-infection intercanopy spaces had no unique species.

All the non-native species were forbs and they were most dominant within canopy patches compared to intercanopy spaces (except for *Tagetes* sp.) (Table 1). Asteraceae was the most common family for forbs (n = 6 species). Common forbs within canopy patches were *Asparagus africanus*, *Bidens pilosa, Sida alba,* and *Hibiscus sidiformis*. *Tagetes minuta* and *Senna italica* were in higher abundance in intercanopies than canopy patches by 31% to 70% and 75% to 97%, respectively (Table 1).

Fabaceae-Mimosoideae was the most common family for trees (n = 5 species). *V. karroo* tree abundance was 72% to 85% higher within intercanopy spaces compared to canopy patches. Whilst, *Z. mucronata* and *Flueggea virosa* were (95% to 99%, and 100%, respectively) higher with canopy patches than intercanopies (Table 1).

Overall, the four microhabitats had significantly different species assemblages as shown by the spatially diverging arrows (Figure 1). The species that had a higher association to each microhabitat (i.e., the shorter the distance away and sharper the angle in relation to the microhabitat) are shown in the colour shaded areas (grey, yellow, red and green). Canopy patches had a higher number of positively associated species compared to intercanopy spaces. High infection canopy patches also had a higher number of associated species compared to the other microhabitats. However, species that shared greater angles with each microhabitat and/or species and microhabitats that were on the opposite sides of each other from the point of origin had a negative relationship (e.g., high mistletoe-infection canopy patches and *V. sieberiana, V. tortilis, S. nigrescens*). Species that were associated with two microhabitats fell on the vector lines that link the microhabitats, and canopy patch microhabitats had more species that were associated with them. Species that were found in relatively similar abundances in all the microhabitats and/or those whose abundances were not differentiated by microhabitats occur near the centre (e.g., *D. eriantha* and some morphospecies).

Species affinity to different microhabitats

The results supported the expectation that the affinity of species would be highest within the canopy patches compared to the intercanopy spaces. There were 27 (34%) species (including some

morphospecies) that showed a strong affinity to canopy patches (>90% of occurrences), whilst intercanopy spaces had 7 (9%) species that had strong affinity to them (<10% of occurrences). When comparing high infection canopy patches and intercanopy spaces, 24 (30%) species showed a strong positive affinity to high infection canopy patches, while 5 (6%) species showed a strong positive affinity to the high infection intercanopy spaces (Table 2, Fig. 2a).

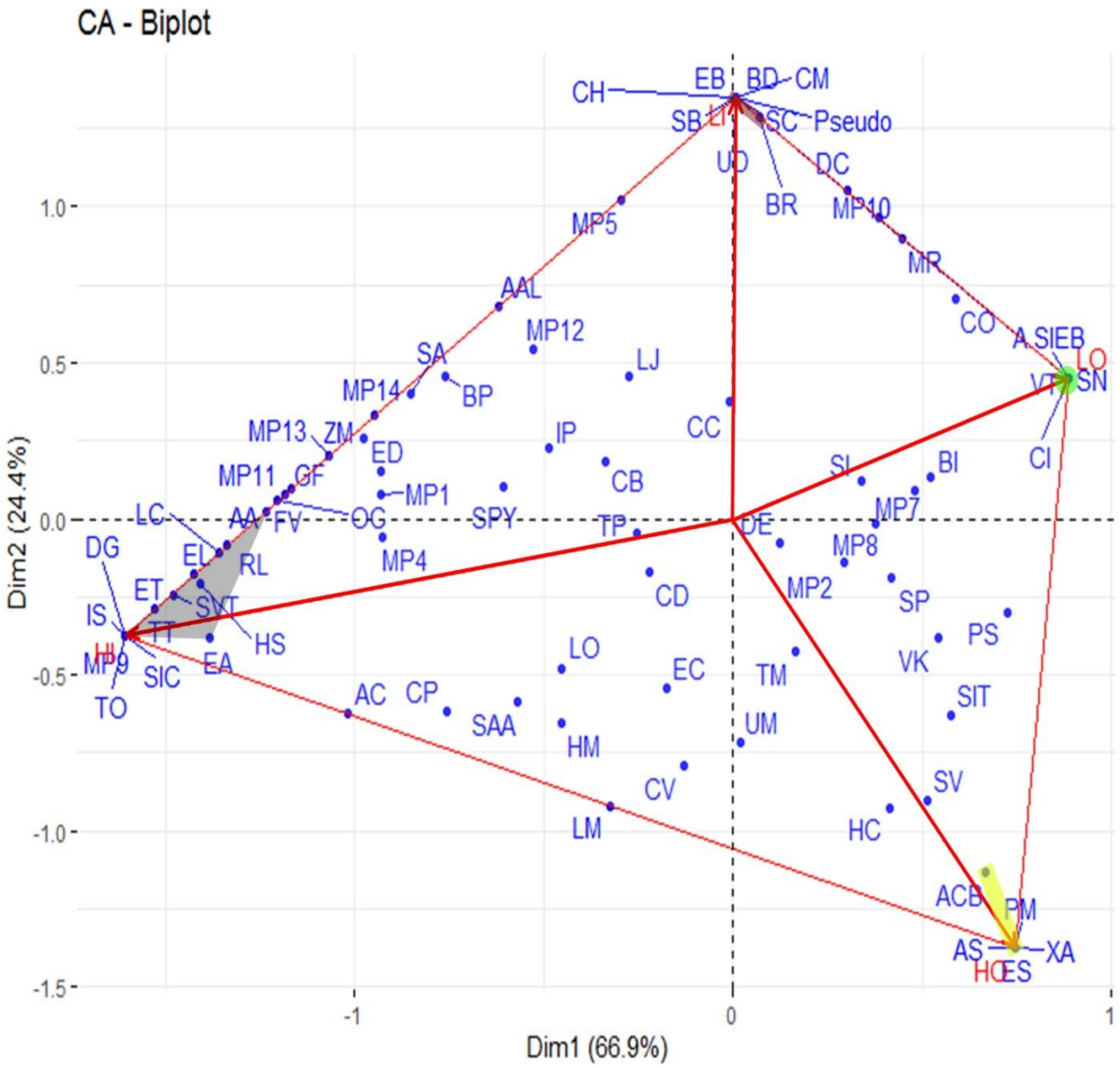


Fig. 1. Correspondence analysis biplot (first two axes explaining 91.3% of total variation), showing the affinity of each species to the four microhabitats. HI- high mistletoe-infection tree canopy patches; HO-high mistletoe- infection intercanopy spaces; LI- low mistletoe- infection tree canopy patches; LO- low mistletoe- infection intercanopy spaces (taxa codes are shown in Table 1). Positions of the species are shown in blue, whilst the microhabitats are shown using red arrows. The grey, yellow, red and green shaded sections show species highly associated with each microhabitat.

Similarly, 24 (30%) species showed a strong positive affinity to low mistletoe-infection canopy patches, whilst 7 (9%) species showed a high positive affinity with low mistletoe-infection intercanopy spaces (Fig. 2b). When species affinity was compared for high- or low mistletoe-infection canopy patches, 12 (15%) species showed a strong positive affinity towards high infection canopy patches, whilst 8 (10%) species showed a strong positive affinity to low infection canopy patches (Fig. 2c). Additionally, on comparing high- and low mistletoe-infection intercanopy spaces, 11 (14%) species showed a positive affinity to high infection intercanopy spaces and 7 (9%) species showed a high affinity to low infection intercanopies (Table 2, Figure 2d).

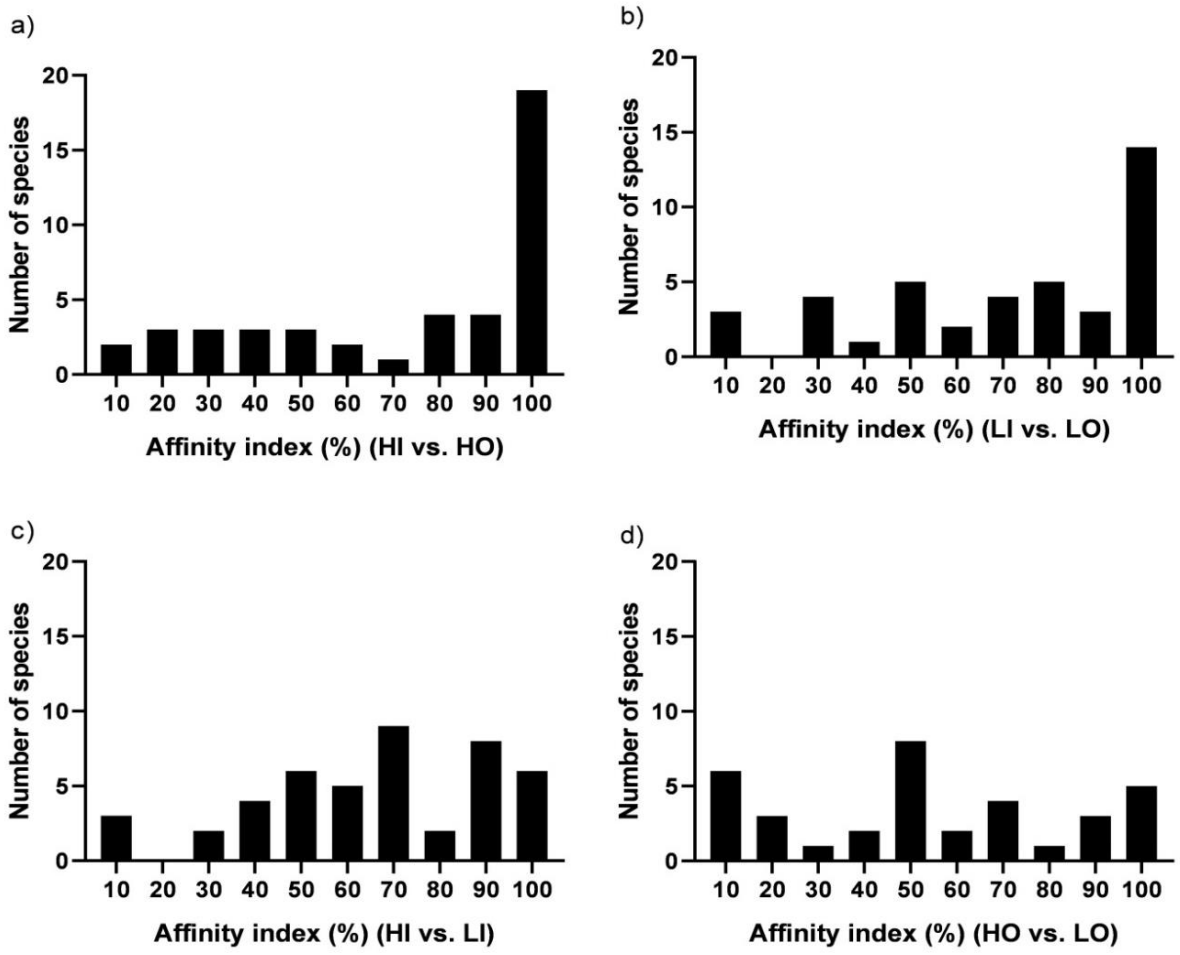


Fig. 2. Pair-wise comparisons of the distribution of the affinity index of species in the four microhabitats, the higher the affinity index the more it is related to a microhabitat.

Species diversity indices

Rarefaction curves for each microhabitat reached their asymptotes (except for low mistletoe-infection canopy patches) indicating that sampling was sufficient (Fig. 3). The ANOSIM results for the comparisons of all the four microhabitats combined showed that there was a significant difference in the species composition (R = 0.19, *P* = 0.001). The pair-wise comparisons showed significant dissimilarity (Table 3) and each microhabitat pair had an ANOSIM R statistic of < 0.35 and *P* = 0.01, showing that each microhabitat had a distinct species composition.

Table 2: Pair-wise comparisons using Fisher exact test of the affinity (mistletoephily index) of each plant species to high versus low mistletoe-infected canopy patches and intercanopy spaces. The index percentage shows the percentage of individual species abundances between the two microhabitats. Empty spaces represent species with <5 individuals, whilst NS represents no significant differences ($P > 0.05$). * $P < 0.05$, ** $P < 0.01$, ***$P < 0.001$.

	Canopy vs. Intercanopy				**Infection high vs low**			
	High infection		**Low infection**		**Canopy patch (CP)**		**Intercanopy (IC)**	
Species	**Affinity**	**Index (%)**	**Affinity**	**Index (%)**	**Affinity**	**Index (%)**	**Affinity**	**Index (%)**
Grasses								
Aristida congesta barbicollis	IC***	4	IC***	0	H***	100	H***	90
Aristida congesta congesta								
Aristida scabrivalis	IC***	0					H***	100
Bothriochloa insculpta	IC***	5	NS	47	L***	5	NS	51
Cenchrus ciliaris	CP***	93	IC***	31	NS	57	L***	4
Chloris pycnothrix	CP*	67			H***	97	H**	89
Chloris virgata	IC***	40	NS	37	H***	91	H***	89
Cynodon dactylon	NS	53	CP***	68	H***	59	H***	73
Dactyloctenium giganteum								
Digitaria eriantha	NS	41	NS	46	NS	49	NS	54
Eragrostis aspera	CP***	94	IC*	31	H***	99	H*	65
Eragrostis biflora			CP***	100	L***	0		0
Eragrostis curvula	NS	47	IC***	35	H***	83	H***	75
Eragrostis lehmanniana	CP***	100	CP*	100	H***	89		
Eragrostis superba	IC***	0		0			H***	100
Heteropogon contortus	IC***	15	NS	49	H***	64	H***	91
Heteropogon melanocarpus	NS	55	NS	55	H***	90	H***	90
Melinis repens			NS	50				
Morphospecies1	CP*	89			NS	67		
Panicum maximum	IC***	0		0			H***	100
Pogonarthria squarrosa	IC***	10	IC***	0			NS	41
Setaria incrassata	IC***	29	IC***	42	L***	30	L***	43
Setaria pumila	IC***	27	IC***	22	NS	52	NS	46
Setaria verticillata	CP***	100	CP***	100	H***	92		
Sorghum versicolor	IC***	13	IC***	5	H***	92	H***	79
Sporobolus ioclados								
Sporobolus pyramidalis	CP***	80	CP***	76	H***	62	NS	56
Themeda triandra								
Urochloa mosambicensis	IC***	38	IC***	7	H***	96	H***	75
Forbs								
Achyranthes aspera	CP***	100	CP***	100	L***	39		
Asparagus africanus	CP***	100	CP***	100	H***	77		
Berkheya radula			CP***	93	L***	0	L***	0
Bidens pilosa	CP***	97	CP***	97	NS	50	NS	53
Commelina benghalensis	CP***	74	NS	63	NS	54	NS	42

Corchorus olitorius	NS	17	IC**	30	L***	5	L***	10
Hibiscus sidiformis	CP***	99	CP***	100	H***	89	H**	100
Indigofera schimperi	CP***	100			H*	100		
Ipomoea plebeia	CP***	71	CP***	95	NS	47	H**	88
Lantana camara	CP***	100	CP***	100	H***	85		
Leucas martinicensis	NS	45			H*	100	H*	100
Lippia javanica	CP***	76	CP***	77	L***	38	NS	40
Lippia oatessi	NS	55	CP***	100	H*	74	H***	100
Morphospecies2	NS	43	NS	13			NS	36
Morphospecies3								
Morphospecies4	CP***	84	CP***	100	H***	72	H**	100
Morphospecies5	CP***	100	CP***	100	L***	19		
Morphospecies6								
Morphospecies7	IC***	21	IC***	31	L***	27	L***	39
Morphospecies8								
Morphospecies9	CP***	100			H*	100		
Morphospecies10			NS	57				
Oxalis corniculata								
Schkuhria pinnata								
Senna italic	IC***	13	IC***	5	H***	84	H***	64
Sida alba	CP***	98	CP***	100	NS	54	H*	100
Solanum aculeastrum	CP***	60	CP***	64	H***	89	H***	91
Solanum catombelense			CP**	100	L***	0		
Tagetes minuta	IC***	32	NS	41	H*	61	H***	70
Tagetes patula	NS	57	NS	23	H*	80	NS	47
Taraxacum officinale	CP***	100			H***	100		
Woody plants								
Berchemia discolor					NS	0		
Combretum hereroense			CP***	100	L***	0		
Combretum imberbe								
Combretum molle								
Dichrostachys cinerea			NS	67	L*	0		
Elaeodendron transvaalense	CP***	100		100	H***	95		
Euclea divinorum	CP***	100	CP**	70	H***	72	L***	0
Flueggea virosa	CP***	100	CP***	100	H***	75		
Grewia flavescens	CP***	100	CP***	100	H***	73		
Morphospecies11	CP***	100	CP***	100	H***	74		
Morphospecies12	CP***	98	CP***	81	L**	45	L***	7
Morphospecies13	CP***	100	CP**	100	NS	67		
Morphospecies14	CP***	100	CP***	100	NS	59		
Pseudolachnostylis maprouneifolia			CP***	100	L***	0		
Rhus lancea	CP***	100	CP***	100	H***	83		
Sclerocarya birrea								
Senegalia nigrescens			IC***	0			L***	0
Vachellia karroo	IC***	13	IC***	22	L*	43	H***	58

Vachellia sieberiana			IC***	0			L***	0
Vachellia tortilis								
Ximenia americana								
Ziziphus mucronata	CP***	99	CP***	96	H***	63	L***	19

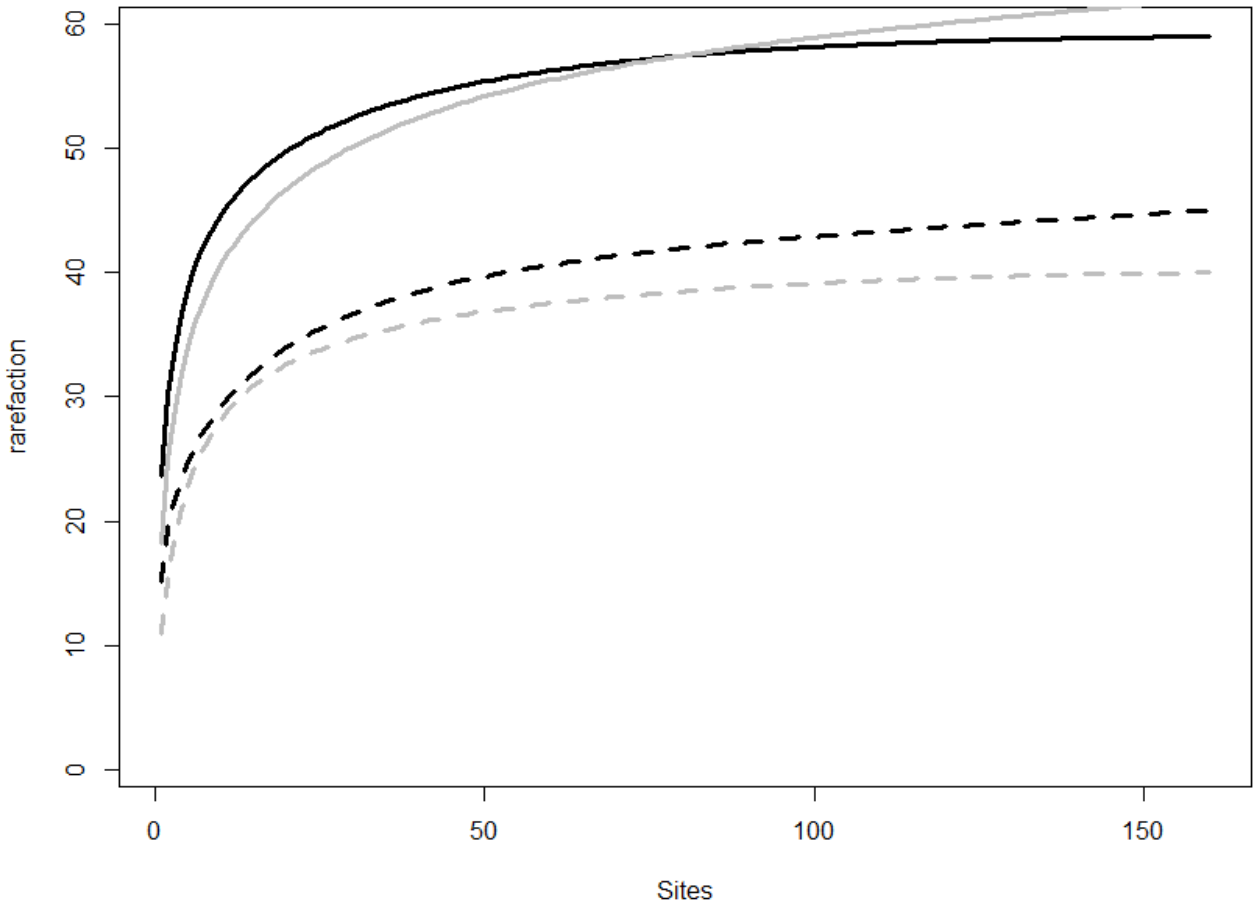


Fig. 3. Rarefaction curves for species assemblages for high mistletoe-infection canopy patches (solid–black), high infection intercanopy spaces (black-dashed), low mistletoe-infection canopy patches (solid-grey), low infection intercanopy spaces (grey-dashed). Values represented as rarefaction are the expected species richness for each microhabitat and their standard deviation derived from sampling individuals.

Table 3: Pairwise comparisons of species composition among the four microhabitats using ANOSIM. Values in bold indicate significant differences ($P < 0.05$).

Pairwise comparisons	ANOSIM R	*P*
High infection canopy patches *vs*. High infection intercanopy spaces	0.32	**0.01**
Low infection canopy patches *vs*. Low infection intercanopy spaces	0.11	**0.01**
High infection canopy patches *vs*. Low infection canopy patches	0.24	**0.01**
High infection intercanopy spaces *vs*. Low infection intercanopy spaces	0.04	**0.01**

Effect of canopy and mistletoe-infection on species diversity indices

Canopy *vs*. intercanopy had the most significant effects on species richness and diversity (highest F values), but infection level was most significant for evenness. The number of individuals/m^2 between canopy patch microhabitats (97.03±1.17) and intercanopy space microhabitats (99.38±1.17), and amongst high- (98.16±1.17) and low mistletoe-infection microhabitats (98.25±1.17) was not significantly different ($P > 0.05$; Table 4). Species richness/m^2 was 1.64-fold higher within canopy patches (8.91±0.14) compared to the respective intercanopy space microhabitats (5.44±0.14). High mistletoe-infection tree microhabitats (8.17±0.14) were 1.32-fold more species-rich compared to the corresponding low mistletoe-infection tree microhabitats (6.18±0.14). However, mistletoe-infection was the only factor that significantly influenced the species evenness/m^2, with higher species evenness (by 5%) in high mistletoe-infection microhabitats (0.60±0.01) compared to low mistletoe-infection microhabitats (0.57±0.01). Species diversity/m^2 was 1.47-fold higher in canopy patches (1.56±0.02) compared to intercanopy spaces (1.06±0.02). Species diversity/m^2 was also 1.26-fold higher within high mistletoe-infection microhabitats (1.46±0.02) compared to low mistletoe-infection microhabitats (1.16±0.02).

Table 4: General linear model (GLM) analyses of the effects of canopy (canopy *vs*. intercanopy), mistletoe infection (high *vs.* low), and their interactions on species abundance, richness, evenness and Shannon–Wiener diversity index at the 1 m^2 scale. Values in bold show significant effects ($P < 0.05$), $df = 1,636$ in every case.

Variable	Effects					
	Canopy Canopy *vs*. Intercanopy		Infection High *vs*. Low		Canopy × Infection	
	F	Sig.	F	Sig.	F	Sig.
Abundance	2.01	0.156	0.004	0.947	0.09	0.760
Richness	301.24	**<0.001**	98.43	**<0.001**	26.06	**<0.001**
Evenness	0.17	0.684	6.74	**0.010**	2.61	0.107
Shannon diversity	253.06	**<0.001**	91.45	**<0.001**	18.85	**<0.001**

Species diversity indices across the four microhabitats

The number of individuals/m^2 in each of the four microhabitats was not different (Kruskal-Wallis $\chi^2 = 1.47$, $df = 3$, $P = 0.689$; Fig. 4a). The results supported the hypothesis that species richness, evenness, and diversity would be highest below canopies of high mistletoe-infected trees. High mistletoe-infection canopy patches had 52% higher species richness than low mistletoe-infection intercanopy spaces which had the lowest species richness (Kruskal-Wallis $\chi^2 = 255.49$, $df = 3$, $P < 0.001$; Fig. 4). Species evenness was 8% higher in high mistletoe-infection canopy compared to low mistletoe-infection canopy patches which had the lowest species evenness (Kruskal-Wallis $\chi^2 = 11.44$, $df = 3$, $P = 0.010$, Fig. 4c). High mistletoe-infection canopy patches were between 25% to 45% more diverse compared to the other microhabitats (Kruskal-Wallis $\chi^2 = 229.17$, $df = 3$, $P < 0.001$, Fig. 4d).

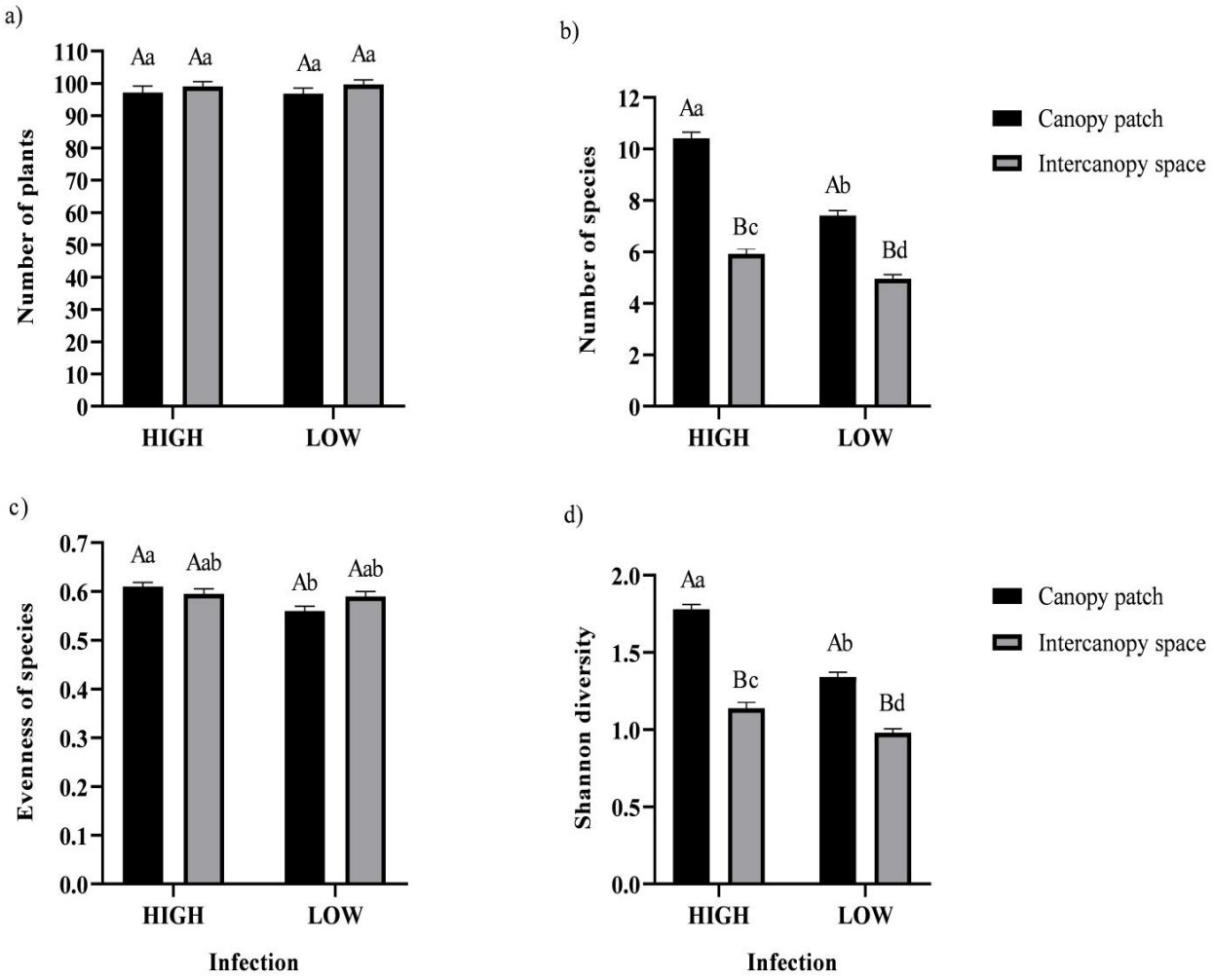


Fig. 4. Comparisons of mean (± SE) abundance (a), richness (b), evenness (c), and (d) Shannon-Wiener diversity index at the 1 m^2 scale among the four microhabitats. Capital letters show differences within infection microhabitats (canopy *vs*. intercanopy). Lower-case letters show differences among the four microhabitats (Kruskalmc, $P < 0.05$).

Comparison of species diversity for each growth form (grass, forbs, trees)

Grass diversity was between 13% and 29% higher within high mistletoe-infection canopy patches compared to the other three microhabitats (Kruskal-Wallis $\chi^2 = 70.47$, $df = 3$, $P < 0.001$, Fig. 5a). Likewise, forb diversity was between 35% to 75% higher (Kruskal-Wallis $\chi^2 = 163.70$, $df = 3$, $P < 0.001$; Fig. 5b) within high mistletoe-infection canopy patches than the other microhabitats, both intercanopy microhabitats had the lowest forb diversity. Canopy patches of high mistletoe-infected trees had 43% to 99% more tree diversity compared to the intercanopies of both high and low mistletoe-infected trees, which had the lowest tree species diversity (Kruskal-Wallis $\chi^2 = 163.7$, $df = 3$, $P < 0.001$; Fig. 5c).

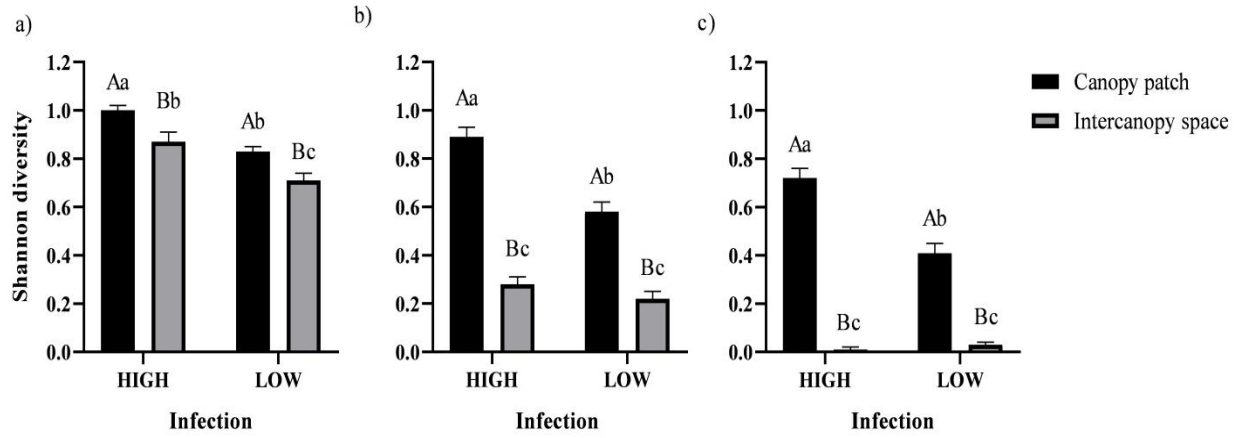


Fig. 5. Comparisons of grass (a), forb (b) and tree (c) Shannon–Wiener diversity index among four microhabitats. Capital letters show differences within infection microhabitats (canopy patch vs. intercanopy spaces). Lower-case letters show differences among the four microhabitats (Kruskalmc, $P < 0.05$).

Functional diversity indices across the four microhabitats

Effect of canopy and mistletoe-infection on functional diversity indices

The comparisons between canopy versus intercanopy microhabitats showed larger effects on functional diversity indices compared to the degree of infection (Table 5). Functional diversity indices were significantly higher within canopy patches (FDis: 0.15±0.003; FEve: 0.61± 0.01; FRic: 0.13±0.004; RaoQ: 0.03±0.001) than intercanopy patches (FDis: 0.07±0.003; FEve: 0.46±0.01; FRic: 0.10±0.004; RaoQ: 0.02±0.001). High mistletoe-infection microhabitats (FDis: 0.15±0.003; FEve: 0.55±0.01; FRic: 0.13±0.004; FDiv: 0.71±0.01; RaoQ: 0.03±0.001) had higher functional diversity compared to low mistletoe-infection microhabitats (FDis: 0.10±0.003; FEve: 0.51±0.01; FRic: 0.10±0.004; FDiv: 0.69±0.01; RaoQ: 0.02±0.001).

Table 5: General linear model (GLM) analyses of the effects of canopy (canopy *vs*. intercanopy), mistletoe infection (high *vs.* low), and their interactions on functional dispersion (FDis), evenness (FEve), richness (FRic), divergence (FDiv), and Rao's quadratic entropy (RaoQ). Values in bold show significant effects ($P < 0.05$) df = 1,636 in every case.

	Effects					
FD indices	Canopy (Canopy *vs*. Intercanopy)		Infection (High *vs*. Low)		Canopy × Infection	
	F	Sig.	F	Sig.	F	Sig.
FDis	322.34	**<0.001**	39.76	**<0.001**	14.96	**<0.001**
FEve	90.55	**<0.001**	7.31	**0.01**	0.02	0.89
FRic	29.43	**<0.001**	32.11	**<0.001**	2.89	0.09
FDiv	2.82	0.09	1.97	0.16	1.35	0.25
RaoQ	244.30	**<0.001**	32.15	**<0.001**	13.08	**<0.001**

Functional diversity indices acrosss the four microhabitats

High mistletoe-infection canopies displayed 59% higher FDis compared to low mistletoe-infection intercanopy spaces (Kruskal-Wallis $\chi^2 = 236.71$, $df = 3$, $P < 0.001$; Fig. 6a). FEve was 24% to 30% lower within intercanopy spaces compared to canopy patches (Kruskal-Wallis $\chi^2 = 97.25$, $df = 3$, $P < 0.001$; Fig. 6b). High mistletoe-infection canopy patches had the highest FEve (0.63±0.01), whilst low mistletoe-infection intercanopy spaces had the lowest FEve (0.44±0.02). Likewise, FRic was between 27% to 47% greater in high mistletoe-infection canopy patches than all the other microhabitats (Kruskal-Wallis $\chi^2 = 64.87$, $df = 3$, $P < 0.001$; Fig. 6c). Similarly, high mistletoe-infection canopies (0.04±0.001) had 26% to 59% higher RaoQ (Kruskal-Wallis $\chi^2 = 205.84$, $df = 3$, $P < 0.001$) compared to low mistletoe-infection canopies (0.03±0.001), and high- and low mistletoe-infection intercanopy spaces (0.02±0.001, 0.01±0.001, respectively). However, FDiv did not significantly vary (Kruskal-Wallis $\chi^2 = 3.29$, $df = 3$, $P = 0.350$; Fig. 6d) across the four microhabitats.

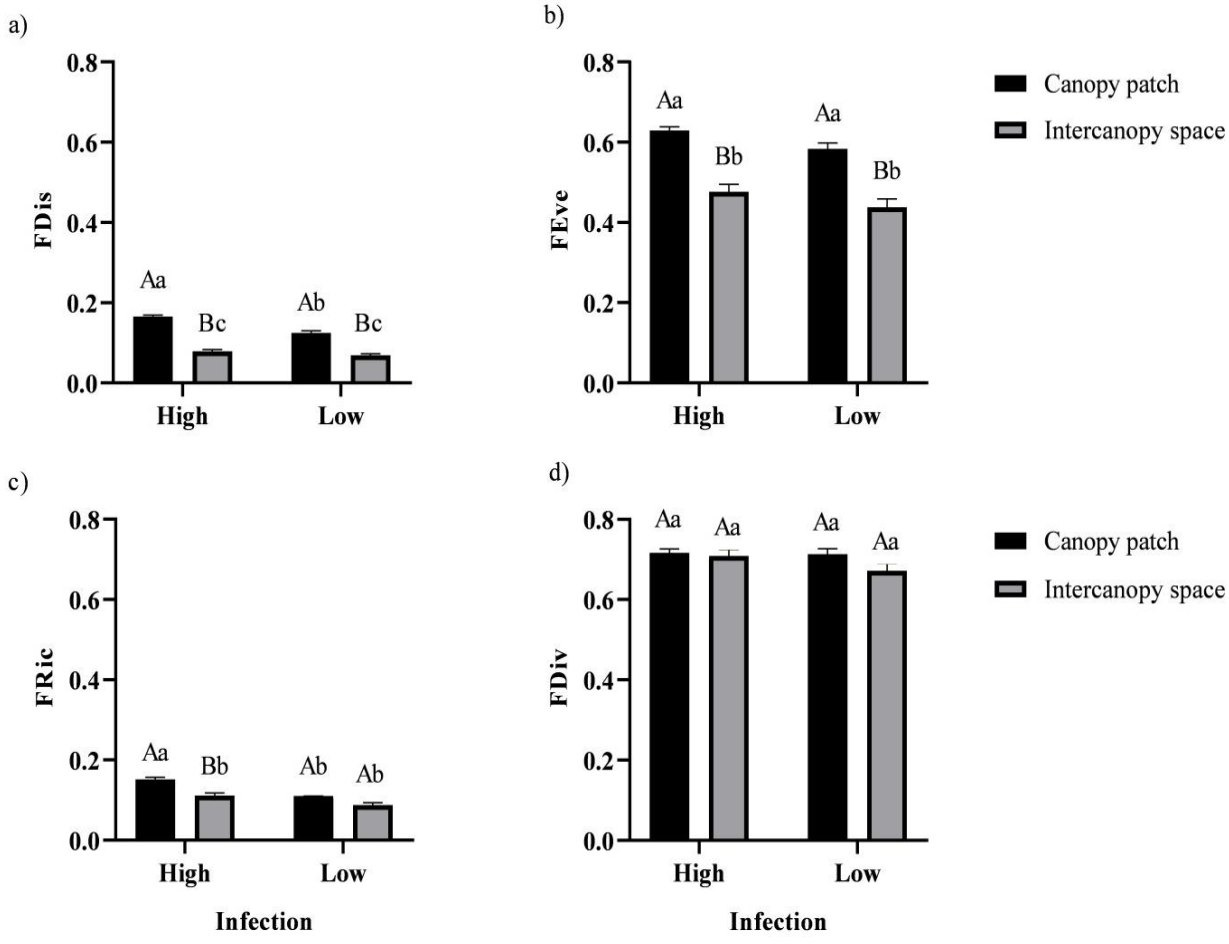


Fig. 6. Comparisons of (a) functional dispersion (FDis), (b) evenness (FEve), (c) richness (FRic) and (d) divergence (FDiv) between the four microhabitats (mean ± standard error). Capital letters show differences within infection microhabitats (canopy *vs*. intercanopy). Lower-case letters show differences among the four microhabitats (Kruskalmc, $P < 0.05$)

Relationship between species diversity and functional richness

Species diversity and functional richness were positively correlated (Spearman's rank correlation: r_s = 0.48, $P < 0.001$) within high mistletoe-infection canopy patches (Appendix 1), but not the other microhabitats. Furthermore, species diversity and functional diversity were not significantly correlated in the other microhabitats ($P > 0.05$).

Specific trait measurements

Contrary to the predictions that there would be significant differences in growth and leaf measurements of *Z. mucronata*, most of the mean trait measurements did not differ between high- and low mistletoe-infection subcanopies. However, height and chlorophyll content were significantly higher by 27% and 6%, (respectively) within high- compared to low mistletoe-infection canopy patches (Table 6).

Table 6: *Ziziphus mucronata* trait measurements (mean ± standard error) within high- and low mistletoe-infection tree canopy patches. Numbers in bold indicate significant differences ($P < 0.05$).

Trait	**Mistletoe-infection**		***P*. value**
	High	Low	
Height (cm)	23.34±2.34	17.03±0.39	**0.04**
Stem Diameter (cm)	0.36±0.05	0.33±0.04	0.11
Canopy area (CA) (cm^2)	215.3±2.95	197.0±2.54	0.50
Leaf area (LA) (cm^2)	7.42±0.83	6.60±0.74	0.47
Specific Leaf Area (SLA) ($m^2.kg^{-1}$)	2.03±0.13	1.77±0.12	0.18
Leaf Dry Matter Content (LDMC) ($mg.g^{-1}$)	543.2±12.29	552.0±15.26	0.65
Whole Leaf Thickness (µm)	276.8±1.05	328.1±1.56	0.14
Chlorophyll content	33.52±0.06	31.41±0.07	**0.001**

Discussion

A proliferation in degree of mistletoe infections on a tree resulted in changes in species composition and functional traits, increases in species richness and diversity, and plant sizes, consequently leading to greater spatial variability of plant assemblages. There were even larger differences between subcanopy and intercanopy microsites in species and functional diversity indices compared with infection degree. This suggests that canopy effects are more important in determining overall vegetation composition and structure, whilst mistletoe-infection effects are additive to the already existing spatial heterogeneity in these semi-arid savanna systems. Canopy patches also had significantly higher species richness, diversity and a greater number of species that had a strong affinity to them compared to the intercanopy spaces.

Species composition and diversity

High soil fertility and the weakening of mistletoe-infected *V. karroo* trees could have increased species diversity by supporting growth of a diverse suite of understory competitors within the canopy patches compared to intercanopy spaces (Ndagurwa *et al*., 2020; Monteiro *et al*., 2020; Chapter 2).

Furthermore, birds and animals visiting the mistletoe-infected trees could have also transported and deposited seeds from other trees, forbs and grasses into the canopy patches, thus increasing species diversity (Dean, *et al*., 1999; Mellado *et al*., 2016; Mellado and Zamora, 2016; Hódar *et al*., 2018). Grasses and forbs such as *S. verticillata* and *B. pilosa* easily attach to animal fur, resulting in high levels of seed deposition under the tree canopies (Dean *et al*., 1999), particularly under the frequently visited high mistletoe-infection trees. Therefore, shading from tree canopies, seed deposits, and availability of limiting resources, could have led to niche complementarity and competitive inclusion of slower growing species such as some of the forb and tree species (Spasojevic and Suding, 2011; Ndagurwa *et al*., 2013; Ramalho *et al*., 2018).

Intercanopy spaces were dominated by grasses with average to high grazing value (*S. incrassata* and *H. contortus*) indicating that these spaces were less disturbed by livestock (Sankaran *et al*., 2008; Chapter 2). Similarly, low mistletoe-infection microhabitats had a higher abundance of high grazing value grass species compared to high infection microhabitats that had mixed species ranging from low-high grazing value (Van Oudtshoorn, 2014). Higher abundance of decreaser grasses within low mistletoe-infection microhabitats is associated with low-intermediate disturbance (Proulx and Mazumder, 1998; Kioko *et al*., 2012; Solbrig, *et al*., 2017; Chapter 2) and intermediate disturbance often results in higher species diversity (Peterson and Reich, 2009; Török *et al*., 2016). However, high mistletoe-infection canopy patches had the highest species diversity despite having greater animal disturbance (Chapter 2). This could be due to higher soil fertility as shown by the presence of decreaser grasses (*S. incrassata, D. eriantha, C. ciliaris* and *T. triandra*) (Sankaran *et al*., 2008; Riginos, 2009; Van Oudtshoorn, 2014; Seymour *et al*., 2016). Regardless, high mistletoe-infection canopy patches had a high occurrence of grasses such as *S. pyramidalis, E. curvula* and forb invasives such as *Bidens pilosa, Sida alba*, and *Lantana camara* which are indicators of disturbed sites. The presence of grasses that can tolerate trampling and defoliation (e.g., *C. dactylon,* and *S. verticillata*) within high infection canopy patches could be demonstrating the negative impacts of long-term animal disturbances (Davies *et al*., 1998; Muvengwi *et al*., 2017). In this regard, high infection canopy patches may have the potential for an even greater species diversity than observed in this study, but higher animal disturbances could have led to the loss of some unique species (Seymour *et al*., 2016). Consequently, if overgrazing is not monitored, there may be long-lasting negative effects on species composition and forage quality (Kioko *et al*., 2012).

The abundance of grasses compared to other growth forms in the intercanopy spaces could result from them being superior better competitors in spaces with lower nutrients and water, and also being better adapted to higher fire intensity in the open. Munzbergova and Ward (2002) and Linstädter *et al*. (2016) also reported low species richness and diversity within the intercanopy spaces of *Acacia* trees and *Vachellia bussei* respectively, compared to their canopy patches. Moreover, high grass productivity (Chapter 2) could have also led to a decline in species richness due to exclusion of

woody plants that rely on light for growth but are killed by more intense fires with the higher fuel load in the intercanopy (Suding *et al*., 2005; Riginos, 2009).

Intercanopy spaces also have higher temperatures and lower moisture contents, conditions that can significantly lower the abundances of many plant species. Subsequently, intercanopy spaces were mostly dominated by grasses that preferred full sunlight, grasses such as *H. contortus* that are water efficient (Van Oudtshoorn, 2014) and *V. karroo* juveniles. Similarly, the dominance of grasses and *V. karroo* juveniles within intercanopy spaces has also been reported by Proulx and Mazumder (1998). Leguminous species favour areas with significantly higher concentrations of available soil phosphorus (Hódar *et al*., 2018). However, mistletoe-infected tree canopy patches have even higher concentrations of soil nitrogen, phosphorus and potassium combined (Suding *et al*., 2005; Muvengwi *et al*., 2015; Mellado *et al*., 2016; Ndagurwa *et al*., 2016; Hódar *et al*., 2018). Therefore, *V. karroo* juveniles could have been outcompeted by other growth forms within canopy patches (Hódar *et al*., 2018), thus their higher abundance in the intercanopy spaces. Regardless, higher presence of trees in both high and low mistletoe-infection canopy patches could mean that some tree species were capable of out-competing grasses (Davies *et al*., 1998) due to the canopy patch conditions. Eventually, if there is an increase in understory tree density coupled with tree root biomass which accelerates competition for soil resources, grasses are less likely to flourish within canopy patches (Treydte *et al*., 2010; Randle *et al*., 2018).

Studies have also shown that due to higher availability of nutrients, mistletoe-infected trees have different species assemblages and higher diversity compared to uninfected trees (Ndagurwa *et al*., 2016, 2018; Hódar, *et al*., 2018; Monteiro *et al*., 2020). Similarly, high- relative to low mistletoe infection canopy patches had higher soil moisture content, temperature, and litter contents (Chapter 2) which are associated with higher microbial activity, accelerated litter decomposition, and elevated nutrient cycling rates (Solbrig *et al*., 2017; Al-Rowaily *et al*., 2020; Ndagurwa *et al*., 2020). These conditions boost productivity of different growth forms and potentially mask negative effects that arise within high mistletoe-infection canopy patches. This is shown by the higher affinity and abundance of species such as *S. pyramidalis, A. africanus, Z. mucronata* and *F. virosa* for these patches. These species are usually found either on soil with higher moisture content or on nutrient-rich patches, including termite mounds (Van Oudtshoorn, 2014; Muvengwi *et al*., 2016).

Higher species richness and diversity in high mistletoe-infection canopy patches can also be attributed to a change in the canopy structure (Mellado *et al*., 2016) which may have increased light incidence (Mellado and Zamora, 2017). Consequently, these conditions could have led to the high affinity (mistletophily) of herbaceous species that are tolerant to the semi-shaded high mistletoe-infection canopy patches (Hódar *et al*., 2018) leading to the successful establishment of unique species such as *C. dactylon, E. lehmanniana, T. triandra, S. ioclados, and D. aegyptium*. The results in Chapter 2 also

show that in contrast to low mistletoe-infected trees, high mistletoe-infection trees were larger, thus, providing a greater spatial area for a variety of plants to establish (Ludwig *et al*., 2004; Treydte *et al*., 2009; Linstädter *et al*., 2016; Ndagurwa *et al*., 2018). These variations in resources and space show the importance of varying mistletoe infection intensities in ensuring plant species heterogeneity.

Functional diversity

The prediction that high species diversity and richness would lead to high FRic is partially supported in this study. Canopy patches had higher FRic, FDis and RaoQ compared to their intercanopy spaces. Similarly, Mitchell and Bakker (2016) found that forb-rich microhabitats had higher FRic compared to grass-rich microhabitats. Certainly, high nutrient availability underneath tree canopy patches could have increased niche occupancy, thus increasing FRic and FDis (Joseph *et al*., 2015; Schellenberger *et al*, 2017). Consequently, due to the presence of different growth forms, there is high dissimilarity within canopy patches, which shows that resources within the fertility islands are being efficiently utilized even in the presence of competition due to spatio-temporal partitioning (Cooke *et al*., 2019). Such partitioning ensures a more complete range of nutrient acquisition strategies and niche complementarity which significantly improves the canopy patch communities' stability and responses towards environmental and animal disturbances compared to intercanopy spaces (Schirmel *et al*., 2012; Mason *et al*., 2013; Ibarra and Martin, 2015; Mitchell and Bakker, 2016; Cooke *et al*., 2019).

Although canopy patches (high *vs*. low mistletoe-infection) had almost the same number of species and higher FRic and RaoQ, the species assemblages were different and their trait attributes varied, thus they could be functionally different communities (Spasojevic and Suding 2012; Laliberté *et al*., 2013). Additionally, grazing within the canopy patches compared to the intercanopy spaces could have produced gaps for subordinate species to grow alongside the dominant species hence increasing coexistence of species and the functional trait variation (Proulx, and Mazumder, 1998; Grime, 2006; Laliberté *et al*., 2013; Seymour *et al*., 2015). However, RaoQ may also increase with a decrease in grazing intensity (Török *et al*. 2016). Therefore, it is expected that over time, persistently grazed areas will eventually support species with traits that are better adapted to heavy disturbances and shaded areas (habitat generalists). This is to the detriment of those that take longer to re-establish under high disturbances within the shaded patches (habitat specialists) thereby altering the plant assemblages and functional richness within the semi-arid savanna (Ibarra and Martin, 2015).

Intercanopy spaces had lower FRic and FDis which can be attributed to environmental filtering caused by high temperatures and low moisture availability. Therefore, only species with traits that could survive in open spaces were supported leading to the dominance of one growth form (and species with similar traits), and thus lower species diversity (Joseph *et al.*, 2015; Seymour, *et al*., 2015; Cooke *et al*., 2019). Lower species diversity, FRic, and FDis could also have led to poor microhabitat occupation and resource utilisation. Such communities are often adversely affected by

stochastic events, and they are also susceptible to invasions by invasive species, non-native species, and weeds (Mason *et al*., 2005; Joseph *et al*., 2015; Suter *et al*., 2017). For instance, Suter *et al*. (2017) found that in mixtures of ley (grass) species with high dispersion there was effective weed suppression and this was attributed to species having different plant functional traits. Consequently, increasing the number of species with varying traits on a given functional niche space leaves little or no space for weeds (Suter *et al*., 2017). Further, the lower FRic in intercanopy spaces could have been due to the high biomass within these spaces. According to Laliberté *et al*. (2013), areas with high productivity often have lower FRic, and the biomass results show that there was higher productivity within intercanopy spaces (Chapter 2), hence the lower FRic.

The results also show that there was no difference in the FDiv across the four microhabitats but the values were high for all the microhabitats. Contrary to the expectations, FDiv in the intercanopy spaces did not show any trait convergence which is often linked to environmental filtering (Mitchell and Bakker, 2016; Ramalho *et al*., 2018). This suggests that grasses in intercanopy spaces are competing for the limited below ground resources; hence they could have been forced to use different methods of resource acquisition and utilisation leading to high trait divergence (Laliberté *et al*., 2013). Often, in areas with either high point of environmental variation, little competition for resources or proficient resource utilisation, high FDiv values are expected (Mason *et al*., 2005; Török *et al*., 2016). This could mean that in all the microhabitats, despite the differences in the species composition, plants were able to efficiently utilise resources. Furthermore, in high mistletoe-infection canopy patches, it was anticipated that high disturbance would mask the high resources in these patches, and only support species with traits that were able to adapt to animal disturbances, thus, resulting in trait convergence (Laliberté *et al*., 2013). However, FDiv was highest within high mistletoe-infection canopy patches. The reduction of host tree performance due to high mistletoe-infection could have increased the available niche space, enabling more new species to establish, and hence the higher FDiv (Press, 1998; Ndagurwa *et al*., 2016, 2018).

FEve was higher within the canopy patches compared to adjacent intercanopy spaces. Lower FEve implies that there is lower productivity, coupled with high functional homogeneity, and underutilisation of occupied niche space and this supports the FRic and FDis results obtained for the intercanopy spaces. These lower values could mean that there is high functional redundancy within intercanopy spaces (Magnago *et al*., 2014; Joseph *et al*., 2015). Therefore, in the event of a stochastic event resulting in the loss of one species, the high redundancy exhibited in the intercanopy space may improve the resilience of that community since the species are functionally similar (Magnago *et al*., 2014). In contrast, higher FEve implies that there is an even distribution of traits, functional heterogeneity and higher productivity and this has also been shown by canopy patches having higher FRic and FDis (Mason *et al*., 2005; Katovai *et al*., 2011; Magnago *et al*., 2014; Joseph *et al*., 2015;

Török *et al*., 2016).

Influence of canopy patch and mistletoe-infection extent on *Ziziphus mucronata*

Only two (height and chlorophyll content) of the eight traits assessed showed significant differences between high and low mistletoe-infection canopy patches. Saplings within high infection canopy patches were taller than those in low mistletoe-infection canopy patches. *Z. mucronata* within high mistletoe-infection canopy patches could have, rather invested in growth as a strategy to persist in the disturbed areas. In contrast, *Z. mucronata* plants within low mistletoe-infection canopy patches could have opted for defence mechanisms as shown by lower SLA and higher LDMC. These characteristics are associated with conservative resource use, slow growth, longer-lasting and tougher leaves with high leaf cellular components, thus resulting in low palatability due to greater investment in defence (Rodriquez *et al*. 2016; Yan *et al*., 2013; Zheng, *et al*., 2015; Niu *et al*., 2016; Török *et al*., 2016; Wehn *et al*., 2017; Cuma Mushagalusa *et al*., 2019).

High mistletoe-infection canopy patches tended to have plants with higher SLA, thinner leaves and low LDMC. Plants with these characteristics are associated with resource-rich patches (Yulin, *et al*., 2005). These plants have higher rates of photosynthesis and productivity and their leaves are less costly to produce and they have short lifespans (Rodriquez *et al*. 2016; Yan *et al*., 2013; Zheng, *et al*., 2015; Niu *et al*., 2016; Török *et al*., 2016; Wehn *et al*., 2017; Twala, 2019; Cuma Mushagalusa *et al*., 2019). This is supported by the significantly higher chlorophyll content which would have likely increased their photosynthetic potential and primary productivity. This could also further explain why high infection canopy patches had taller plants, compared to low mistletoe-infection canopy patch plants. Therefore, these plants could have had a greater tolerance towards herbivory and environmental stress, and more able to quickly recover after being grazed (Zheng, *et al*., 2015; Niu *et al*., 2016; Ferreira de Melo Junior and Boeger, 2016; Wehn *et al*., 2017). These findings could also be showing that *Z. mucronata* may opt for greater resource acquisition within high mistletoe-infection canopy patches, whilst in low mistletoe- infection tree canopy patches; it opts for a resource conservative strategy (Zheng *et al*., 2015).

Overall, these results suggest that differences in trait measurements may depend on the stage of growth, nutrient levels, animal disturbances or other environmental conditions (e.g., Ndagurwa *et al*., 2016; Mitchell and Bakker, 2016). In the future, it is suggested that more measurements are done on a range of different species, to have a broader scale appreciation of how the two patches influence plant growth. The lack of a significant difference between the plant traits of high and low mistletoe-infection canopy patches could mean that the differences in the nutrient levels in these patches were not sufficiently different (Yulin, *et al*. 2005), although this needs further investigation.

Conclusion

This study investigated how mistletoe infected trees influenced the understory species composition and function within and beyond their canopies in a semi-arid savanna. Mistletoe infection impacts are additive and they are highly centred on canopy presence. Consequently, they further enhance the effects that tree canopies have on the spatial heterogeneity of nutrients and vegetation structure and composition. These results show that all four microhabitats had significantly different species compositions. High mistletoe-infection canopy patches had higher species richness, FRic, FDis and FEve compared to the other microhabitats. This was attributed to higher availability of limiting resources and the semi-shade conditions within these canopy patches. This possibly makes these patches suitable environments for an array of functionally diverse species, thus increasing the resilience within these semi-arid savannas. However, higher occurrence of species that are associated with high animal disturbance within the resource-rich canopy patches could be an indication that they are also susceptible to a change in species composition towards unfavourable ones. Height and chlorophyll content were the only plant trait measurements that significantly differed between high- and low mistletoe-infection canopy patches. The differences were attributed to investment in growth and defence mechanisms for high and low mistletoe-infection canopy patches, respectively. It is also suggested that grazing could have influenced the variations within each trait.

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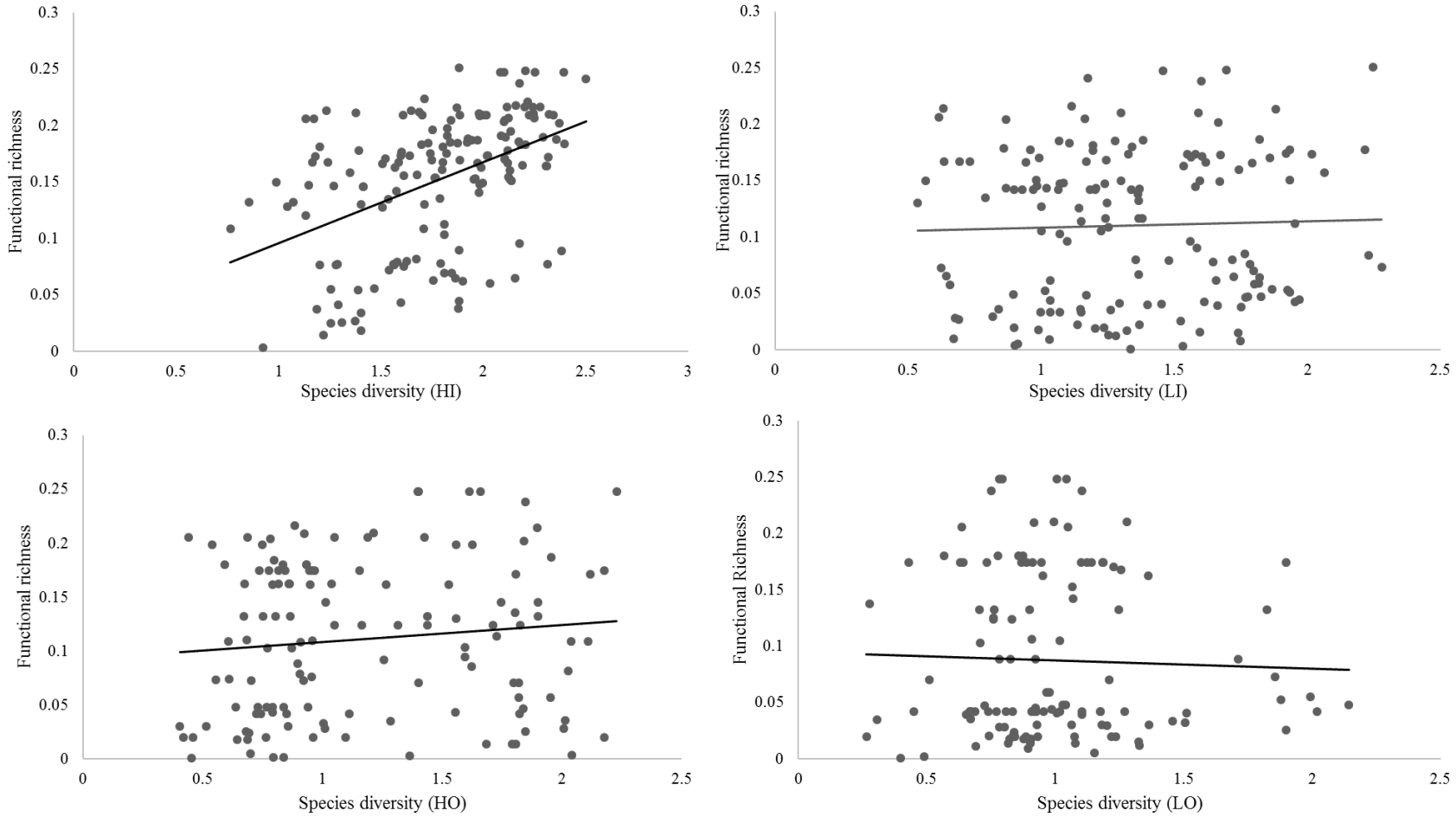
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Appendices

Appendix 1: Correlation between species diversity and functional richness in the four microhabitats. HI- high mistletoe-infection tree canopy patches; HO- high mistletoe- infection intercanopy spaces; LI- low mistletoe- infection tree canopy patches; LO- low mistletoe- infection intercanopy spaces.



CHAPTER 4

Mistletoes negatively impact the regeneration of *Vachellia karroo* trees in a semi-arid African savanna

Abstract

Studies in several environments have shown that mistletoe parasitism can reduce the growth and reproductive capacities of infected hosts. Yet, despite being common and abundant in semi-arid African savannas, the effects of mistletoe parasitism on the regeneration of preferred host tree species has not been fully investigated. Therefore, this study examined the effects of the degree of mistletoe infection (high *vs.* low) on the regeneration of *Vachellia* (*Acacia*) *karroo* trees over three consecutive years. There were a greater number of flower buds, flowers, pods, pod length and seeds/pod in low compared to high mistletoe-infection trees. Similarly, seed and germinable seed production/tree of high mistletoe-infected trees were reduced to only 32% and 20%, respectively, that of low mistletoe-infected trees. There were negative linear relationships between the numbers of flowers, pod length and number of seeds/pod, and seed and germinable seed production/tree with canopy area and number of mistletoes/tree. High mistletoe-infection trees had 13% and 46% more aborted and predated seeds, respectively, whilst low infection trees had 17% more intact seeds. Mass per intact seed was 19% lower for high- (0.030 ± 0.003g) than low mistletoe-infection trees (0.037 ± 0.003g). Consequently, seed and germinable seed production/tree were significantly lower in high- (2273 ± 820; 599 ± 216, respectively) than low mistletoe-infection trees (7088 ± 905; 2947 ± 376). The total percentage germination was 42% and 26% for low- and high mistletoe-infection trees, respectively, but overall germination rates were very similar (36.3 ± 2.5, 36.1 ± 2.6 days). Greenhouse germinated seedlings were 19% taller with 24% larger canopies for low- than high mistletoe-infection seedlings. The soil seedbank of high mistletoe-infection trees (14.4 ± 5.75 seed/m^2) was only 31% that of low infection trees (46.7 ± 10.71 seeds/m^2). The number of understory *V. karroo* juveniles was 72% higher below high- (relatively higher subcanopy light) compared to low mistletoe-infection canopies. Juvenile densities tended to be 1.47 to 2.86-fold higher in high mistletoe-infection canopy patches (1813 ± 528 juveniles/ha) compared to other microhabitats (633 ± 218 to 1236 ± 286 juveniles/ha). Similarly, seedling density was between 1.92 and 4.35-fold higher in high mistletoe-infection canopy patches (1275 ± 344 seedlings/ha) compared to other microhabitats (293 ± 160 to 663 ± 121 seedlings/ha).In contrast, sapling density tended to be 1.23 and 2.08-fold higher in low- and high mistletoe-infection intercanopy spaces (706 ± 343; 573 ± 199 saplings/ha, respectively), compared to their adjacent canopy patches (340 ± 142; 538 ± 201 saplings/ha, respectively). Understory *V. karroo* height, stem diameter, canopy area and volume were between 10-43% higher in low- than high-mistletoe-infection canopy patches. Overall, this study revealed that as mistletoe infection increases, flowers, pods, seed and germinable seed production/tree, seedbank density, and understory *V. karroo* juvenile size decreased, but the abundance of understory *V. karroo* juveniles increased. High mistletoe parasitism reduces the hosts' growth and reproductive capacity, but not its overall regeneration capacity, as shown by relatively higher seedling densities in the subcanopy, but greater sapling densities in the adjacent intercanopy spaces. This suggests that subcanopy patches are safe for seeds, but less safe for seedlings, which persist better and grow to sapling size predominantly in the intercanpy spaces. Increasing levels of mistletoe infection results in higher light, moisture and nutrients, with these effects increasing subcanopy plant growth of an increasingly different suite of species, resulting in higher competition, which may explain the smaller subcanopy *V. karroo* seedlings. Moreover, greater herbivory and competition may have also reduced the sizes of subcanopy *V. karroo* seedlings, and will likely negatively impact their survival over the dry winter period.

Keywords: Flowering, pods, germination, mistletoes, recruitment, reproduction, seedlings, seed banks, semi-arid savannas.

Introduction

All plant species need to reproduce in order to persist and maintain their populations (Wilson and Witkowski, 2003; Helm *et al*., 2011). However, disturbances such as fire, herbivory, competition for water and nutrients, and mistletoe parasitism often result in changes in plant population dynamics (Wilson and Witkowski, 2003; Mourão *et al*., 2009; Cruz Neto *et al*., 2017; Mellado and Zamora, 2020). Although the impacts of mistletoes on plant community structure are well-documented, the effect of mistletoe parasitism on host reproduction is still poorly known (Lamien *et al*., 2006). Only a few studies have shown that mistletoe parasitism has deleterious effects on host reproduction and survival by altering (lessening) the physio-morphological structures (photosynthetic areas and branches/shoots) (Silva and del Rio, 1996; Lamien *et al*., 2006; Mourão *et al*., 2009; Arruda *et al*., 2012; Cruz Neto *et al*., 2017; Mellado and Zamora 2020). Therefore, by accessing the vascular system via a haustorium, mistletoes can reduce the resources that are allocated towards the hosts' reproduction, thus lowering their hosts' reproductive and regeneration capacity (Gomes and Fernandes, 1994; Geils and Hawksworth, 2002; Press and Phoenix, 2004; Mourão *et al*., 2009; Arruda *et al*. 2012; Daneshvar *et al*., 2014; Mellado and Zamora, 2020).

Silva and del Rio (1996) found that heavily parasitized hosts (*Echinopsis chilensis* var. *borealis*) tended to reduce flower buds and flowers, whilst fruit production was significantly reduced by the mistletoe *Tristerix aphyllus*. Similarly, Cruz Neto *et al*. (2017) found that *Cuscuta partita* reduced the flowering of *Zornia diphylla* (Fabaceae) by 72%, as well as reducing pollen viability. Furthermore, mistletoe parasitism can also lead to the production of smaller seeds, reduced seed germination and may result in less vigorous seedlings (Geils and Hawksworth, 2002; Daneshvar *et al*., 2014). Arruda *et al*. (2012) reported that *Struthanthus flexicaulis* (Loranthaceae) infection resulted in reduced fruiting and smaller seeds in *Mimosa calendron* (Fabaceae). Although Gomes and Fernandes (1994) found that the parasite, *Pilostyles ingae* (Rafflesiaaceae) did not affect the numbers of fruits and seeds produced, it negatively reduced fruit size (36%), seed mass (20%), and seedling establishment in *Mimosa naguierei* (Leguminosae).

The regeneration output of the host also varies depending on the species and severity of mistletoe-infection. Mistletoe-infection severity has been shown to be negatively correlated to cone size, seed size, and seed germination rates in *Pinus* spp. (Geils and Hawksworth, 2002; Mellado and Zamora, 2020). Mellado and Zamora (2020) found that in parasitized pine trees, cone production and cone dimensions were lower by ~30% in *Pinus nigra* subsp. *salzmanii* and ~90% in *Pinus sylvestris* subsp. *nevadensis*. Furthermore, parasitism effects on seed numbers, seed germination, and emergence varied depending on pine species (Mellado and Zamora, 2020).

Despite these studies, the influence of varying levels of mistletoe infection on the regeneration of

trees in semi-arid arid savannas has not previously been investigated. Therefore, the aim of this study was to investigate the effects of high- and low mistletoe-infection on the regeneration of *V. karroo* trees in a semi-arid savanna. It was hypothesized that high mistletoe-infected trees would have lower numbers of flower buds, flowers, and pods, and smaller pods with fewer and lighter seeds compared to low mistletoe-infected trees. Further, seed and germinable seed production/tree, and percentage germination, were expected to be higher in low compared to high infection trees, with more predated and aborted seeds in high mistletoe infected trees partially explaining this difference. Low infection canopy patches were expected to have a higher number of *V. karroo* juveniles compared to high infection canopy patches. It was also predicted that the resource-rich canopy micro-habitats would have a higher number of *V. karroo* juveniles with larger size measurements compared to the intercanopy micro-habitats.

Methodology

Study area

The study was conducted in a *V. karroo* dominated stand at Matopos Research Station (MRS), (20°22'60" S and 28°31'0" E, elevation: 1,357-1,402 m a.s.l.) south-west Zimbabwe. The mean annual rainfall is 586mm while temperature is 18°C (Campbell *et al*., 1994; Chirara *et al*., 1998; Mupangwa *et al*., 2013). The study species *V. karroo* is one of the most dominant tree species at MRS, and is widely distributed on loamy soils (Chirara, 2002).

Study tree

V. karroo is a leguminous tree species belonging to the family Leguminosae and subfamily Mimosoidae (Chirara, 2002; Robbertse *et al*., 2014; Magandana, 2016). *V. karroo* trees have a lifespan of ~40 years (O'Connor *et al*., 2010; Magandana, 2016) although it may be reduced (~20years) by mistletoe parasitism (Gourlay *et al*., 1996; Csurhers *et al*., 2016). Height ranges between 1-15m, but some trees can grow up to a height of ~25m (Ross, 1971; Csurhers *et al*., 2016). The morphology of *V. karroo* trees tends to differ depending on the geographical range thus they have several biotypes with distinct characteristics (Ross, 1971). *V. karroo* canopies are composed of fern-like green leaves comprised of 2-7 pinnae pairs each with 8-20 pairs of small obovate-oblong leaflets which are ~3.5-8mm and ~1-2.5mm long and wide, respectively (Ross, 1971; Csurhers *et al*., 2016). They have yellow pompom or spherical shaped flower heads during the wet season (between November to late April) and sometimes flowering can occur 3-4 times a year especially after heavy rains (Robbertse *et al*., 2014; Csurhers *et al*., 2016). Flowers are normally pollinated mostly by insects from the order Coleoptera, Diptera, Hymenoptera and Lepidoptera (Barnes *et al*., 1996; Robbertse *et al*., 2014; Csurhers *et al*., 2016).

They produce sigmoid or sickle shaped pods which are brown in colour when mature. The pods (~16cm long, ~1cm wide) are flat, dehiscent, and open when dry, whilst they are still attached to the tree (Chirara, 2002; Robbertse *et al*., 2014; Csurhers *et al*., 2016). Pods mature between February and June (Robbertse *et al*., 2014; Csurhers *et al*., 2016) and they contain seeds that are green in colour. The seeds are dispersed by herbivores and to a lesser extent wind and water (Walters and Milton, 2003; O'Connor *et al*., 2010; Csurhers *et al*., 2016). It is possible that the seeds can remain viable for more than a year and up to 7 years in the soil, but most persist for less than one year (O'Connor *et al*., 1995; Witkowski and Garner, 2000; Weiersbye and Witkowski 2002; Csurhers *et al*., 2016).

Although *V. karroo* seeds have thinner seed coats compared to other *Vachellia* (*Acacia*) seeds (Walters *et al*., 2005), they still exhibit physical dormancy, but the seed coat is water-soluble (Csurhers *et al*., 2016). Seed dormancy can also be broken by soil moisture, animal digestion and by fire nonetheless; fire can cause a decline in the number of seeds in the soil seed bank (Walters and Milton, 2003). Bruchid beetles, other insects, rodents and small and large ungulates are the major predators of *Acacia* pods, seeds, and seedlings (Barnes, 2001; Walters *et al*., 2005; O'Connor *et al*., 2010; Lagerwall, 2016) although seedlings are also said to be resistant to browsing (Chirara *et al*., 1998; Csurhers *et al*., 2016). *V. karroo* seedlings are susceptible to factors such as fire, competition, and shading (Chirara *et al*., 1998; Csurhers *et al*., 2016).

Tree identification

Adult trees with the highest and lowest mistletoe infection (n = 10 in each category) were selected (Table 1). For each tree, the regeneration capacity based on flowering, fruiting, pod production, seed banks, seed germination, and viability were studied. The abundance and height, stem diameter and canopy long (D_1) and short (90^o of D_1; = D_2) diameter of *V. karroo* juveniles within and beyond canopy patches were also recorded (see Chapter 3).

Table 1: Mean (± SE) number of mistletoes, diameter at breast height, tree height, and canopy area of high- and low- mistletoe-infection *Vachellia karroo* trees at Matopos Research Station.

Mistletoe infection	Variables				
	No. trees	No. of mistletoes	DBH (cm)	Tree height (m)	Canopy area (m^2)
Low	10	5.50 ± 0.70	21.35 ± 1.49	6.66 ± 0.37	70.71 ± 5.21
High	10	26.1 ± 1.02	29.10 ± 1.62	8.43 ± 0.61	91.73 ± 10.3

Flowering, pod and seed production

The number of flower buds and flowers on each high- and low mistletoe-infection tree were counted using a hand tally counter, in each of the four cardinal directions (at a 90° angle), and then summed to get the total. This was done in early December, end of December, and end of January, for two consecutive years (2018-2019). Similarly, pods in the canopies were counted between March and May

in 2018 and 2019. On each occasion, pods per tree were counted twice and averaged (Walters and Milton, 2003). For three consecutive years (2018-2020), ±50 dehisced pods were picked at random from the canopy of each tree, placed in marked plastic bags, and fumigated using an insecticide immediately after collection to minimise bruchid attack. Pod diameter and length were measured, and seeds/pod counted. Each seed was weighed to a precision of 0.0001g (Witkowski and Garner, 2000). The number of intact seeds collected from each tree was recorded as the seed production of each tree. Seed production per tree was calculated as follows:

Seed production/tree = [Number of pods/tree]*[Mean intact seeds/pod]

Soil seed bank

Seed bank samples were collected between May and August 2018. Samples were taken in the four cardinal directions at half the distance between the tree bole and the canopy edge beneath each high- and low mistletoe-infected tree, at 0-10cm depth using a steel quadrat (0.3m × 0.3m, 10cm depth). The four samples from each tree were pooled and passed through a 2mm sieve to separate the *V. karroo* seeds from the bulk of the soil. Each sample was then place on a white paper background under high light to aid in the searching and counting of seeds.

Seed characteristics and germination

The proportion of (i) intact seeds, (ii) seeds that showed signs of bruchid beetle predation and (iii) aborted seeds were counted per sample. Intact seeds (±20/tree) were germinated at the University of the Witwatersrand under greenhouse conditions from 10th November 2020 to 21st January 2021 (73 days). Seeds were planted at a depth of ~1cm in free draining seed trays, watered twice daily for 30 days, with seedling emergence monitored daily. The number of days taken for each seed to germinate was recorded to determine percentage and rate of germination over 30 days. Germination rate was the mean days to germinate. Seeds that did not emerge over the 30 days were dug up and separated into decomposed and intact for each tree. Intact seeds were then lightly scarified using a soldering iron (heat point method) on the flat side to break physical dormancy (Weiersbye and Witkowski, 2002) and then replanted as before. The number of days taken for each seed to germinate (emerge) was recorded and then percentage and rate of germination were determined. Percentage germination was compared between high and low mistletoe-infection trees. Germinable seeds/tree were calculated as

Germinable seeds/tree = [Seed production/tree]*[Percentage seed germination/100]

The size of each seedling from each infection category was measured at the end of the trial.

Characteristics of greenhouse seedlings and understory *Vachellia karroo* trees

Beneath and beyond each tree, the number of *V. karroo* juveniles within the whole subcanopy area (between tree bole and canopy edge) and in the intercanopy spaces (whole area at distances between 1-2 times the radii of the canopy of each tree) were counted and measured at the end of April 2020.

Stem diameters were measured using a calliper, height (m) with a calibrated rod (2m), and canopy long (D_1) and short (90^o of D_1; = D_2) diameters using a tape measure. Similarly, stem diameters, height, canopy long and short diameter, of seedlings in the greenhouse were measured after 106 days from seed planting (27^{th} of February 2021).

Data analysis

General linear models (GLM) in SPSS 23 for Windows (SPSS Inc., 2012, Chicago, IL U.S.A) were used to investigate the effect of year and degree of infection on numbers of flower buds, flowers, pods, pod diameter and length, number of seeds/pod, intact seed and germinable seed production/tree. Correlation and regression analysis were used to explore the relationship between host canopy area and number of mistletoes *vs*. flower buds, flowers, pods, pod length, number of seeds in each pod, number of seeds in each seed bank, intact seed and germinable seed production/tree. Correlation analyses were also done for seed mass and percentage germination of seeds (after 30 days, between 31-73 days and after 73days), percentages of intact, predated, aborted and decomposed seeds. Either t-tests or Wilcox signed ranked tests, were used to compare the number of seeds in the seed bank, seed mass, height, canopy area and volume of seedlings between high- and low mistletoe-infection trees. Chi-square contingency tables were used to test if there were associations in the proportions of intact, predated and aborted seeds, and for germinated and ungerminated seeds (after 30 and 73 days) between high- and low mistletoe-infected trees. Data were tested for normality using the Shapiro-Wilk test.

Juvenile density within canopy patches of each tree was calculated using the canopy area of each tree and the densities were compared using a t-test. Height, basal stem diameter, canopy area and canopy volume size-class distributions were constructed for the understory *V. karroo* juveniles and the Kolmogorov-Smirnov tests were used to compare the size classes among the four micro-habitats. Height, basal stem diameter, canopy area and canopy volume and density of the *V. karroo* juveniles, seedlings and saplings were also compared among micro-habitats using Kruskal-Wallis tests in *R* v. 3.1.5 (R Core Team, 2018).

Results

Reproduction – from flowers to seeds

Degree of mistletoe infection (high *vs*. low) had significantly more effects on the reproductive outputs of *V. karroo* trees compared to variation between years (Table 2). Moreover, except for number of seeds/pod, there were no significant interactions between degree of infection and year of collection. There was no significant difference in the number of flower buds, flowers, number of pods, pod length and diameter, seeds/pod, seed and germinable seed production /tree, observed between years (Table 2; Appendix 1).

Pod diameter was the only variable not affected by mistletoe infection degree. However, the number of flower buds in high mistletoe-infection trees (433 ± 92) were only 60% of that in low mistletoe-infection trees (719 ± 70). Flowers were 3.1-fold less in high (288 ± 67) than low mistletoe-infection trees (894 ± 146). Similarly, the number of pods was 2.9-fold lower within high- (298 ± 103) compared to low mistletoe-infection trees (862 ± 109, Table 2). Pods from low mistletoe-infection trees (10.87 ± 0.29 cm) were 14% longer than pods from high mistletoe-infection trees (9.40 ± 0.38 cm, Table 2). As a result, high mistletoe-infection trees (7.20 ± 0.32) had 1.15-fold less seeds/pod compared to low mistletoe-infection trees (8.31 ± 0.26). Seed and germinable seeds production/tree in high mistletoe-infection trees (2273 ± 820; 599 ± 216) were 32% and 20% (respectively) that of low mistletoe-infection trees (7088 ± 905; 2947 ± 376). The soil seed bank was 3.24-fold higher (W = 22, $P = 0.03$) under low- (46.7 ± 10.7 seeds/m^2) than high mistletoe-infection trees (14.4 ± 5.8 seed/m^2).

Table 2: General linear model (GLM) analyses of the effects of year of collection (2018, 2019), mistletoe infection (high *vs.* low), and their interactions on the number of flower buds, flowers, pods, pod diameter, pod length and number of seeds/pod, seed production/tree and germinable seed production/tree. Values in bold show significant effects ($P < 0.05$). *, data collected from 2018-2020.

Variable	Effects								
	Year 2018 *vs*. 2019 (2020)			Infection degree High *vs*. Low			Year × Infection degree		
	df, error	F	Sig.	*df*, error	F	Sig.	*df*, error	F	Sig.
Flower buds/tree	1, 36	0.01	0.927	1, 36	5.28	**0.022**	1, 36	<0.01	0.977
Flowers/tree	1, 36	<0.01	0.985	1, 36	13.50	**0.001**	1, 36	0.06	0.812
Pods/tree	1, 36	0.13	0.720	1, 36	13.37	**0.001**	1, 36	<0.01	0.963
Pod diameter (cm)	1, 36	0.99	0.951	1, 36	0.99	0.327	1, 36	1.87	0.180
Pod length (cm)*	2, 54	1.44	0.247	1, 54	9.65	**0.003**	2, 54	1.14	0.328
Seeds/pod*	2, 54	1.24	0.298	1, 54	8.15	**0.006**	2, 54	4.64	**0.014**
Seed production/tree	1, 36	<0.01	0.974	1, 36	14.81	**<0.001**	1,36	0.23	0.632
Germinable seed/tree*	1, 36	4.15	0.942	1, 36	3.76	**<0.001**	1,36	0.47	0.652

The interaction of year and mistletoe-infection degree significantly influenced the number of seeds/pod only i.e., for 2018 and 2020, high mistletoe-infection trees (6.34 ± 0.58; 6.86 ± 0.59) had 28% and 16%, (respectively) less seeds/pod compared to low mistletoe-infection trees (8.78 ± 0.44; 8.18 ± 0.54) (Table 2). In 2019, high mistletoe-infection trees (8.40 ± 0.24) had 5% more seeds/pod compared to low mistletoe-infection trees (7.97 ± 0.37).

Relationships between canopy area and number of mistletoes with reproductive traits

There tended to be a negative linear relationship between number of mistletoes and reproductive traits (Fig. 1). There was a significant negative correlation between number of flowers (Spearmans's rank correlation: $r_s = -0.52$, $P = 0.02$), pod length ($r_s = -0.58$, $P = 0.01$), number of seeds/pod ($r_s = -0.46$, $P = 0.04$), seeds produced/tree ($r_t = -2.38$, $P = 0.03$), germinable seed production/tree ($r_t = -3.24$, $P = 0.005$) with number of mistletoes/tree. However, there was no significant correlation between the

number of flower buds (Spearmans's rank correlation: r_s = -0.35, *P* = 0.13), number of pods (r_s = -0.38, *P* = 0.10), and number of seeds found in the seed banks (r_s = -0.42, *P* = 0.06) with number of mistletoes/tree.

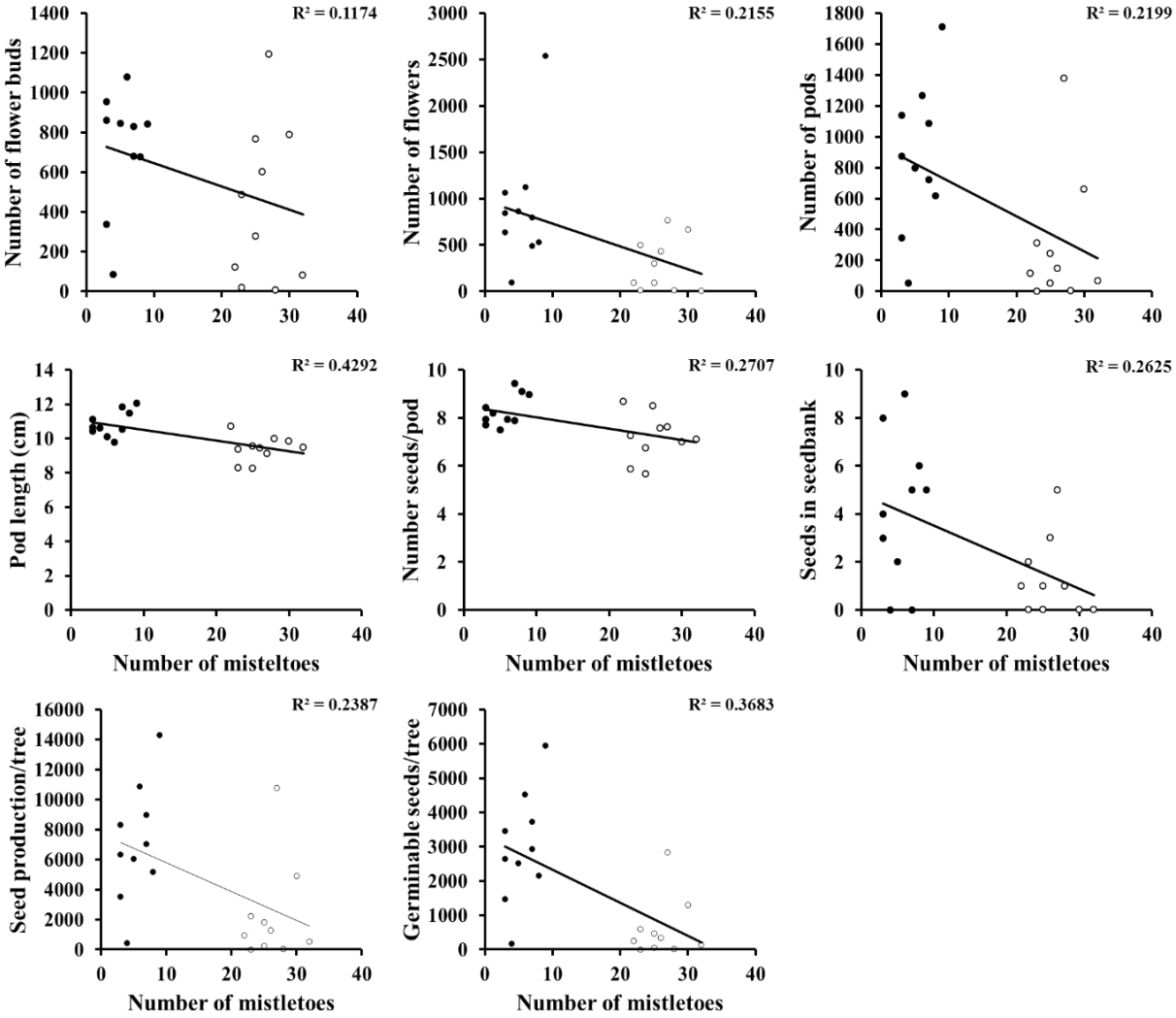


Fig. 1. Relationship between number of flowers buds, flowers, pods, pod length, number of seeds per pod, and number of seeds in each seed bank per tree with number of mistletoes found on *Vachellia karroo* trees in Matopos Research Station, Zimbabwe. Closed and open circles represent low and high mistletoe-infected trees.

Furthermore, canopy areas of low compared to high mistletoe-infected trees had more reproductive traits. Flower buds (r_s = -0.39, *P* = 0.09), number of seeds per pod (r_s = -0.29, *P* = 0.21), and number of seeds in the seed bank (r_s = -0.35, *P* = 0.13) did not have a significant relationship with canopy area. Nonetheless, number of flowers (r_s = -0.63, *P* = 0.004), number of pods (r_s = -0.50, *P* = 0.03) and seed production/tree (r_t = -2.37, *P* = 0.03), germinable seed production /tree (r_t = -2.25, *P* = 0.04) had a significant negative relationship with canopy area (Fig. 2).

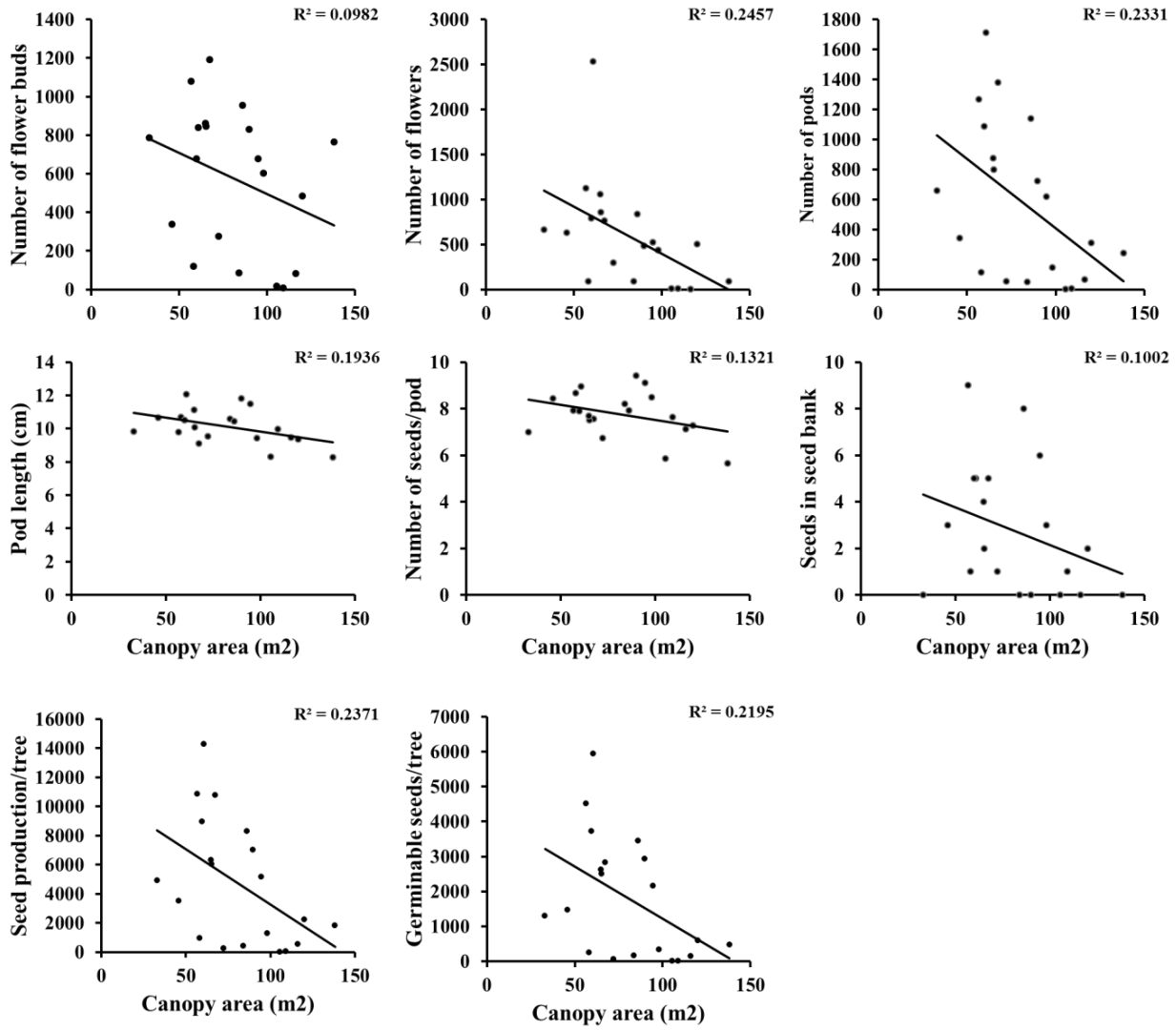


Fig. 2. Relationship between number of flowers buds, flowers, pods, pod length, number of seeds per pod, and number of seeds in each seed bank per tree with canopy areas (m^2) of mistletoe infected *Vachellia karroo* in Matopos Research Station, Zimbabwe.

Seed fate and germination

There was a significant association ($\chi^2_2 = 28.70$, $P < 0.001$) between infection degree (high *vs*. low) and seed fate (intact, predated, and aborted seeds). High mistletoe-infection canopy patches tended to have higher aborted (by 13%) and predated seeds (by 46%), whilst low infection trees were more associated (by 17%) with intact seeds (Fig. 3a). There was also a significant relationship ($\chi^2_1 = 8.97$, $P = 0.003$) between infection degree and germination after 30 days, and germination was 4-fold higher in high- (12%) compared to low mistletoe-infection trees (3%), (Fig. 3b, Fig. 4). Conversely, between 31 and 73 days, low (34%) compared to high infection trees (12%) had 2.83-fold higher germination ($\chi^2_1 = 23.01$, $P < 0.001$). Similarly, after 73 days the overall germination was1.62-fold higher ($\chi^2_1 = 8.47$, $P = 0.004$) for low (42%) compared to high infection (26%) trees (Fig. 3c, Fig. 4). The mean germination time of seeds from high- and low mistletoe infection trees within the 30-day period was

14.5 ± 1.76 and 14.1 ± 1.84 days, respectively, whilst after scarification mean germination time was 21.9 ± 1.95 and 21.9 ± 1.93 days for high and low mistletoe-infection seeds. The overall mean germination time was 36.3 ± 2.54 and 36.1 ± 2.57 days for high and low infection seeds, respectively.

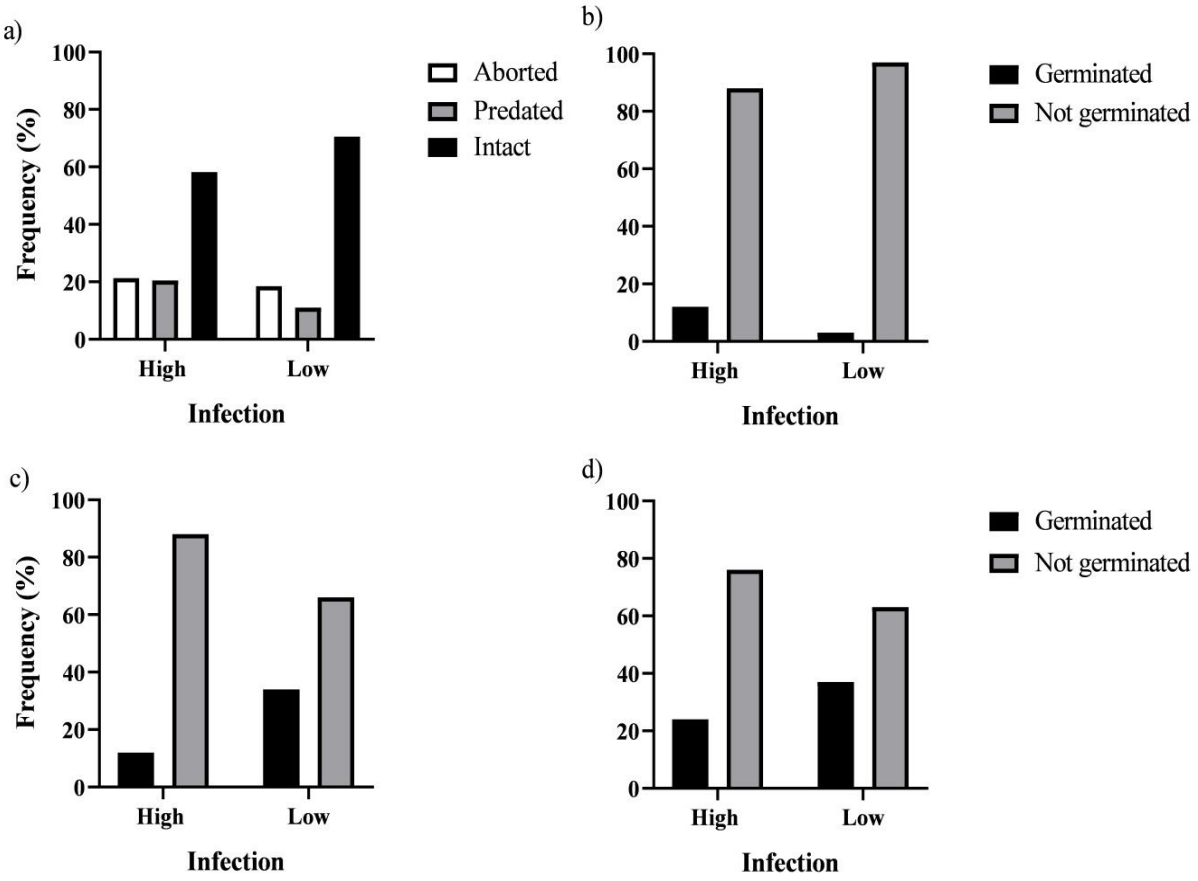


Fig. 3. Fate of high- and low mistletoe-infection seeds (a) before germination (b) after 30 days (c) between 31 and 73 days and (d) total germination after 73 days from planting.

Low mistletoe-infected trees (0.037 ± 0.003g) tended to have a higher seed mass per seed (1.23-fold) than high mistletoe-infected trees (0.030 ± 0.003g), (t = -1.7669, *df* = 18, *P* = 0.09). There was a weak positive correlation between seed mass with percentage germination after 73days, percentage germination after scarification and with bruchid attack (Appendix 2). In contrast, there was a weak negative correlation between seed weight with and percentage of aborted seeds/tree (Appendix 2).

Size of greenhouse seedlings

There was no significant difference in the mean basal stem diameter (t = 0.23118, *df* = 66.184, *P* = 0.818, Fig. 5a) of high- and low mistletoe-infection seedlings. Low mistletoe-infection seedlings were 19% taller than high mistletoe-infection seedlings (W = 945.5, *P* < 0.001, Fig. 5b). Canopy area was 24% larger for low- compared to high mistletoe-infection seedlings (W = 1121, *P* = 0.008, Fig. 5c).

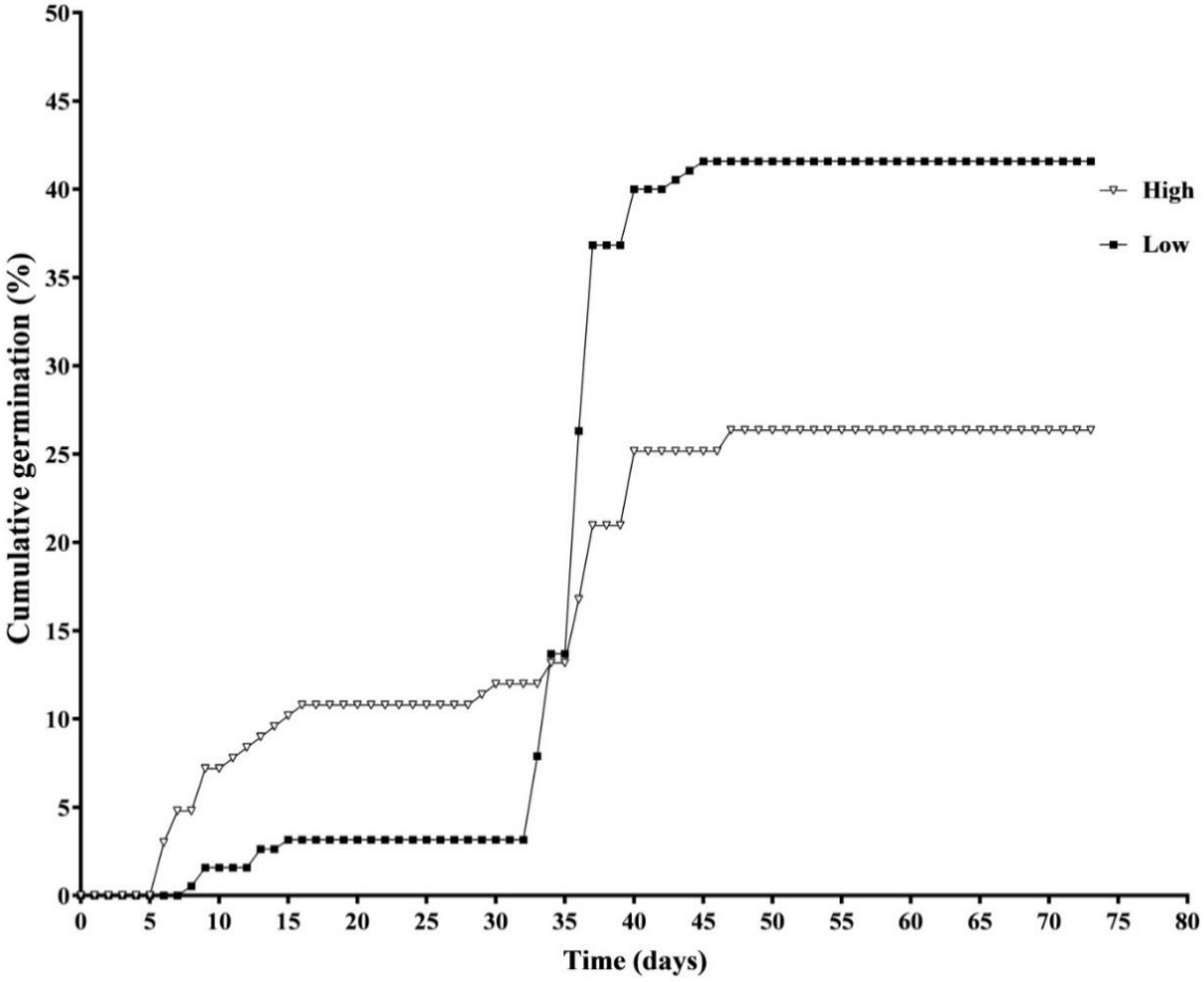


Fig. 4. Comparison of cumulative seed germination between high- and low mistletoe-infection *Vachellia karroo* trees.

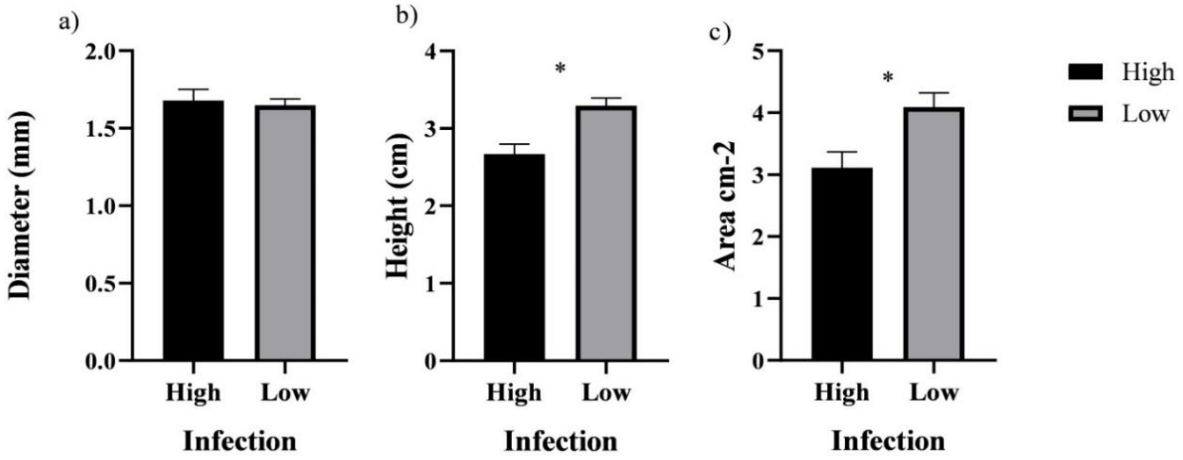


Fig. 5. Comparisons (mean ± SE) of (a) stem diameter (b) height and (c) canopy area greenhouse seedlings from high and low-infected tree seeds. The asterisks show differences between the microhabitats (Kruskalmc, $P < 0.05$).

Size distribution of understory *Vachellia karroo* trees

Canopy versus intercanopy had more significant effects compared to mistletoe-infection effects (high *vs*. low) but there were no significant interactions between them (Table 4). Intercanopy spaces had larger *V. karroo* juveniles than canopy patches in terms of height (23.11 ± 0.59 *vs*. 19.26 ± 0.66 cm), basal stem diameter (0.31 ± 0.01 cm *vs*. 0.29 ± 0.01 cm), canopy area (180 ± 14.10 cm^2 *vs*. 93 ± 15.62 cm^2), and canopy volume (3367 ± 312 cm^3 *vs*. 1476 ± 345 cm^3). Low mistletoe-infected trees (23.11 ± 0.74 cm) had taller *V. karroo* juveniles than high mistletoe-infected trees (18.82 ± 0.48 cm) but basal stem diameter did not differ between high- (0.29 ± 0.01 cm) and low mistletoe-infection micro-habitats (0.31 ± 0.01 cm). *V. karroo* juveniles beneath low mistletoe-infected trees had larger canopies than high mistletoe-infected trees (canopy area: 165 ± 17.67 cm^2 *vs*. 108 ± 11.42 cm^2; volume: 3165 ± 390 cm^3 *vs*. 1678 ± 252 cm^3).

Table 4: General linear model (GLM) analyses of the effects of canopy (canopy *vs*. intercanopy), mistletoe infection (high *vs*. low), and their interactions on basal stem diameter, height, canopy area and canopy volume of *Vachellia karroo* juveniles. Values in bold show significant effects ($P < 0.05$), error $df = 1,415$ in every case.

Variable	Effects					
	Canopy		Infection		Canopy × Infection	
	Canopy *vs*. Intercanopy		High *vs*. Low			
Stem diameter (cm)	5.54	**0.019**	2.23	0.136	0.25	0.620
Height (cm)	18.95	**<0.001**	28.59	**<0.001**	0.59	0.444
Canopy area (cm^2)	17.12	**<0.001**	7.21	**0.01**	0.83	0.363
Canopy volume (cm^3)	16.62	**<0.001**	10.27	**0.001**	2.21	0.138

There was a significant difference in the mean basal stem diameter (Kruskal Wallis $\chi^2 = 12.23$, $df = 3$, $P = 0.01$, Fig. 6a), height (Kruskal Wallis $\chi^2 = 50.61$, $df = 3$, $P < 0.001$, Fig. 6b), canopy area (Kruskal Wallis $\chi^2 = 33.28$, $df = 3$, $P < 0.001$, Fig. 6c) and canopy volume (Kruskal Wallis $\chi^2 = 43.59$, $df = 3$, $P < 0.001$, Fig. 6d) of understory juvenile trees among micro-habitats. Low mistletoe-infection intercanopy spaces had between 16% to 76% larger size measurements compared to high mistletoe-infection canopy patches. Size measurements were 10% to 43% larger in low- compared to high mistletoe-infection canopy patches.

Understory juvenile *Vachellia karroo* distribution pattern

The majority of *V. karroo* juveniles were found in the canopy patches and intercanopy spaces of high mistletoe-infection trees ($n = 172$ and $n = 122$, respectively), whilst low mistletoe-infection canopy patches and intercanopy spaces had the least number of juveniles ($n = 49$ and $n = 76$, respectively). There was no significant difference in the density of *V. karroo* juveniles across the microhabitats (Kruskal-Wallis $\chi^2 = 4.84$, $df = 3$, $P = 0.184$, Table 5). However, juvenile density tended to be higher in high mistletoe-infection canopy patches and intercanopy spaces (1813 ± 528 juveniles/ha; 1236 ±

218 juveniles/ha) compared to low mistletoe-infection canopy patches and intercanopy spaces (633 ± 218 juveniles/ha; 999 ± 218 juveniles/ha), respectively. Seedling density was between 1.92 and 4.35-fold higher (Kruskal-Wallis $\chi^2 = 9.74$, $df = 3$, $P = 0.02$, Table 5) in high mistletoe-infection canopy patches compared to other microhabitats. Sapling density did not vary across microhabitats (Kruskal-Wallis $\chi^2 = 1.33$, $df = 3$, $P = 0.721$, Table 5), however, high and low mistletoe infection intercanopy spaces had 1.07 and 2.08 –fold higher sapling density compared to their adjacent canopy patches (Table 5). Two-sample Kolmogorov-Smirnov tests showed that there were no significant differences in the size class distributions in all the microhabitats ($P > 0.05$, Appendix 3).

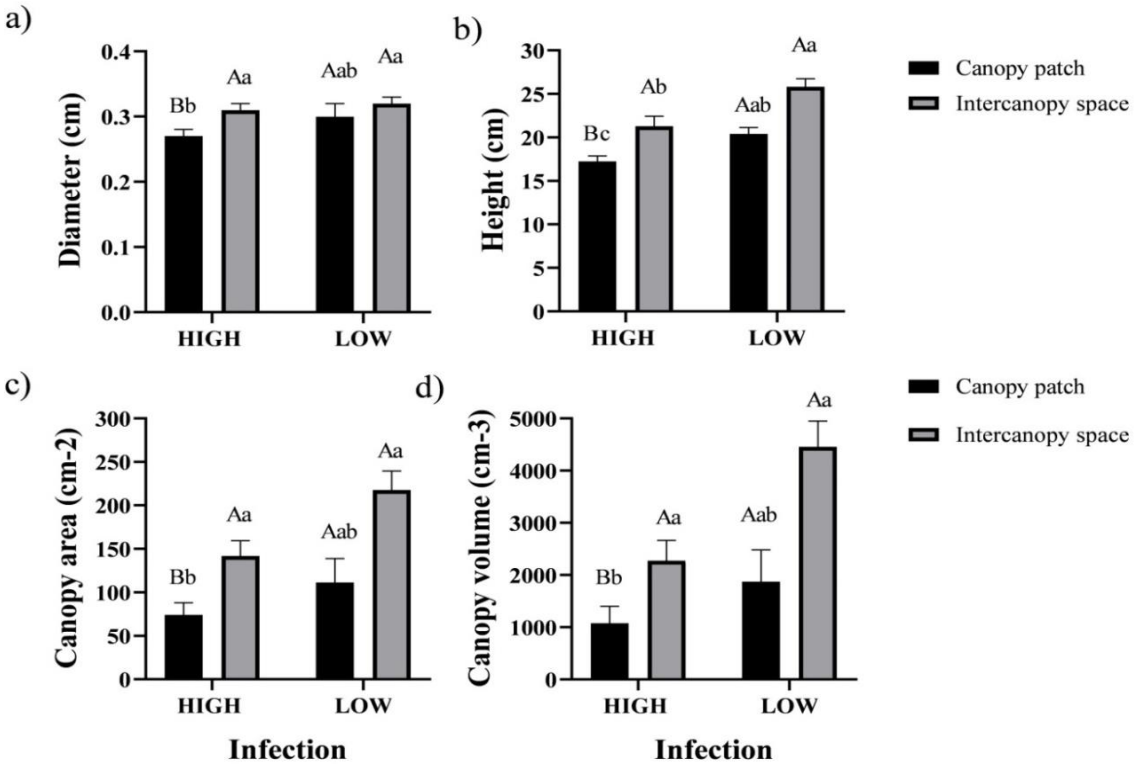


Fig. 6. Comparisons of mean (± SE) stem diameter (a), height (b), canopy area (c), and canopy volume (d) of understory juvenile trees amongst four microhabitats. Capital letters show differences within infection microhabitats (canopy patch and intercanopy spaces) whilst lower-case letters show differences among the four microhabitats (Kruskalmc, $P < 0.05$).

Table 5. Comparison of juvenile, seedling and sapling densities (/ha) among the four microhabitats**.** The proportions of seedlings and saplings are shown inside the brackets. Means in columns not sharing a small common letter (subscript) are significantly different (Kruskalmc, $P < 0.05$).

Micro-habitats	Density (/ha)		
	Total	Seedlings (Proportions)	Saplings (Proportions)
High mistletoe-infection canopy	1813 ± 528^{a}	1275 ± 344^{a} (70%)	538 ± 201^{a} (30%)
Low mistletoe –infection canopy patch	633 ± 218^{a}	294 ± 92^{b} (46%)	340 ± 142^{a} (54%)
High mistletoe-intercanopy spaces	1236 ± 286^{a}	663 ± 121^{ab} (54%)	573 ± 199^{a} (46%)
Low mistletoe-intercanopy spaces	999 ± 423^{a}	293 ± 160^{b} (29%)	706 ± 343^{a} (71%)

The basal stem diameter sizes for the four microhabitats depicted a bell shape and the diameter classes ranged between 0-0.7cm. Most of the plants recorded under high mistletoe-infection canopy patches trees ranged from 0.2-0.3cm (73%) in basal stem diameter (Fig. 7a), whilst high mistletoe- infection intercanopy space basal stem diameters were mostly 0.3cm (44%), (Fig. 7b). Similarly, basal stem diameters of low mistletoe-infection canopy patches and intercanopy spaces were mostly 0.3cm (51% and 49%, respectively) (Fig. 7c, d).

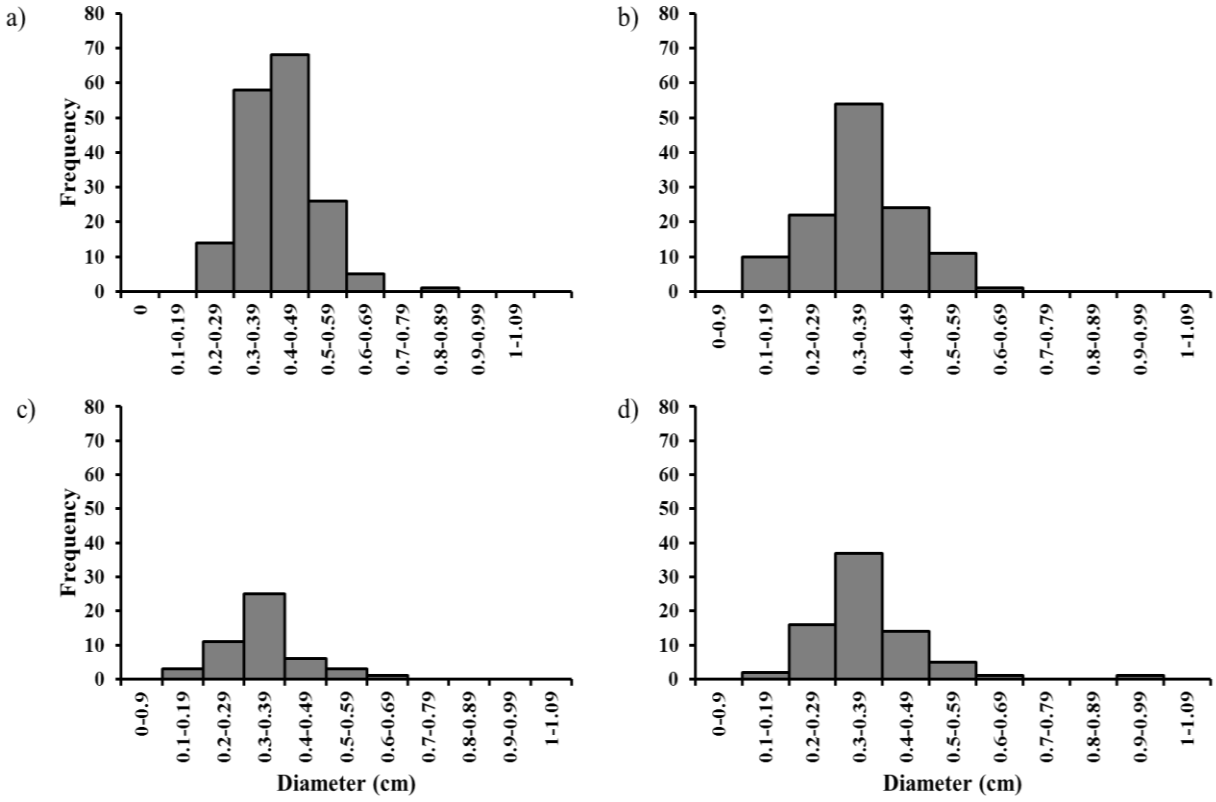


Fig. 7. Distribution of basal stem diameter sizes of *Vachellia karroo* juveniles within (a) high mistletoe-infection canopy patches, (b) high mistletoe-infection intercanopy spaces, (c) low mistletoe-infection canopy patches and (d) low mistletoe-infection intercanopy spaces.

Height size-class distribution also followed a bell shape for all the microhabitats, and high mistletoe-infection canopy patches and intercanopy space plants were mostly between10-20cm (55%) and 10-30cm (76%) in height, respectively (Fig. 8a, b). However, in low mistletoe-infection canopy patches and intercanopy spaces (Fig. 8c, d) the frequency of *V. karroo* juveniles was mostly highest between heights of 15-25cm (57% and 41%, respectively).

In contrast, canopy area and canopy volume both showed an inverse J shape for the four micro-habitats (Appendix 4 and 5), with juveniles within high mistletoe-infection canopy patches mostly ranging between 0-200cm^2 and 0-1000cm^3, respectively. For the other micro-habitats canopy area and

canopy volume ranged from 0-50 cm^2 and 0-1000cm^3, respectively, and then tended to be evenly distributed throughout the other size classes.

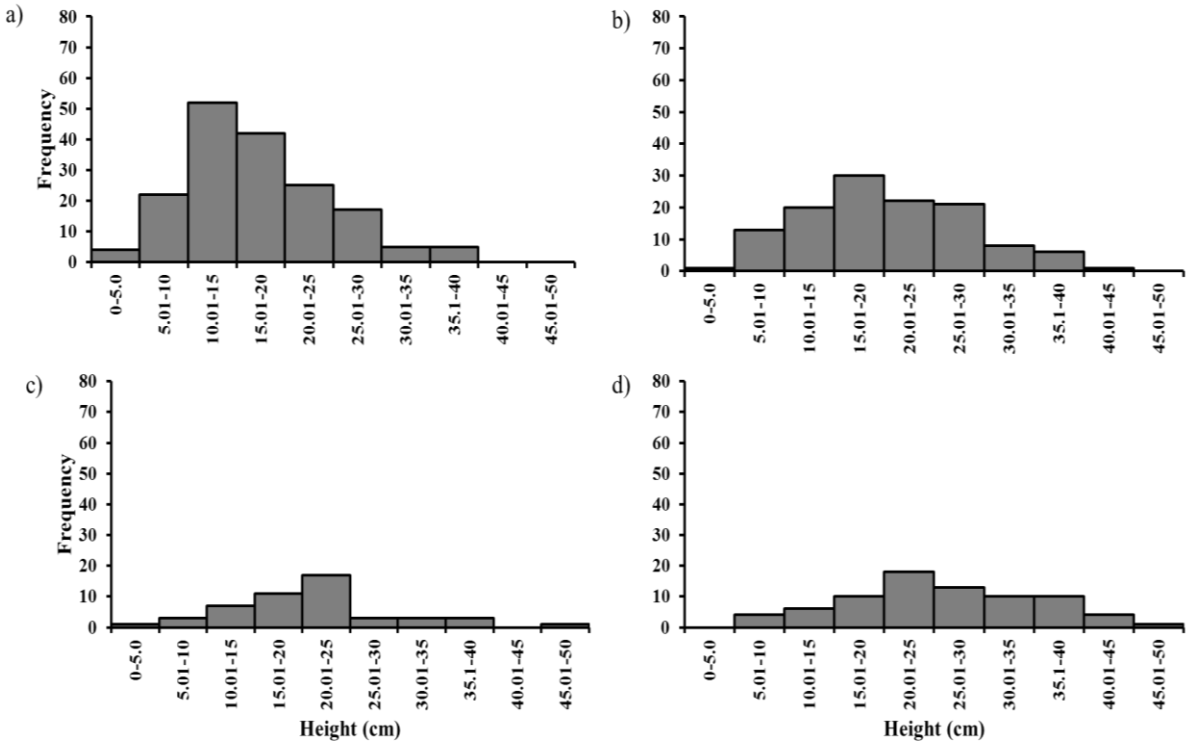


Fig. 8. Distribution of height sizes of *Vachellia karroo* juveniles within (a) high mistletoe-infection canopy patches, (b) high mistletoe-infection intercanopy spaces, (c) low mistletoe-infection canopy patches, and (d) low mistletoe-infection intercanopy spaces.

Discussion

In support of the initial hypothesis, the results show that in this semi-arid savanna the regeneration capacity of *V. karroo* trees is negatively correlated to high mistletoe infection intensity. High mistletoe-infected trees had significantly lower reproductive capacity compared to low mistletoe-infected trees. For instance, flower production was significantly lower on high mistletoe-infection canopies compared to low mistletoe-infection canopies similar to findings elsewhere (Silva and del Rio, 1996; Cruz Neto *et al.*, 2017). Mistletoe parasitism reduces flower production due to significantly higher competition for both water and nutrients between hosts and mistletoes (Silva and del Rio, 1996; Daneshvar *et al.*, 2014; Cruz Neto *et al.*, 2017). However, when mistletoe infection is less severe, the negative effects (water and nutrient demand) of mistletoes are less deleterious as compared to when mistletoe intensity is high (Geils and Hawksworth, 2002; Daneshvar *et al.*, 2014). Consequently, because high mistletoe-infected *V. karroo* trees significantly reduce the number of flower buds, flowers and seeds, the impacts will cascade to other organisms that rely on these flowers. For example, *V. karroo* flowers have tangible amounts of pollen (high protein content) and nectar,

which facilitate bird and insect pollination (Dingaan and du Preez, 2017). Therefore, reduction of flower production can affect other processes higher up in the trophic level or ecosystem. Moreover, poorly developed flowers negatively impede pollination (non-viable pollen and reduction of pollen grains) and fertilization thus resulting in deformed fruits with aborted or fewer viable seeds (Daneshvar *et al*., 2014; Cruz Neto *et al*., 2017). Indeed, pod production was significantly lower in high mistletoe-infected trees relative to low mistletoe-infected trees and the shorter pod lengths reduced the number of seeds/pod as well.

Seed banks

The seed bank below low mistletoe-infected trees had 3.2-fold more seeds compared to high mistletoe-infected trees. This can be attributed to variations in canopy structure, soil temperatures and moisture (Chapter 2), and higher numbers of aborted seeds. High mistletoe-infection canopy patches also had higher animal visitation (Chapter 2) which could have also fed on pods and seeds that were on the tree and then dispersed onto the ground, thus reducing the seedbank.

Low mistletoe-infection canopy patches, due to lower light incidence from closed canopies have lower soil temperatures compared to high mistletoe-infection canopy patches with semi-open canopies (Mellado and Zamora, 2017). These low temperatures may not promote seed desiccation (and/or death) (O'Connor *et al*., 2010) enabling better persistence of seeds within low compared to high mistletoe-infection canopy patches.

Larger trees with rounded canopies are expected to have more seeds in contrast to smaller, slender and sparingly branched trees (Witkowski and Garner, 2000; Walters and Milton, 2003; O'Connor *et al*., 2010). However, in this study, the smaller low mistletoe-infected trees produced a higher number of pods, with seeds that were larger than those of high mistletoe-infected trees. It is possible that the reduction in physio-morphological attributes of the high mistletoe-infected trees due to high mistletoe parasitism could have significantly reduced the number of pods and seeds (Silva and del Rio, 1996; Sala *et al.*, 2001; Press and Phoenix, 2004; Lamien *et al*., 2006; Mourão *et al*., 2009; Arruda *et al.* 2012; Cruz Neto *et al*., 2017). In contrast, low mistletoe-infected trees have intact canopies and mistletoe effects may not be significant enough to result in deformations that cascade down to specific reproductive traits. Nevertheless, low mistletoe-infection trees had relatively lower number of seeds (by 2.4-fold) compared to a study by Walters and Milton, (2003) who found 110 seeds m^{-2} underneath the *V. karroo* canopy in KwaZulu-Natal, South Africa. The variation could be due to their study area having higher rainfall (760-1250mm) compared to MRS. Also, they did not indicate any impacts from mistletoe parasitism. Moreover, the smaller seedbank from low mistletoe-infection trees in this study could have been due to these trees allocating resources towards defending against mistletoe infection rather than to reproduction (see for example Dzerefos and Witkowski, 1997).

High mistletoe-infected trees could be producing smaller (in mass) and fewer seeds in order to compensate for loss in resources to the mistletoes (Daneshvar *et al*., 2014). It had been expected that larger seeds would have more bruchid bettle attack due to larger resources; however, the smaller high infection seeds were more susceptible to bruchid attack compared to low infection seeds, thus contributing to reducing the seedbank. As a result, the low number of seeds coupled with their smaller weight and susceptibility to predation could reduce the recruitment of seedlings underneath high mistletoe-infected trees. However, low mistletoe infection trees had higher stored seeds which may eventually, successfully establish as seedlings as mistletoe infection intensifies and light and soil resources increase (Witkowski and Garner, 2000). Indeed, seed banks are stores for genes or gene complexities which ensure the existence of a species and certainly the dynamics of the plant life cycle is also dependent on the seed stage (Witkowski and Garner, 2000; Wilson and Witkowski, 2003). Therefore, smaller seed banks within high mistletoe canopy patches might limit the encroachment and successful establishment of *V. karroo* trees in the long run. However, despite higher mistletoe infection and low production of seeds, high mistletoe-infected trees still produced viable seeds (Geils and Hawksworth, 2002).

Germination

High mistletoe-infected trees with fewer and smaller seed with limited food and nutrient reserves were expected to have lower germination compared to low mistletoe-infected trees with higher seed weight and larger food and nutrient reserves (Gomes and Fernandes, 1994; Daneshvar *et al*., 2014; Lagerwall, 2016). In agreement, these results show that low mistletoe-infection seeds had significantly higher overall percentage germination compared to high mistletoe-infection seeds. Nonetheless, there were differences in the way that the seeds germinated. For instance, after scarification the larger and apparently harder-coated low mistletoe-infection seeds had higher percentage germination compared to those from high mistletoe-infection canopy patches, with scarification breaking physical dormancy, allowing these seeds to imbibe water, leading to earlier germination. Nonetheless, the percentage germination and germination time before scarification was significantly higher for seeds from high- compared to low mistletoe-infected trees. This could be due to high mistletoe-infected trees having smaller and softer seeds with thinner seed coats that support higher water permeability, and thus higher initial germination rates (O'Connor *et al*., 2010; Souza and Fagundes, 2014). Therefore, high mistletoe-infected trees could be producing smaller, softer-coated seeds, which germinate as soon as moisture becomes available as a strategy to facilitate quicker germination rates and possibly increase survival (O'Connor 1995; O'Connor *et al*., 2010; Souza and Fagundes, 2014). However, this strategy can be a disadvantage as seeds become susceptible to high rates of decomposition if exposed to high moisture levels and a prolonged period in the soil. Regardless, due to higher nutrient reserves, seedlings that originated from the larger low mistletoe-infection seeds were bigger and more competitive than those from high mistletoe-infection seeds.

Understory *Vachellia karroo* distribution

High mistletoe-infection canopy patches had higher numbers of *V. karroo* juveniles compared to the other micro-habitats. Higher numbers of *V. karroo* juveniles within these canopy patches could be indicating the weakening of the large mistletoe-infected trees thus reducing their competitive edge. Diverse subordinate trees thrive within canopy patches of high mistletoe-infected trees due to increased light penetration coupled with the host becoming a poor competitor (Mellado and Zamora, 2017; Chapter 3). Mellado and Zamora (2020) also found high tree diversity within mistletoe-infected tree canopy patches (Chapter 3). Therefore, high mistletoe infections play a facilitative role in recruitment of *V. karroo* juveniles by reducing the host physio-morphological structures structures, thereby improving subcanopy light conditions (Mellado and Zamora, 2017) that favour the establishment of understory *V. karroo* juveniles.

Generally, woody seedling establishment is also influenced by moisture and nutrient availability (O'Connor 1995, Barnes, 2001; Bhadouria *et al*., 2016). Therefore, higher juvenile numbers within canopy micro-habitats could be attributed to high nutrient and water availability especially within high mistletoe-infection canopy patches (Geils and Hawksworth, 2002; Bhadouria *et al*., 2016; Magandana, 2016; Chapter 2), thus compensating for the low number of seeds produced by these trees. Joubert *et al*. (2013) also report that subcanopies had three times more *S. mellifera* seedlings (per m^2) than in intercanopy spaces. However, intercanopy spaces had a higher seedling survival rate (Joubert *et al*., 2013), agreeing with the results where the juveniles in the intercanopy spaces were larger than in the canopy patches. This suggests that higher percentage germination does not necessarily translate to greater seedling vigour and larger juvenile sizes over time (Souza and Fagundes, 2014). Furthermore, despite these patches having higher soil moisture, temperature and nutrients (Chapter 2), the diverse suite of mistletophilous woody and herbaceous species (Chapter 3) could have increased competition for resources, thus explaining the smaller size of *V. karroo* juveniles within these patches. Therefore, when the high mistletoe-infected tree eventually dies, the increase in available space and increased nutrients, may result in enhanced recruitment from juveniles to larger size classes, of a diverse range of species, including *Ziziphus mucronata* Willd., and *Flueggea virosa* (Roxb. ex Willd.) Voigt *subsp. Virosa,* which are dominant within the canopy patches (Chapter 3, 5). This may shift the dominant *V. karroo* stands into more diverse mixed woodland with a higher functional diversity. Alternatively, this will most likely result in a change in the *V. karroo* population, especially if *V. karroo* seedlings and saplings are poor competitors.

Poor seedling recruitment to larger size classes within high mistletoe-infection canopy patches can also be attributed to higher herbivory (Chapter 2), or to most of the seedlings dying during the cold winter months, hence the dominance of small sized plants. In contrast poor seedling establishment in the intercanopy spaces is probably due to fewer seed dispersing here as its further from the parent

trees, or lack of critical soil resources for successful germination and seedling recruitment within these spaces.

However, high mistletoe-infection intercanopy spaces also had significantly higher numbers of juvenile *V. karroo* trees showing that they can successfully germinate further away from the canopy (Chapter 5). These results agree with O'Connor (1995) who reported that *V. karroo* seedlings can be both facultative sciophytes and heliophytes that thrive in shaded or unshaded areas, (respectively). This has been observed in other *Vachellia* (*Acacia*) seedlings, for example *V. tortilis* have been reported to have seedlings further away from the canopy of the trees as their germination is better in the open rather than inside the canopy patches (Witkowski and Garner 2000, Barnes, 2001; Bhadouria *et al*., 2016). Abundance of seedlings in high mistletoe-infection intercanopy spaces could be due to effective seed dispersal by livestock to open areas, where seedlings can establish and progress to larger stage classes (O'Connor *et al*., 2010). Nonetheless, if soil nutrients are high in the presence of grasses, seedling growth is likely to be hindered by competition because grasses are better competitors and can suppress seedling growth, establishment and performance (Bhadouria *et al*., 2016). Similar to the findings by Lamont *et al*. (1993) high mistletoe-intercanopy spaces like high infection-canopy patches are also safe sites for juveniles and even safer sites for saplings.

Although *V. karroo* trees are long-lived and they have low adult mortality rates, the reproduction of *V. karroo* trees in MRS is evidently negatively affected by high mistletoe-parasitism. On average, there are 193 mistletoe-infected *V. karroo* trees/ha (Chapter 5) and if mistletoe parasitism causes high tree mortality, then lower reproductive capacities is a concern. Moreover, with mature *V. karroo* trees being harvested for firewood (personal observation), the *V. karroo* population is under serious threat from a number of disturbances and stress factors at the study site. Therefore, there is a need to further investigate mortality and persistence of mistletoe-infected *V. karroo* trees in this semi-arid savanna.

Conclusion

The study shows that indeed high mistletoe-infection can reduce the regeneration capacity of the host *V. karroo* trees by reducing the number of flowers, pods and pod length, number of seeds/pod, and geminable seed production/tree, as well as the seed bank after seed dispersal. This was attributed to physio-morphological changes to the canopy and competition between the host and the mistletoes for resources. However, high mistletoe-infection canopy patches had more juveniles compared to the other microhabitats showing that mistletoes are facilitating heterogeneity in the recruitment of *V. karroo* trees. Intermediate light conditions, higher soil moisture, and increased nutrients compared to low mistletoe-infection canopy patches could have facilitated this greater establishment of seedlings and saplings below the high mistletoe-infected trees. Thus, high mistletoe-infection canopy patches were safe for *V. karroo* juveniles, compared with low infection canopy patches being safer sites for

seed banks. Furthermore, high mistletoe-infection seeds dispersed further away into the intercanopy spaces, landing on suitable germination sites, increasing regeneration success, with saplings being abundant here. Therefore, high mistletoe-infection intercanopies were safer for saplings, whilst their adjacent canopy patches were safer sites for seedlings especially before the cold-dry season.

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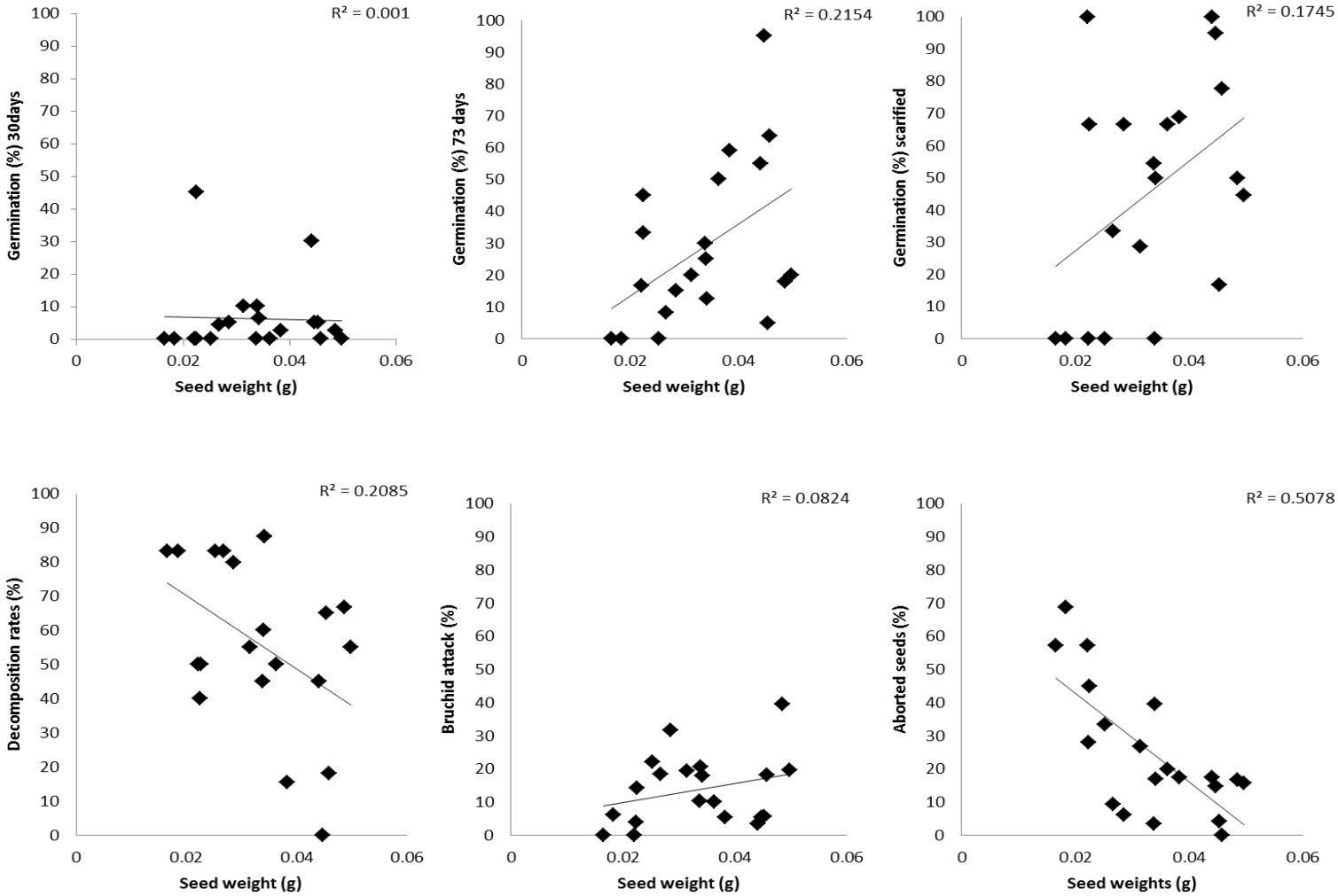
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Appendices

Appendix 1. Mean (standard error) from the General linear model (GLM) analyses of the effects of year of collection (2018, 2019), mistletoe infection (high *vs.* low), and their interactions on flower buds/tree, flowers/tree, pods/tree, pod diameter (cm), pod length (cm), seeds/pod, seed production/tree, Germinable seed/tree collected. *, data collected from 2018-2020.

Variable	**Year (Mean ± SE)**			**Infection degree (Mean ± SE)**		**Year × Infection degree (Mean ± SE)**					
	2018	2019	2020	High	Low	High 2018	Low 2018	High 2019	Low 2019	High 2020	Low 2020
Flower buds/tree	571 ± 75.11	582 ± 99.66		433 ± 92.20	719 ± 70.22	426 ± 103	715 ± 92.36	441 ± 159	723 ± 111		
Flowers/tree	593 ± 121	589 ± 144		288 ± 67.35	894 ± 146	309 ± 86.56	876 ± 191	267 ± 108	912 ± 231		
Pods/tree	552 ± 111	608 ± 136		298 ± 103	862 ± 109	274 ± 101	830 ± 156	322 ± 186	893 ± 161		
Pod diameter (cm)	0.66 ± 0.03	0.65 ± 0.01		0.64 ± 0.03	0.67 ± 0.02	0.62 ± 0.05	0.69 ± 0.03	0.66 ± 0.02	0.65 ± 0.02		
Pod length (cm)*	10.07 ± 0.55	10.65 ± 0.32	9.68 ± 0.42	9.40 ± 0.38	10.87 ± 0.29	8.92 ± 0.87	11.22 ± 0.47	10.37± 0.40	10.93 ± 0.50	8.90 ± 0.58	10.45 ± 0.53
Seeds/pod*	7.56 ± 0.45	8.19 ± 0.22	7.52 ± 0.42	7.20 ± 0.32	8.31 ± 0.26	6.34 ± 0.58	8.78 ± 0.44	8.40 ± 0.24	7.97 ± 0.37	6.86 ± 0.59	8.18 ± 0.54
Seed production/tree	4660 ± 1020	4701 ± 1031		2273 ± 820	7088 ± 905	1950 ± 760	7370 ± 1477	2596 ± 1497	6806 ± 1124		
Germinable seed/tree*	1789 ± 429	1756 ± 386		599 ± 216	2947 ± 376	514 ± 200	3065 ± 614	684 ± 395	2830 ± 467		

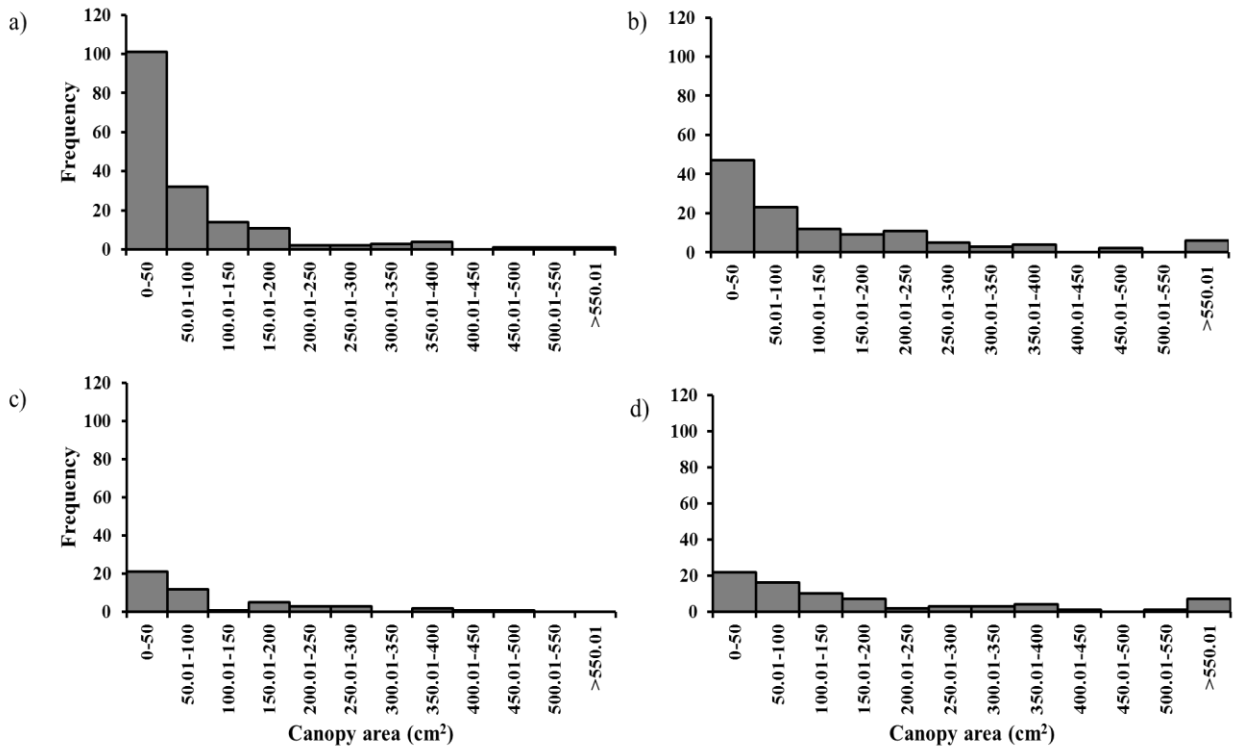
Appendix 2. Regression between mean seed weight/tree and (a) percentage germination after 30 and (b) 73 days, (c) percentage germination of scarified seeds, (d) percentage decomposition, (e) proportion of seeds attacked by bruchids and (f) proporion of aborted seeds



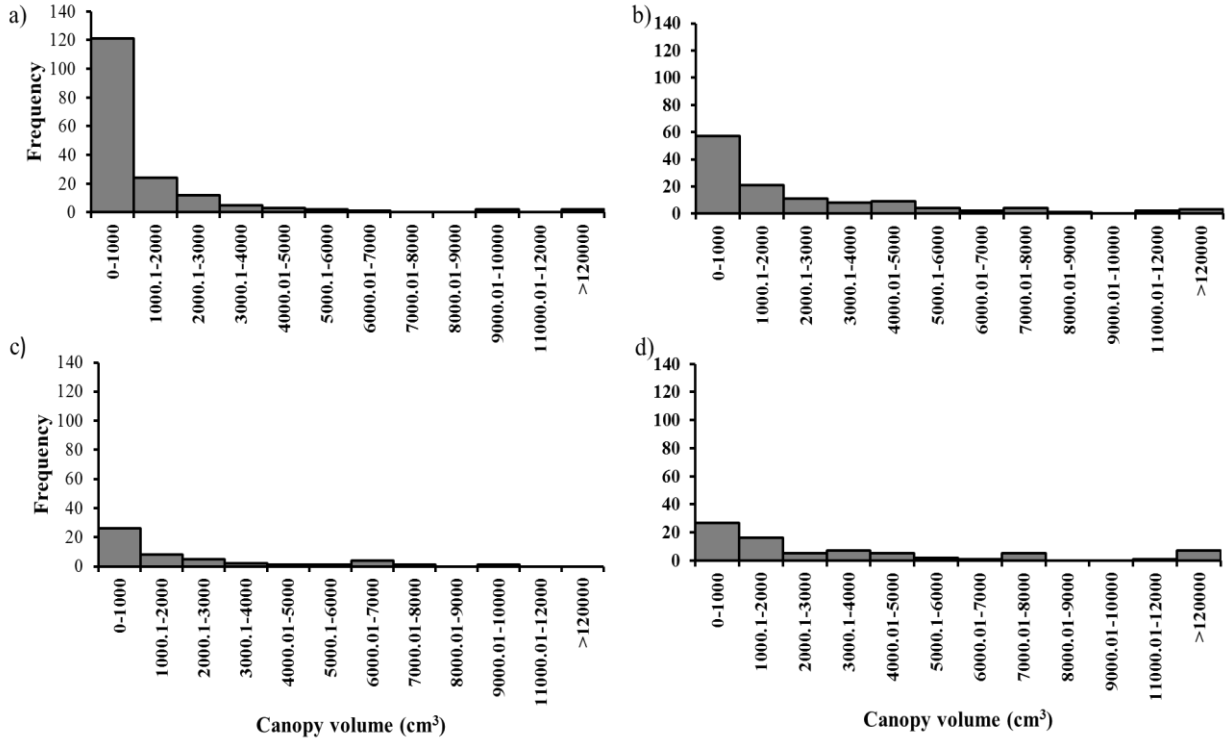
Appendix 3. Comparisons of stem diameter, height size, canopy area and volume classes among microhabitats using the two-sample Kolmogorov-Smirnov test.

Microhabitat	Stem diameter (cm)		Height (cm)		Canopy area (cm^2)		Canopy volume (cm^3)	
	D	***P***	**D**	***P***	**D**	***P***	**D**	***P***
High mistletoe-infection								
Canopy patch *vs*. Intercanopy	0.2	0.988	0.16	0.996	0.25	0.847	0.25	0.847
Low mistletoe-infection								
Canopy *vs*. Intercanopy	0.2	0.988	0.42	0.249	0.25	0.848	0.33	0.518
Canopy patches							0.25	0.847
High *vs*. Low	0.3	0.759	0.42	0.249	0.17	0.996		
Intercanopy spaces								
High *vs*. Low	0.2	0.988	0.33	0.518	0.17	0.996	0.25	0.847

Appendix 4. Distribution of canopy area sizes of *Vachellia karroo* juveniles within (a) high mistletoe-infection canopy patches, (b) high mistletoe-infection intercanopy spaces, (c) low mistletoe-infection canopy patches and (d) low mistletoe-infection intercanopy spaces.



Appendix 5. Distribution of canopy volume sizes of *Vachellia karroo* juveniles within (a) high mistletoe-infection canopy patches, (b) high mistletoe-infection intercanopy spaces, (c) low mistletoe-infection canopy patches, and (d) low mistletoe-infection intercanopy spaces.



CHAPTER 5

Spatial distribution analysis of woody plants in relation to mistletoe-infected trees in a semi-arid African savanna

Abstract

Mistletoe-infected trees increase resource heterogeneity within semi-arid savannas and hence can restructure plant community processes and distribution patterns. Little or no information is available on how mistletoe infected *Vachellia karroo* trees are spatially distributed and on how they influence the spatial patterns of their surrounding conspecifics and heterospecifics. In this study, each woody individual was stem mapped using a Cartesian plane (x, y) within three 50 × 50m plots (each with >30 mistletoe-infected trees) located within *V. karroo* dominated stands in a semi-arid savanna, in South-West Zimbabwe. Second order statistics such as pair correlation $g(r)$ and the $L(r)$ function were used for univariate and bivariate analysis, whilst the mark correlation function ($k_{mm}(r)$) was used to analyse correlation of canopy area and mistletoe infection intensity. Mistletoe-infected tree density ranged from 128-244 trees/ha and the univariate analysis of mistletoe-infected trees was consistent with a random pattern. This pattern could have been a result of unsystematic mistletoe seed dispersal patterns by birds on the already randomly distributed mature trees. Nonetheless, the high abundance of clustered seedlings and saplings resulted in the univariate analysis of all the woody species and *V. karroo* trees (independently) thus exhibiting aggregation at small scales. Likewise, the bivariate analysis between mistletoe-infected trees and all the woody seedlings, saplings, and shrubs, predominantly displayed significant clustering at small scales (<5m) and random patterns as distances increased. However, mistletoe-infected trees and their conspecific seedlings and saplings in the plot with intermediate mistletoe-infected tree density showed significant repulsion at shorter distances. Lastly, the bivariate analysis between mistletoe-infected trees and their conspecific shrubs and both heterospecific and conspecific uninfected mature trees was consistent with a random pattern. These results show that there are differences in the ways in which mistletoe-infected trees influence the heterospecific and conspecifics woody species, which may also vary among different stage classes. Heterospecific seedlings (and/or saplings) largely benefit from facilitation or nurse protégé interactions within the shaded and resource-rich canopy patches of mistletoe-infected trees. In contrast, although *V. karroo* juveniles may also benefit from high resource patches, they seem to be repelled by inter- and intraspecific competition. Thus, juvenile conspecifics may benefit by being dispersed further away from putative parent plants, to where there is higher light and less competition. However, the random patterns shown between mistletoe-infected trees and their conspecific shrubs and mature uninfected trees could imply that there is both facilitation and competition, depending on the extent of mistletoe weakening of host trees. Hence, there is facilitation arising from high mistletoe parasitism, with low competition and high litter quantities in contrast to high competition when mistletoes are fewer as hosts try to compensate for losses to the mistletoes. This changes the regular distribution often exhibited by large savanna trees. Thus, these results show that mistletoes alter distribution patterns and spatial heterogeneity in semi-arid savannas.

Key words: Bivariate analysis, competitive exclusion, facilitation, mistletoes, semi-arid savannas, spatial patterns, stage-classes, univariate analysis, *Vachellia karroo*.

Introduction

A spatial point pattern within plant communities is a unique order or arrangement of individual plants in a set of points or in patches within a mapped area (Philips and MacMahon, 1981; Dale, 2000; Fortin and Dale, 2005). A spatial point pattern uses distance between individuals as a measure of the spatial relationship within a population (Pablo and Gusman, 2017) and it computes the distribution and coexistence of plants within an observational space (Martinez *et al*., 2010; Velázquez *et al*., 2014). It is useful in showing the scales at which a particular pattern is significant and the scale at which plant size classes are negatively or positively correlated (Fortin and Dale, 2005; Martinez *et al*., 2010). In plant communities, spatial patterns are often shaped by morphological (size and growth patterns of plants), environmental (biotic and abiotic), and phytosociological factors, which are linked to how one species influences the occurrence of another (allelopathy and nurse protégé interactions) (Barot, 1999; Dale, 2000; Cheng *et al*., 2014; Jiang *et al*., 2017; Muvengwi *et al*., 2018; Tamjidi and Lutz, 2020). Furthermore, the spatial distribution of plants can also influence the presence or absence of species and can change how resources are utilised (Barot, 1999).

In semi-arid savannas, plant diversity, structure and community spatial patterns are negatively or positively influenced by heterogeneity in soils, topography, termite mounds, large trees, and fire (Dale, 2000; Meyer *et al*., 2008; Rayburn *et al*., 2011; Schleicher *et al*., 2011; Muvengwi *et al*., 2017, 2018, 2020; Svatek *et al*., 2018). Large-sized trees are keystone as they accumulate nutrients and increase available water within their subcanopies, both limiting resources in semi-arid environments (Belsky, 1994; Kanz, 1996; Muvengwi *et al*., 2015). These localised contributions result in large trees becoming fertility islands, with relatively higher subcanopy establishment of seedlings of different woody species expected, leading to aggregation of small size classes (Belsky, 1994). Pillay and Ward (2012) showed that *Vachellia karroo* (Hayne) Banfi & Galasso seedlings were aggregated at small scales and limited to the zone of influence (10m) of mature *V. karroo* trees. This results in large trees depicting nurse plant syndrome (Flores and Jurado, 2003).

Many studies have looked at the spatial patterns and distribution of mistletoes on their hosts particularly at large scales (Overton, 1996; Aukema, 2004; Kavanagh and Burns, 2012; Sayad *et al*., 2017). However, few of these studies have focused on how mistletoe-infected trees can influence the spatial patterns of the understory trees in their localized environments. Studies have focused on how mistletoe-infected trees improve soil physical and biochemical properties through; 1) depositing litter with high nutrient levels; 2) higher leaf turnover; 3) highly decomposable leaf litter; 4) facilitation of host litter decomposition; 5) and through droppings and other debris from birds and animals visiting the mistletoes (Ndagurwa *et al*., 2013, 2014, 2015, 2020; Mellado *et al*., 2016; Mellado and Zamora, 2017; Chapter 2). Because mistletoes are aerial, their litter falls below the host, leading to small and localized nutrient patches (Press and Phoenix, 2004; Ndagurwa *et al*., 2014, 2016). Thus, they are likely to alter the immediate understory spatial patterns by permitting facilitation of different tree

species of smaller size classes (Press and Phoenix, 2004). Therefore, through further enhancing soil nutrients and occasionally soil moisture (Ndagurwa *et al*., 2013, 2014, 2015, 2020; Chapter 2), mistletoe-infected *V. karroo* canopy patches have the potential to become 'safe sites', which allow regeneration and enhanced local recruitment for certain species i.e., mistletophily (Chapter 3). Hence, due to facilitation clustering of both conspecific and heterospecific woody species of smaller stage classes at shorter distances rather than at longer distances from the mistletoe-infected tree is expected (Fortin and Dale, 2005).

Mistletoes are also hemi-parasitic, obtaining resources through a haustorium, which penetrates and transports solutes from the host plant tissue (Preston *et al*., 2010; Arruda *et al*., 2012). They can kill the host or supress the host's morphology and physiology through high transpiration rates, interfering with the host's biomass allocation patterns (Sala *et al*., 2001; Press and Phoenix, 2004; Arruda *et al*., 2012; Sayad *et al*., 2017).These negative changes reduce the dominant hosts' competitiveness thus facilitating the establishment and growth of subordinate understory plants and neighbouring shrubs and mature trees, subsequently leaning the spatial pattern towards aggregation (Pennings and Callaway, 1996; Spasojevic and Suding, 2011; Muvengwi *et al*., 2015; Ndagurwa *et al*., 2016, 2018). However, studies have shown contradictory results on soil moisture levels within mistletoe-infected tree canopy patches i.e., decrease in soil moisture (Ndagurwa *et al*., 2014b) and increase in soil moisture due to mistletoe parasitism (Chapter 2). Therefore, since soil moisture is critical for seed germination and seedling establishment (Wilson and Witkowski 1998), aggregation is uncertain underneath mistletoe-infected trees as competition for water can still occur. For that reason, there is need to investigate the distribution patterns linked to mistletoe-infected trees.

No known study has investigated how mistletoe-infected *V. karroo* trees in semi-arid savannas influence the spatial distribution of their surrounding con- and hetero-specifics of different size classes. Therefore, this study assessed the spatial distribution of mistletoe-infected *V. karroo* trees in a semi-arid savanna in South-west Zimbabwe, using spatial point pattern analysis. Due to dispersers revisiting the same host trees it was hypothesized that host trees will have a high intensity of mistletoes within their canopies (Mora-Pinto, 2005; Mellado and Zamora, 2017). It was also expected that mistletoe-infected trees will exhibit clustering due to transmission by dispersers visiting trees that are closer together. In order to ascertain the probable relationship and patterns of mistletoe-infected *V. karroo* trees with different stage classes, infected tree canopy patches were assumed to be 'safe sites' for a diversity of nurse plants due to high nutrients, and it was hypothesized that seedlings and saplings would aggregate at small scales around infected *V. karroo* trees, due to weakening of the competitive edge of the host despite possible soil moisture limitation. Additionally, it was hypothesised that the inter-tree distances of mistletoe-infected mature trees with uninfected shrubs and mature trees would increase and result in a regular spatial pattern due to density dependent thinning.

Methodology

Study area

The study was carried out at the 28 000 ha Matopos Research Station (MRS) South-West Zimbabwe (20º 31'S, 28º 31'E, 1340 m a.s.1.), 30 km from Bulawayo (Chirara *et al*., 1998; Moyo *et al*., 2011; Mupangwa *et al*., 2013). MRS receives a mean annual precipitation of 586 mm whilst the mean annual temperature is 18ºC, and has both loamy sand and clay loam soils. These fine textured soils are dominated by *V. karroo* trees that are indigenous to southern Africa. At MRS, *V. karroo* is one of the main hosts of mistletoes such as *Viscum verrucosum* Harv (Viscaceae), *Pliocosepalus kalachariensis* (Schinz) Danser (Loranthaceae) and *Erinthemum ngamicum* (Sprague) Danser (Loranthaceae) (Ndagurwa and Dube, 2013; Ndagurwa *et al*., 2013, 2016). Consequently, the focus of this study was on *V. karroo*, a host that has been observed to offer high resource longevity to mistletoes. Therefore, stands with large, tall and older *V. karroo* trees were targeted as these tree characteristics due to their greater ability of these trees to provide resources (Press and Phoenix, 2004; Ndagurwa *et al*., 2012).

Woody species mapping and measurements

Sampling was done between March and April in 2018. Three relatively homogenous (little different in physical environmental (very flat slope) or herbivory) plots measuring 50 × 50 m (0.25 ha) were randomly selected within *V. karroo* dominated patches. These large plots ensured sampling of at least 30 mistletoe-infected *V. karroo* trees, which is the minimum number for detecting significant spatial autocorrelation (Fortin and Dale, 2005). Using the point-pattern analysis, the location of every woody plant was stem-mapped (x–y coordinates) on a Cartesian plane. The Cartesian plane specifies each point uniquely in space by a pair of numerical coordinates, i.e., distances to the point from two fixed perpendicular directed lines (Dale, 2000; Pillay and Ward, 2012). The plots were subdivided into ten, 5 × 50 m transects to ensure the accuracy of the coordinates. For trees with a single stem base, the coordinates of the stem base were taken whilst for multistemmed plants the canopy centroid coordinates were recorded. For each tree, the species and its size (height, stem diameter, long and perpendicular crown diameter (D_1 and D_2), canopy area ($\pi(D_1/2)(D_2/2)$), presence and species of mistletoes) were measured. Individual woody species were classified using height and stem diameter into four stage classes, seedlings (stem diameter, ≤ 0.19; height, ≤ 64cm), saplings (stem diameter, ≥ 0.20 ≤ 1cm; height, ≥ 0.65 ≤ 2m), shrubs (≥ 1.01 ≤7cm; height, ≥ 2 ≤ 3m) and mature trees (stem diameter, ≥ 4cm; height, ≥ 3.01m).

Data analysis

Statistical analyses were conducted using *R* software (www.r-project.org). Data were first tested for normality using the Shapiro-Wilk test prior to any analysis. Most of the data were not normally distributed therefore the non-parametric alternatives were used.

Analyses of mistletoe infected Vachellia karroo structural attributes

Among the four plots the height, stem diameter, canopy area of all the mistletoe-infected trees were compared using the Kruskal-Wallis test followed by a post hoc Kruskalmc for non-parametric data ($P = 0.05$). Regressions between mistletoe counts with tree height, stem diameter, and canopy area were analysed using the Spearman correlation test.

Voronoi tessellations

Voronoi tessellations were used to determine the closest surrounding area of influence of each mistletoe-infected tree, with the respective infected tree at the centre of the area, compared to any other infected tree in each plot (Getzin *et al*., 2015; Muvengwi *et al*., 2018). The importance of the voronoi tiles is based on the distribution of the tile areas and occurrence of hexagonal tiles in each plot, i.e., the greater the number of tiles with six sides the more regular the pattern (Getzin *et al*., 2015; Muvengwi *et al*., 2018). Only tiles that were not found at the edges of the plots were included in this analysis due to the area and number of sides of tiles found at the edges being inflated (Muvengwi *et al*., 2018). The package *deldir* was used to compute the voronoi tessellations. The differences in the number of sides, tile area and density and nearest neighbour distances between the three plots were analysed using Kruskal-Wallis tests, followed by a post hoc Kruskalmc.

Nearest neighbour distance analysis

In order to supplement second order statistics and to detect competition between mistletoe-infected trees, the nearest neighbour analysis was used (Muvengwi *et al*., 2018b). The nearest neighbour analysis estimates the likelihood of finding the nearest mistletoe-infected tree within a distance r of the representative mistletoe-infected tree in each plot, using the package *spatstat*.

Univariate analysis

The package *spatstat* (Baddeley *et al*., 2015) was used to analyse the univariate distribution within each plot of (1) all the different woody plant species (2) *V. karroo* only, (3) mistletoe-infected *V. karroo* trees and (4) all mature trees (inclusive of mistletoe-infected trees). Both the pair correlation $g(r)$ and the Ripley's $K(r)$ functions were used to determine the spatial patterns. The Ripley's K function $K(r)$ includes first order effects such as environmental attributes, it is accumulative and it muddles effects at larger scales with effects at finer scales (Barot, 1999; Condit *et al*., 2000; Getzin, 2006; Jiang *et al*., 2017, Muvengwi *et al*., 2018). K(r) is defined as the estimated number of points in a circle of radius r positioned at a random point, divided by the intensity (λ) of the pattern. However, in this study, the Ripley $K(r)$ function was incorporated by using the transformed L-function which can stabilize the variance of the Ripley $K(r)$ function and makes the visualisation of the graph simpler for evaluating deviations (Velázquez *et al*., 2014; Baddeley *et al*., 2015). The *L(r)* function is also apt when confirming null models. The $L(r)$ function is presented as:

$$L(r) = \sqrt{\frac{K(r)}{\pi}} - r \qquad (1)$$

However, it is difficult to interpret the range and intensity of the interaction between trees using the Ripley's *K* function, therefore, the pair correlation function was also used. Unlike the *K*(*r*) function, the pair correlation function uses standardised density to describe patterns (Getzin, 2006) and it is non-cumulative, provides better interpretation of neighbourhood density and can detect and remove specific distances wherein there are violations to the null hypothesis (Velázquez *et al*., 2014; Muvengwi *et al*., 2018b). Furthermore, the pair correlation unlike the Ripley's *K*-function uses the probability density function which is intuitive in evaluating scale dependent effects. Consequently, the pair correlation function does not muddle effects at shorter with those at longer distances thus reveals the different scales of a pattern at all distances (Moustakas *et al*., 2008; Muvengwi *et al*., 2018). The pair correlation approximates the probability of discovering a tree at distance *r* from a representative focal point, normalized by divided by the intensity (λ) of the pattern (Dohn *et al*., 2017). Therefore, *g*(*r*) details the spatial structure i.e., aggregation and regularity at known radius *r* based on inter tree distances:

$$g(r) = \frac{K`(r)}{2\pi r} \; for \; \geq 0 \qquad (2)$$

For a pair correlation, a regular pattern has fewer neighbours than in a completely random pattern shown by $g(r) < 1$, whilst a clustered pattern is shown by a larger number of trees within distance *r*, than expected under complete spatial randomness i.e., $g(r) > 1$, and a complete spatial randomness shown by $g(r) = 1$. For all the point pattern analyses, Ripley's isotropic correction which accounts for edge effects was applied. Furthermore, in order to determine if there is a significant difference between the actual observed pattern and null model of complete spatial randomness (CSR), all the summary statistics (univariate and bivariate pattern analyses) were assessed using 199 Monte Carlo simulations (5^{th} lowest and 5^{th} highest value). These generated 95% simulation envelopes from which assessment of departure of the observed pattern from the null model was quantified (Baddeley *et al*., 2014, 2015). Furthermore, the Diggle-Cressie-Loosmore-Ford test (DCLF) was used to investigate whether the observed spatial patterns were significantly different from the null hypothesis of complete spatial randomness since envelopes are susceptible to Type 1 error (Velázquez *et al*., 2014; Baddeley *et al*., 2014; Dohn *et al*., 2017).

Bivariate analysis

The bivariate point-pattern analysis is used when analysing two species of plants or two stage classes of the same species, on whether their distribution is independent of each other (Dale, 2000; Wiegand and Moloney, 2013). Bivariate analysis can describe the patterns of each of the types of species or stage classes, and how they are related (Dale, 2000). Clustering shows a positive correlation between

two groups of plants, whilst a regular pattern shows repulsion between the two groups of plants and lastly patterns exhibiting no correlation between the groups are shown by a random pattern. Thus, the patterns of interaction between infected trees and all the other woody species and between mistletoe-infected trees and the varying stage classes (seedlings, saplings, shrubs and uninfected mature trees) were tested using bivariate statistics i.e. bivariate estimator for pair correlation, $g_{12}(r)$ function:

$$g_{12}(r) = \frac{K'_{12}(r)}{2\pi r} \qquad (3)$$

and the *L*-function bivariate estimator:

$$L_{12}(r) = \sqrt{\frac{K_{12}}{\pi}} - r \qquad (4)$$

Wherein $g_{12}(r)$ and $L_{12}(r)$ are the probable densities of points of pattern 2 at distance *r* of a random point of pattern 1, divided by the intensity λ_2 of pattern 2 (Getzin *et al*., 2006).

Mark correlation function

The mark correlation function $k_{mm}(r)$ for quantitative marked patterns was used to investigate if canopy area or mistletoe counts on pairs of infected trees exhibited any spatial correlation dependent on their distance *r*. The relationship between the mistletoe-infected trees (real valued marks) and their characteristics (canopy area, mistletoe numbers) was quantified for positive real-value marks, as *f* (m, m′) = mm′, (Baddeley *et al*., 2015). There were three possible outcomes of the relationship between the marks, i.e., if m × m′ of canopy area or mistletoe numbers of two trees separated by distance *r* were equal to the overall marks mean μ, i.e., if $k_{mm}(r) = 1$, then there is no spatial correlation, if $k_{mm}(r) > 1$, there is a positive association (correlation), and lastly if $k_{mm}(r) < 1$ there is a negative association (Muvengwi *et al*., 2018). Furthermore, the Poisson null model which assumes complete spatial randomness was used to generate pointwise envelopes for the summary function $k_{mm}(r)$, from 39 simulations of random labelling of the mistletoe-infected trees (Baddeley *et al*., 2014).

Results

The total number of woody plants and the stand density was highest in $plot_2$ and lowest in $plot_1$ (Table 1). The distribution and location of all the woody plants within each plot is shown in Fig. 1a-c. $Plot_3$ had the lowest species richness, whilst $plot_2$ had the highest, with *V. karroo* being the most dominant species for $plot_1$ (86.9%) and $plot_2$ (74.1%), but not $plot_3$ (23.3%), which was dominated by smaller stage classes of *Ziziphus mucronata* (49.1%), (Appendix 2). All the plots were dominated by saplings whose percentage abundance across the plots ranged from 73.9% to 79.5% (Appendix 1). The stem diameter and height across the plots ranged from 0.1-14.27cm and 0.11-5.83m (Table 1, Appendix 1).

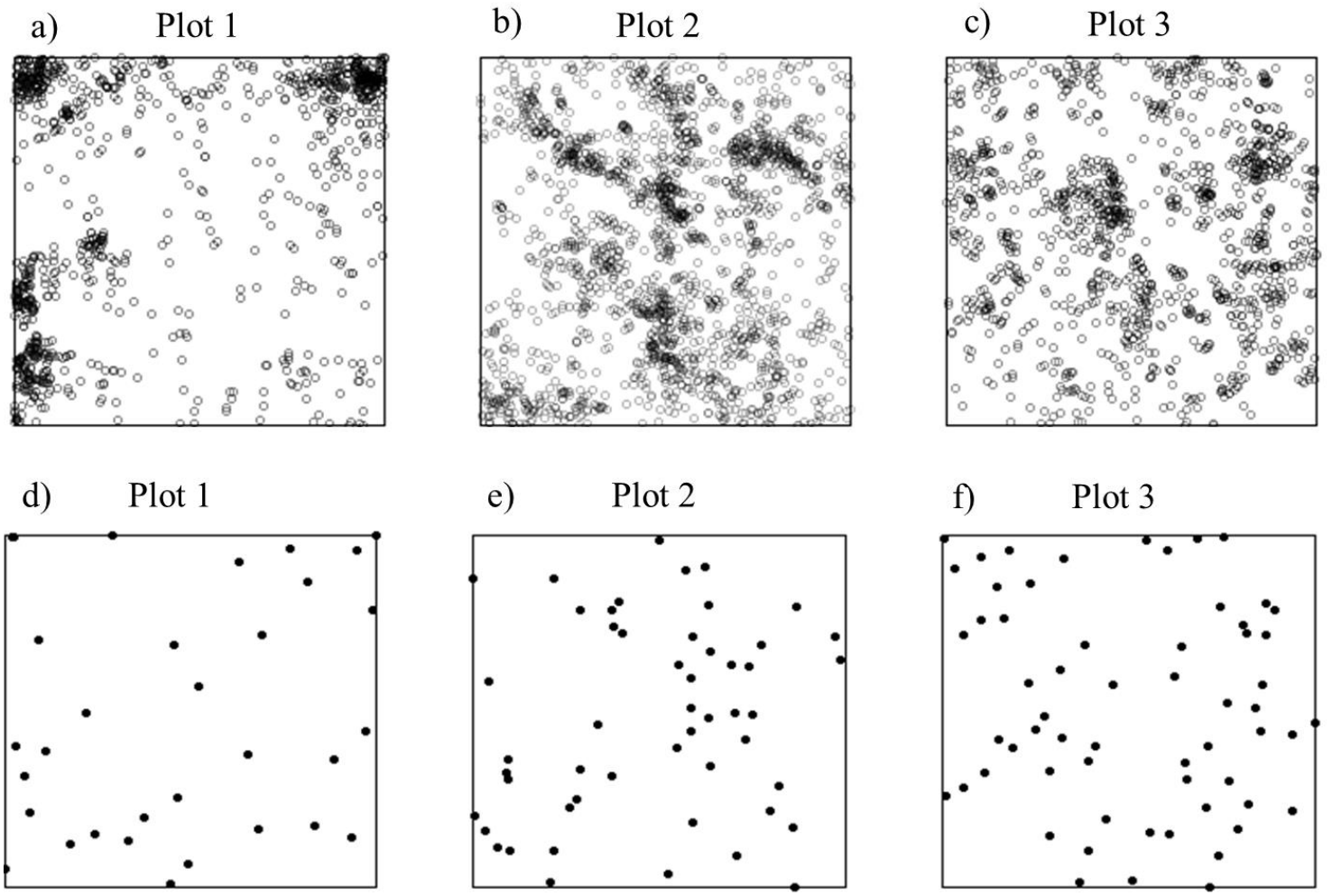


Fig. 1. Spatial distribution maps of all the woody species (a-c) and mistletoe-infected trees (d-f) recorded within the three 0.25 ha (50 × 50m) plots, for plot 1 to 3, respectively.

Table 1. Number of woody plants, stand density/ha, stem diameter and height ranges/plot and number of woody species in plot 1 to 3, respectively.

Parameter	Plot number		
	Plot 1	Plot 2	Plot 3
Total woody plants	1100	2174	1273
Stand density/ha	4400	8588	5092
Stem diameter range/plot (cm)	0.10-14.27	0.10-12.56	0.10-10.00
Height range/plot (m)	0.11-5.19	0.15-5.83	0.12-5.38
Number of species	11	13	10

Mistletoe-infected tree structural attributes

The number (Fig. 1d-f) and density of mistletoe-infected trees was generally higher in $plot_3$ and lowest in $plot_1$ (Table 2). There was a significant difference in the stem diameter (Kruskal Wallis χ^2 = 9.33, *df* = 2, *P* = 0.009), canopy area (Kruskal Wallis χ^2 = 7.03, *df* = 2, *P* = 0.03) and mistletoes/tree counts (Kruskal Wallis χ^2 = 8.42, *df* = 2, *P* = 0.01) on mistletoe-infected *V. karroo* trees across the three plots (Table 2). However, mean height was not significantly different (Kruskal Wallis χ^2 = 0.35, *df* = 2, *P* = 0.83). Uninfected mature trees tended to be smaller in height and DBH than mistletoe-infected mature trees (Fig. 2). In each of the three plots there was a significant (*P* < 0.05) positive correlation between mistletoe counts and tree height, diameter, and canopy area (Appendix 3a-b).

Table 2. Total number and density of all mature *V. karroo* trees, number and density of mistletoe-infected *Vachellia karroo* trees (Proportion of infected *V. karroo* trees in relation to the total of all the mature trees) and mistletoe-infected *V. karroo* stem diameter, height, canopy area, mistletoes/tree (mean ± S.E, and range) in the three plots. Means in rows not sharing a small common letter are significantly different (Kruskalmc, *P* < 0.05).

Parameter	Plot number		
	Plot 1	Plot 2	Plot 3
Total number of mature trees	42	77	120
Total density of mature *V. karroo* trees	168	308	480
Number of infected trees	32	52	61
Density of infected trees/ha	128 (76%)	208 (68%)	244 (51%)
Stem diameter (cm)	15.76 ± 1.40^{a}	13.85 ± 0.89^{ab}	11.80 ± 0.67^{b}
Height (m)	5.67 ± 0.37^{a}	6.20 ± 1.67^{a}	6.18 ± 0.22^{a}
Canopy area (m^2)	28.11 ± 3.42^{a}	21.06 ± 2.19^{ab}	18.76 ± 1.80^{b}
Mistletoes/tree counts	3.21 ± 0.42^{b}	5.37 ± 0.76^{a}	4.12 ± 0.72^{ab}
Mistletoes/tree (range)	1-9	1-25	1-31

Voronoi tessellations

The mean number of edge counts surrounding each tree location did not significantly differ (Kruskal Wallis χ^2 = 0.69, *df* =2, *P* = 0.71) across the three plots (Table 3, Fig. 3). All the three plots were dominated by hexagonal shapes around each mistletoe-infected tree, and $plot_1$ had the highest percentage of tiles with hexagonal shape (50% of all observed tiles) compared to $plot_2$ and $plot_3$ (39%,

38% of all observed tiles, respectively). $Plot_1$ had a greater mean tile area (Kruskal Wallis $\chi^2 = 18.39$, $df = 2$, $P < 0.001$) than $plot_2$ and $plot_3$. However, the mean tile density was higher in $plot_2$ and $plot_3$ compared to $plot_1$.

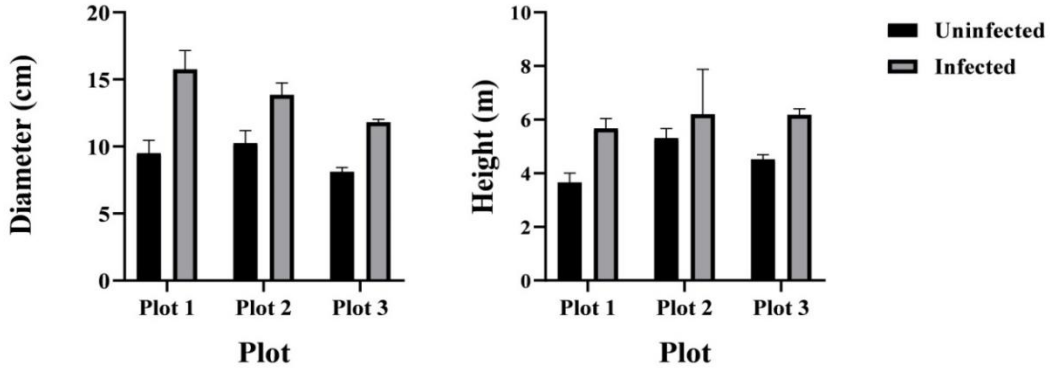


Fig. 2. Comparison (mean ± SE) of a) stem diameter and b) height of mistletoe-infected and uninfected mature trees within each plot.

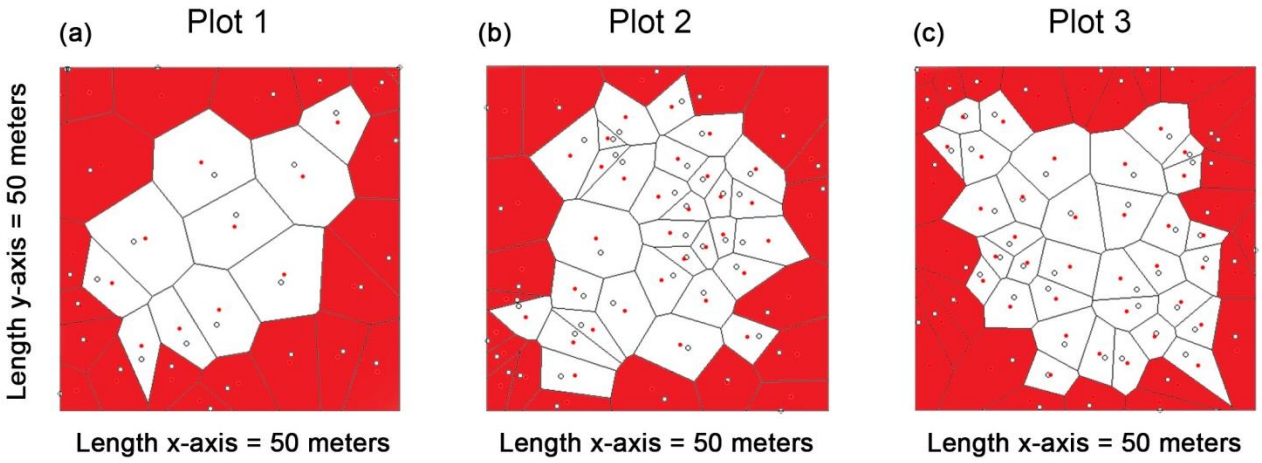


Fig. 3. Voronoi tessellations (analysed without edge tiles coloured in red) around each mistletoe-infected *Vachellia karroo* trees within 0.25ha (50 × 50 m) plots, plot 1 to 3, (a-c, respectively). The open circles and red dots are showing the data points and tile centroids respectively. The tile for each point is the closest surrounding area around each infected tree compared to any other infected tree.

Table 3. The mean ± SE of number of tile sides, tile area, tile density and nearest neighbour distances compared across the three plots. Means in rows not sharing a small common letter are significantly different (Kruskalmc, $P < 0.05$).

Parameter	Plot number		
	Plot 1	Plot 2	Plot 3
Number of sides	6.00 ± 0.30^a	5.74 ± 0.24^a	5.79 ± 0.20^a
Tile area m^2	107.76 ± 1.40^a	41.63 ± 4.15^b	47.18 ± 8.09^b
Tile density (tiles/ha)	128	204	240
Nearest neighbour distances (m)	5.77 ± 0.52^a	3.84 ± 0.33^b	3.68 ± 0.16^b

Nearest neighbour

There was a significant difference (Kruskal Wallis test: $\chi^2 = 19.90$, $df = 2$, $P < 0.001$) in the mean nearest neighbour distances of mistletoe infected trees across the three plots. $Plot_1$ had the longest mean nearest neighbour distance, whilst both $plot_2$ and $plot_3$ had the shortest and similar nearest neighbour distances (Table 3).

Univariate analysis

The univariate analysis of all the woody species in each of the three plots showed strong evidence of interspecific aggregation ($g(r)>1$), and the pattern significantly deviated from the null model of spatial randomness (Table 4) at distances from 0m-11m, 0-7.2m, and 0-6m for plots 1-3, respectively (Fig. 4a-c). Thereafter, $plot_1$ was regular whilst plot 2 and 3 tended to be random. Likewise, the $L(r)$ function in all the three plots showed significant clustering at distances between 0-12m for all woody species and for all *V. karroo* trees (Appendix 4).

Similarly, the spatial pattern (g(r)) for all the *V. karroo* trees (Fig. 4d-f) confirmed intraspecific aggregation at distances 0-11m, 0-6m, and 0-5.7m for plots 1-3, respectively, and the patterns deviated significantly (DCLF test: $P < 0.05$, Table 4) from the null model of complete spatial randomness (CSR). However, all the mature trees (Appendix 6) and mistletoe-infected trees showed a completely random pattern ($g(r) = 1$) across the three plots (Fig. 4g-i, Table 4) as the patterns did not deviate from the null model of CSR (DCLF test: $P > 0.05$, Table 4). Consistently, the $L(r)$ function showed random spatial distribution of all the mature and mistletoe-infected trees across the three plots.

Table 4. Results of the Diggle-Cressie-Loosmore-Ford Test (DCLF) of complete spatial randomness under Monte Carlo, based on 199 simulations with fixed number of points

	Plot number	u-value	*P*	rank
All woody species	1	131.98	0.01	1
All woody species	2	6.28	0.01	1
All woody species	3	4.82	0.01	1
V. karroo only	1	150.71	0.01	1
V. karroo only	2	17.08	0.01	1
V. karroo only	3	29.23	0.01	1
V. karroo with mistletoes	1	21.33	0.08	8
V. karroo with mistletoes	2	0.50	0.95	95
V. karroo with mistletoes	1	21.33	0.08	8
All mature trees	1	24.90	0.07	7
All mature trees	2	4.91	0.12	12
All mature trees	3	0.53	0.74	74

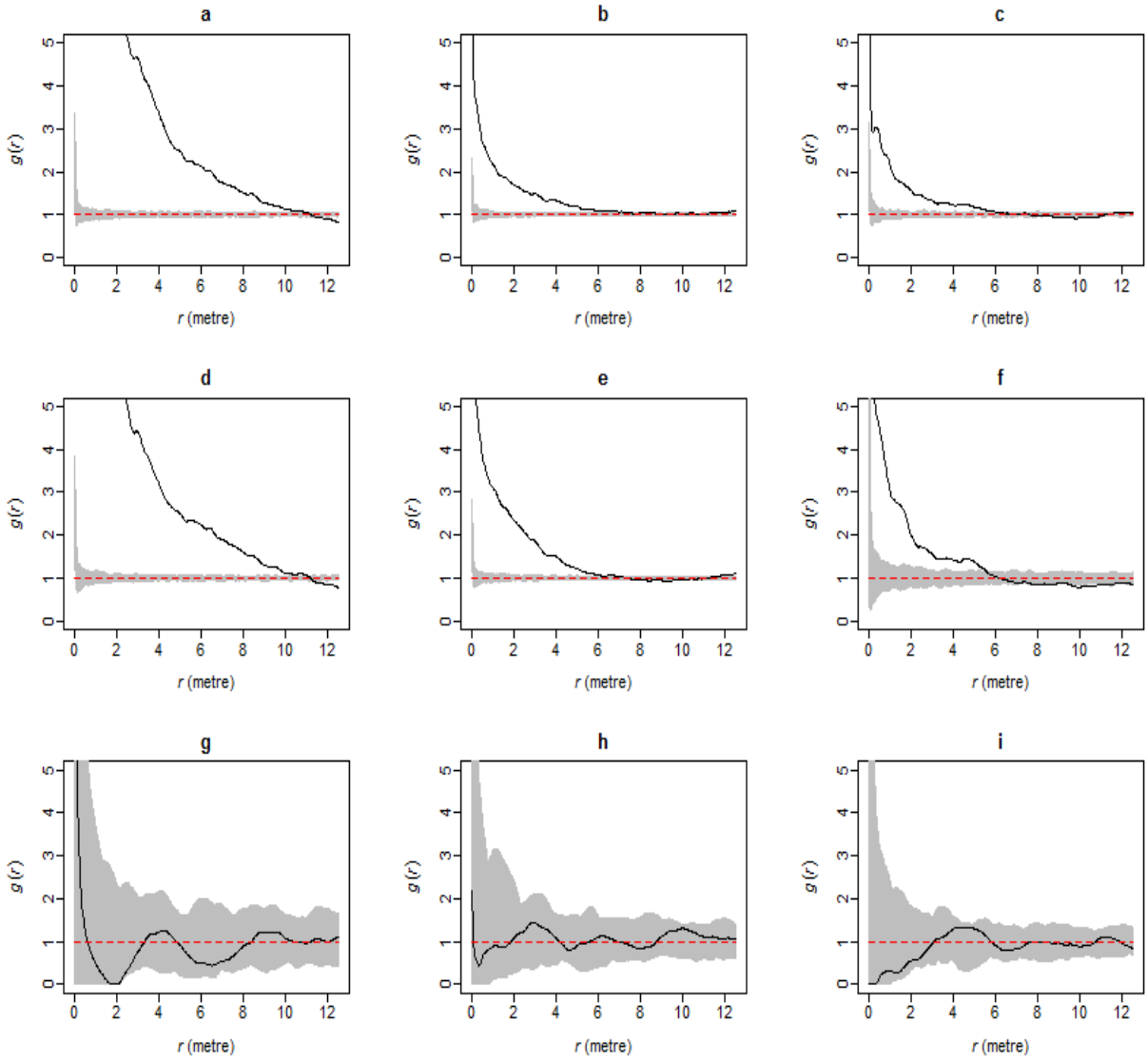


Fig. 4. Summary of univariate analysis showing the spatial patterns of all woody trees occurring in each plot (a-c), all the *Vachellia karroo* trees only (d-f), mistletoe-infected trees (g-i) within the three plots (1-3 respectively) using the pair correlation function g(*r*). The grey shaded areas show the upper and lower critical boundaries of the 95% point-wise envelopes, i.e., the 5th lowest and 5th highest values of the pair correlation function estimated from 199 Monte Carlo simulations of the CSR null model (black lines). If the black line is above the grey area, then the pattern is significantly clustered: if the black line is below the grey shaded area, the pattern is regular, and both patterns will be deviating from the null model of CSR.

Bivariate analysis

In plot 1-3 (Fig. 5a-c), the bivariate point pattern analysis detected a significant positive association between mistletoe-infected (pattern 1) and all the uninfected trees (pattern 2) at shorter distances i.e., 0-1.8m, 0.5-1.0m, and 0-1.8m, respectively, and at these distances the patterns deviated significantly from the null model of CSR ($P < 0.05$, Table 5). In $plot_2$ and $plot_3$ infected trees showed a negative association with all the uninfected trees at distances between 2-2.6m and 7-7.2m ($P < 0.05$, Fig. 5b-c).

However, as the distances increased, all the plots generally exhibited a random pattern. Similarly, the $L_{12}(r)$ bivariate function showed clustering at distances between 3-7.8m, 0.4-1.4m, and 0-4m in plot 1-3, respectively, (Appendix 6).

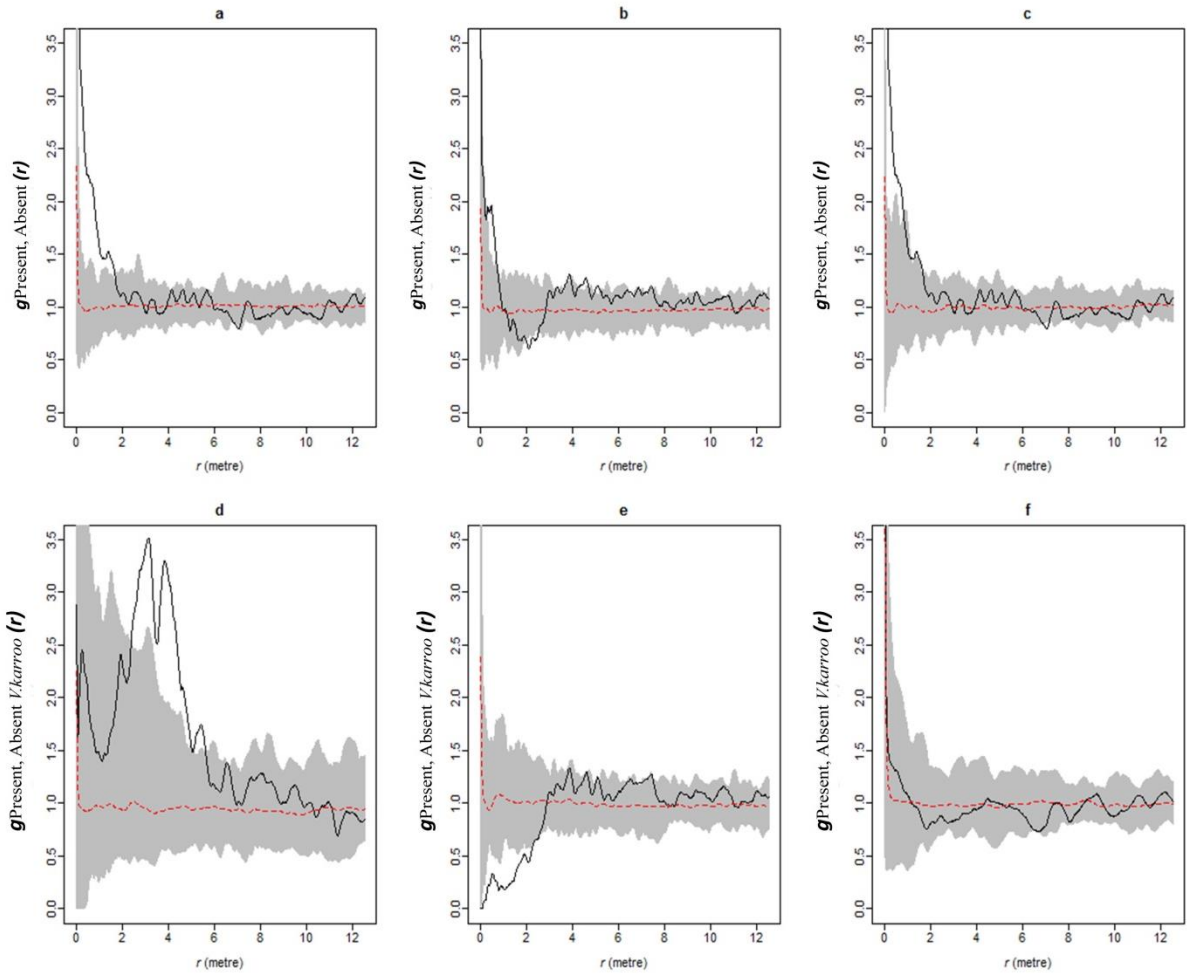


Fig. 5. Summary of the bivariate pair correlation analysis between infected trees and all the woody plants in plots 1-3 (a-c, respectively) and between infected trees and all the *V. karroo* trees within each plot (d-f, respectively). The grey shaded areas show the upper and lower critical boundaries of the 95% point-wise envelopes i.e., the 5^{th} lowest and 5^{th} highest values of the pair correlation function estimated from 199 Monte Carlo simulations of the CSR null model (black lines). The position of the black line across the scale, determines the pattern, if it is either inside, below or above the simulation envelopes the pattern is regarded as uncorrelated, negatively, or positively correlated, respectively.

The bivariate pair correlation function between mistletoe-infected and uninfected *V. karroo* trees in $plot_1$ showed a significant positive association at distances between 2.4-5.8m and 1.8-5m ($P < 0.05$) (Fig. 5d, Table 5). In $plot_2$ (Fig. 5e), the pattern showed significant repulsion ($P < 0.05$) between mistletoe-infected and uninfected *V. karroo* trees at small scales of 0-2.8m, and a non-significant correlation for the rest of the distance. $Plot_3$ (Fig. 5f) showed no significant correlation or deviation from the null model of complete spatial randomness between infected and uninfected *V. karroo* trees.

Likewise, the L_{12}(r) function showed a positive association between mistletoe-infected trees and uninfected *V. karroo* trees at distances from 2-8m in $plot_1$ (Appendix 6). However, $plot_2$ showed a negative association between 0-3m, whilst $plot_3$ showed a complete random spatial pattern.

Table 5. Bivariate analysis ($g_{12}(r)$) for the relationship between infected trees and all the uninfected woody plants, infected and the uninfected *V. karroo* trees and infected trees and different stage classes using the Diggle-Cressie-Loosmore-Ford Test (DCLF) of CSR under Monte Carlo, based on 99 simulations with fixed number of points.

Infected *vs*. Uninfected trees	Plot number	u-value	*P*	rank
Infected trees/All species uninfected	1	21.08	0.01	1
Infected trees/All species uninfected	2	1.20	0.01	1
Infected trees/All species uninfected	3	5.54	0.01	1
Infected/Uninfected *V. karroo* trees	1	11.62	0.01	1
Infected/Uninfected *V. karroo* trees	2	1.42	0.01	1
Infected/Uninfected *V. karroo* trees	3	0.79	0.28	1
Stage Classes				
Infected/Seedlings (All species)	1	70.56	0.01	1
Infected/Seedlings (All species)	2	0.94	0.21	21
Infected/Seedlings (All species)	3	5.62	0.09	9
Infected/Seedlings (*V. karroo*)	1	56.06	0.01	1
Infected/Seedlings (*V. karroo*)	2	1.53	0.14	14
Infected/Seedlings (*V. karroo*)	3	2.33	0.50	50
Infected/Saplings (All species)	1	18.77	0.01	1
Infected/ Saplings (All species)	2	0.76	0.02	2
Infected/ Saplings (All species)	3	8.88	0.01	1
Infected/ Saplings (*V. karroo*)	1	10.66	0.01	1
Infected/ Saplings (*V. karroo*)	2	1.52	0.01	1
Infected/ Saplings (*V. karroo*)	3	8.98	0.02	2
Infected/Shrubs (All species)	1	145.17	0.01	1
Infected/ Shrubs (All species)	2	142.46	0.01	1
Infected/ Shrubs (All species)	3	10.33	0.01	1
Infected/ Shrubs (*V. karroo*)	1	35.00	0.12	12
Infected/ Shrubs (*V. karroo*)	2	59.70	0.03	3
Infected/ Shrubs (*V. karroo*)	3	6.32	0.36	36
Infected/Mature (All species)	1	12.36	0.47	47
Infected/ Mature (All species)	2	18.37	0.06	6
Infected/ Mature (All species)	3	0.73	0.78	78
Infected/ Mature (*V. karroo*)	1	12.36	0.41	41
Infected/ Mature (*V. karroo*)	2	19.06	0.12	12
Infected/ Mature (*V. karroo*)	3	0.37	0.98	98

There was a significant positive relationship ($P < 0.05$) between mistletoe-infected trees and all surrounding woody seedlings in $plot_1$, particularly at distances between 1.8-3.8m (Fig 6a, Table 5). $Plot_2$ and $plot_3$ displayed a predominant random pattern throughout the distances; however, $plot_2$ showed a negative association between all the woody seedlings and mistletoe-infected trees at distances of 1-2.4m, though this pattern was not significantly different from the null model (Fig. 6b-c, Table 5). The $L_{12}(r)$ function also exhibited clustering of all the woody seedlings around mistletoe-

infected trees in $plot_1$ and $plot_3$ at distances of 2.2m-9m and 4.2-7.2m respectively, (Appendix 7a and c). In contrast, the pattern in $plot_2$ was predominantly random but exhibited aggregation between 1.8-2.3m.

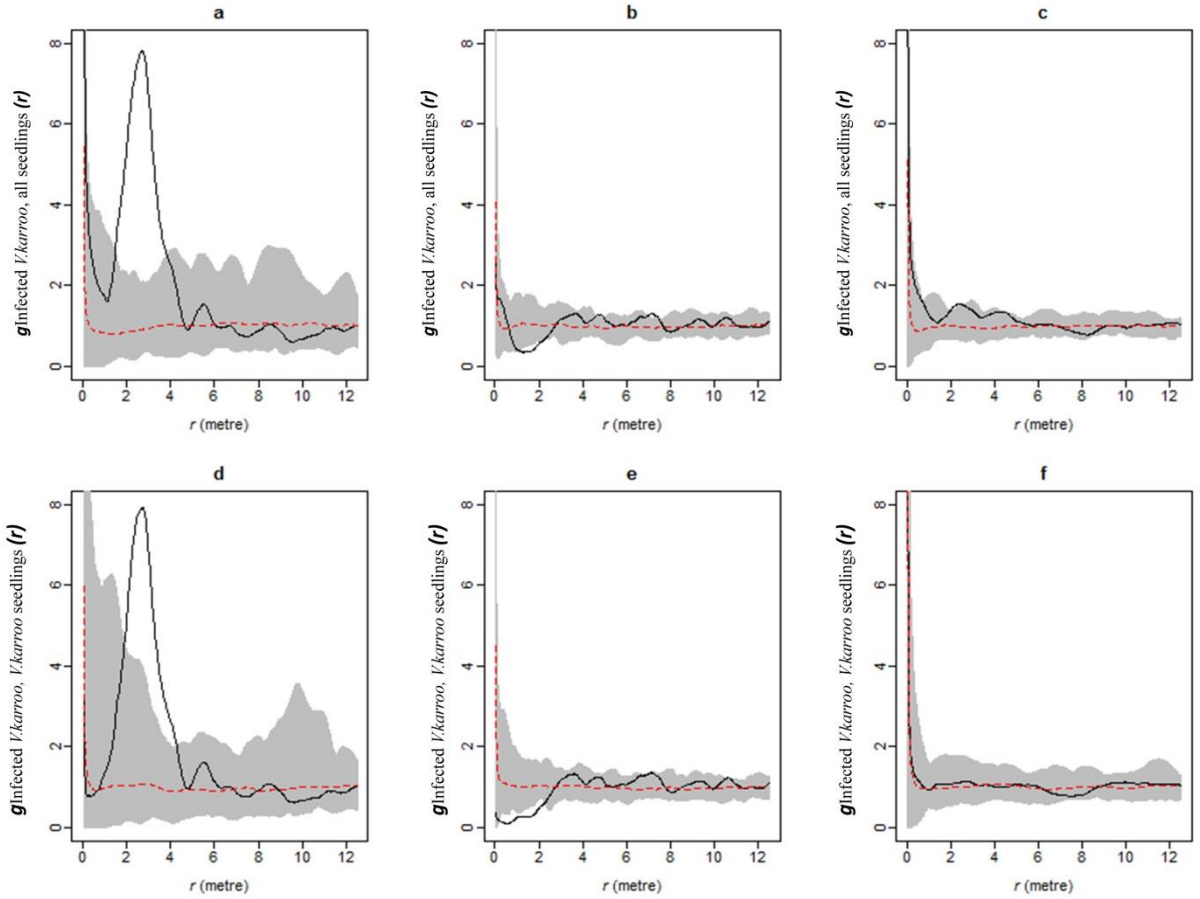


Fig. 6. The bivariate ($g_{12}(r)$) analysis between mistletoe-infected trees and all the seedlings within plot 1-3 (a-c), and between mistletoe-infected trees and *Vachellia karroo* seedlings (d-f), respectively. The grey shaded areas show the upper and lower critical boundaries of the 95% point-wise envelopes i.e., the 5^{th} lowest and 5^{th} highest values of the pair correlation function estimated from 199 Monte Carlo simulations of the CSR null model (black lines). The position of the black line across the scale, determines the pattern, if it is either inside, below or above the simulation envelopes the pattern is regarded as uncorrelated, negatively, or positively correlated, respectively.

$Plot_1$ displayed a significant positive correlation between infected trees and *V. karroo* seedlings at distances of 1.8-4.3m. However, mistletoe-infected trees in $plot_2$ showed a negative association with *V. karroo* seedlings at shorter distances between 0.2-2.2m, but the pattern did not significantly differ from the null model according to the DCLF test (Fig. 6e, Table 5). Conversely, $Plot_3$ showed no deviation from null model of CSR (Fig. 6f). Furthermore, Appendix 7d-e showed aggregation from 2m-7m for $plot_1$ whilst $plot_2$ showed repulsion between mistletoe-infected trees with *V. karroo* seedlings from 1-3.2m. Similar to the pair correlation $plot_3$ showed a random pattern (Appendix 7e).

The bivariate pair correlation in $plot_1$ and $plot_3$ (Fig. 7a, c), showed a significant positive relationship between infected trees and all the saplings at distances between 0.1-5m and 0-1.8m, respectively. In contrast, $plot_2$ showed a random pattern at shorter distances below 2m and significant repulsion at distances from 2-2.4m (Fig. 7b). The $L_{12}(r)$ function also showed clustering of woody saplings around mistletoe-infected trees in $plots_1$ and $plot_3$ at distances between 0-7.9m and 0.2-3.9m, respectively (Appendix 8a, c). In contrast, $plot_2$ showed a random pattern throughout the distances.

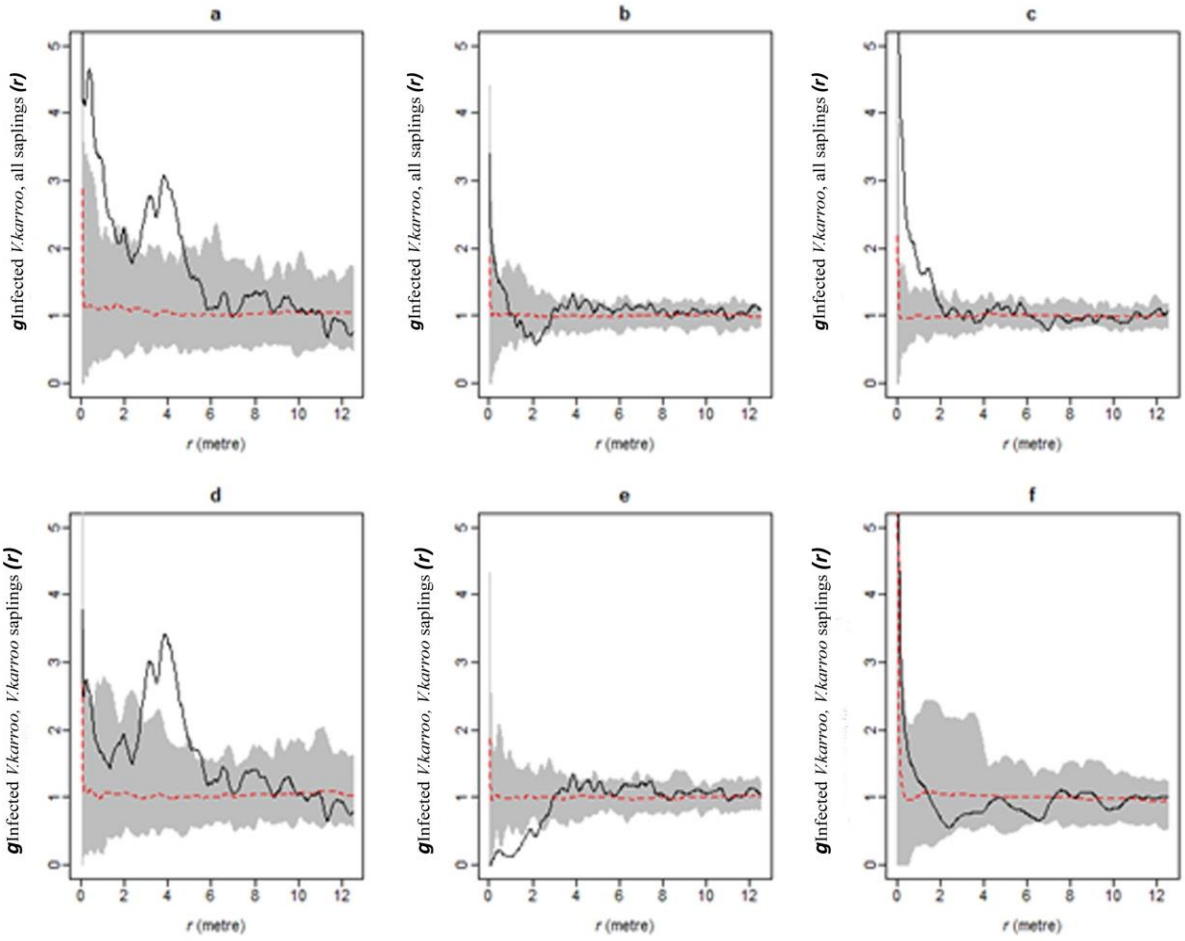


Fig. 7. Summary of the bivariate $g_{12}(r)$ spatial association patterns between mistletoe-infected trees and all the saplings (a-c) and *Vachellia karroo* saplings (d-f) for plots 1-3 respectively. The grey shaded areas show the upper and lower critical boundaries of the 95% point-wise envelopes i.e., the 5^{th} lowest and 5^{th} highest values of the pair correlation function estimated from 199 Monte Carlo simulations of the CSR null model (black lines). The position of the black line, determines the pattern, if it is either inside, below or above the simulation envelopes the pattern is regarded as uncorrelated, negatively, or positively correlated, respectively.

The bivariate pair correlation displayed a significant positive association between mistletoe-infected trees and *V. karroo* saplings at distances between 2.5-5.6m and thereafter a random pattern (Fig. 7d, Table 5). Conversely, $plot_2$ showed significant repulsion at distances between 0.2-2.3m whilst in $plot_3$,

it was consistent with a random pattern (Fig. 7e-f). Furthermore, the relationship between mistletoe-infected trees and *V. karroo* saplings, showed clustering between 0.3-7.9m, repulsion from 0-3.4m, and a random pattern for plots 1-3, respectively (Appendix 8d-f).

The bivariate pair correlation showed significant clustering of shrubs around mistletoe-infected trees at short distances i.e., 0-3.2m, 0-1.4m, and 0-1.2m for plots 1-3, respectively, and then a random pattern for the rest of the distances (Fig 8a-c). The bivariate $L_{12}(r)$ function also showed clustering at distances around 0.6-2m and 4.6-7m, 0-7m and 0.5-1.7m for plots 1-3 (Appendix 9a-c).

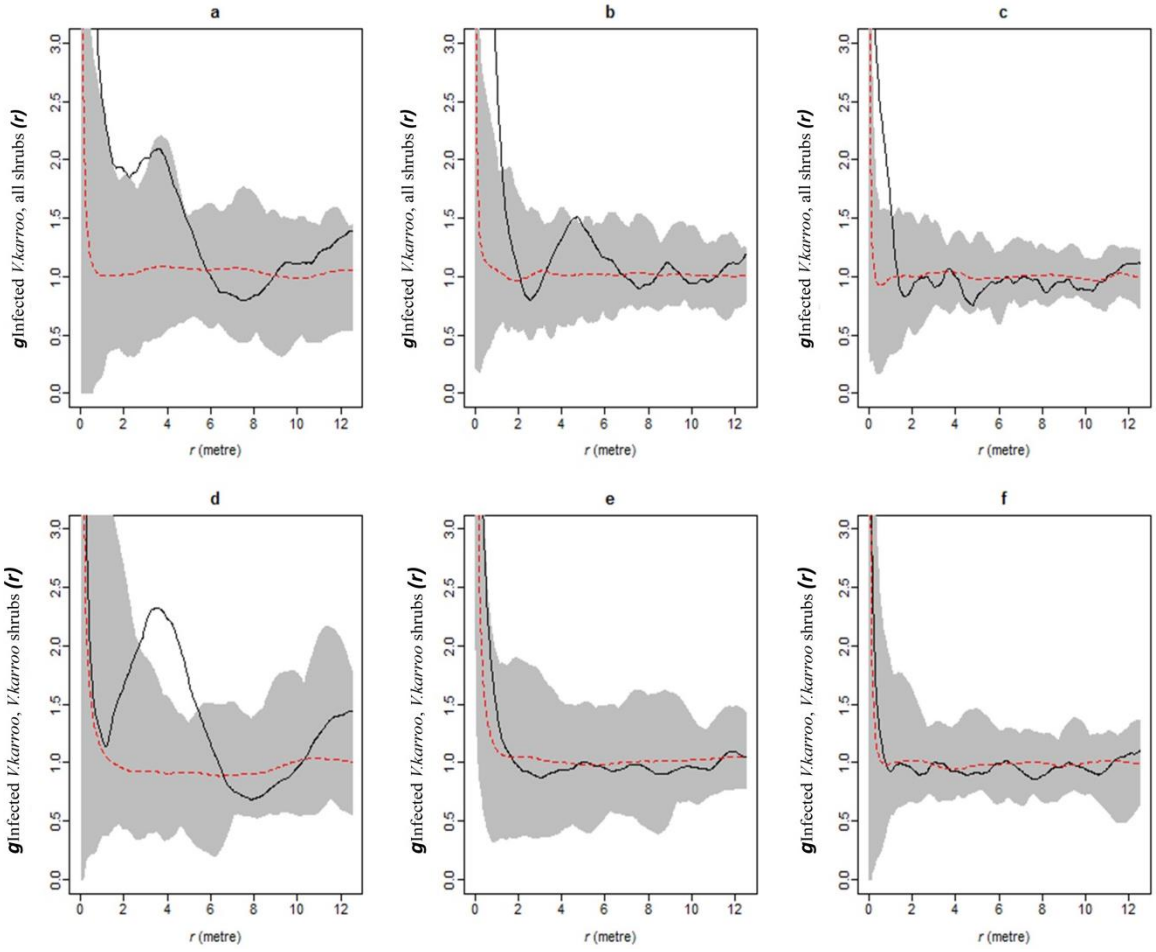


Fig. 8. Summary statistic of bivariate $g_{12}(r)$ patterns of mistletoe-infected trees with all shrubs (a-c), and with *Vachellia karroo* shrubs only (d-f), for plots 1-3 respectively. The grey shaded areas show the upper and lower critical boundaries of the 95% point-wise envelopes i.e., the 5th lowest and 5th highest values of the pair correlation function estimated from 199 Monte Carlo simulations of the CSR null model (black lines). The position of the black line, determines the pattern, if it is either inside, below or above the simulation envelopes the pattern is regarded as uncorrelated, negatively, or positively correlated respectively.

Further, the pair correlation bivariate and the $L_{12}(r)$ function for plot$_1$ showed aggregation of *V. karroo* shrubs around mistletoe-infected trees from distances of 2.4-5.8m and 4.6-7m, respectively (Fig.8d,

Appendix 9d). In contrast, for $plot_2$ and $plot_3$ both the pair correlation bivariate and the $L_{12}(r)$ function exhibited patterns consistent with a random pattern at all distances, but the $L_{12}(r)$ in $plot_2$ showed clustering between 0.8-1.7m (Fig. 8e-f, Appendix 9e-f).

The pair correlation bivariate between mistletoe-infected trees and mature trees of all the species did not deviate from the null model of CSR (Fig. 9a-c, Appendix 10a-c). Although the relationship between mistletoe-infected trees and mature *V. karroo* trees in $plot_1$, showed clustering between 3.2 and 5.2m, according to the DCLF test the pattern did not significantly differ from the null model (Fig. 9d, Appendix 10d). $Plot_2$ and $plot_3$ exhibited patterns that were consistent with a random pattern for both the pair correlation and the $L_{12}(r)$ function (Fig. 9e-f, Appendix 10e-f).

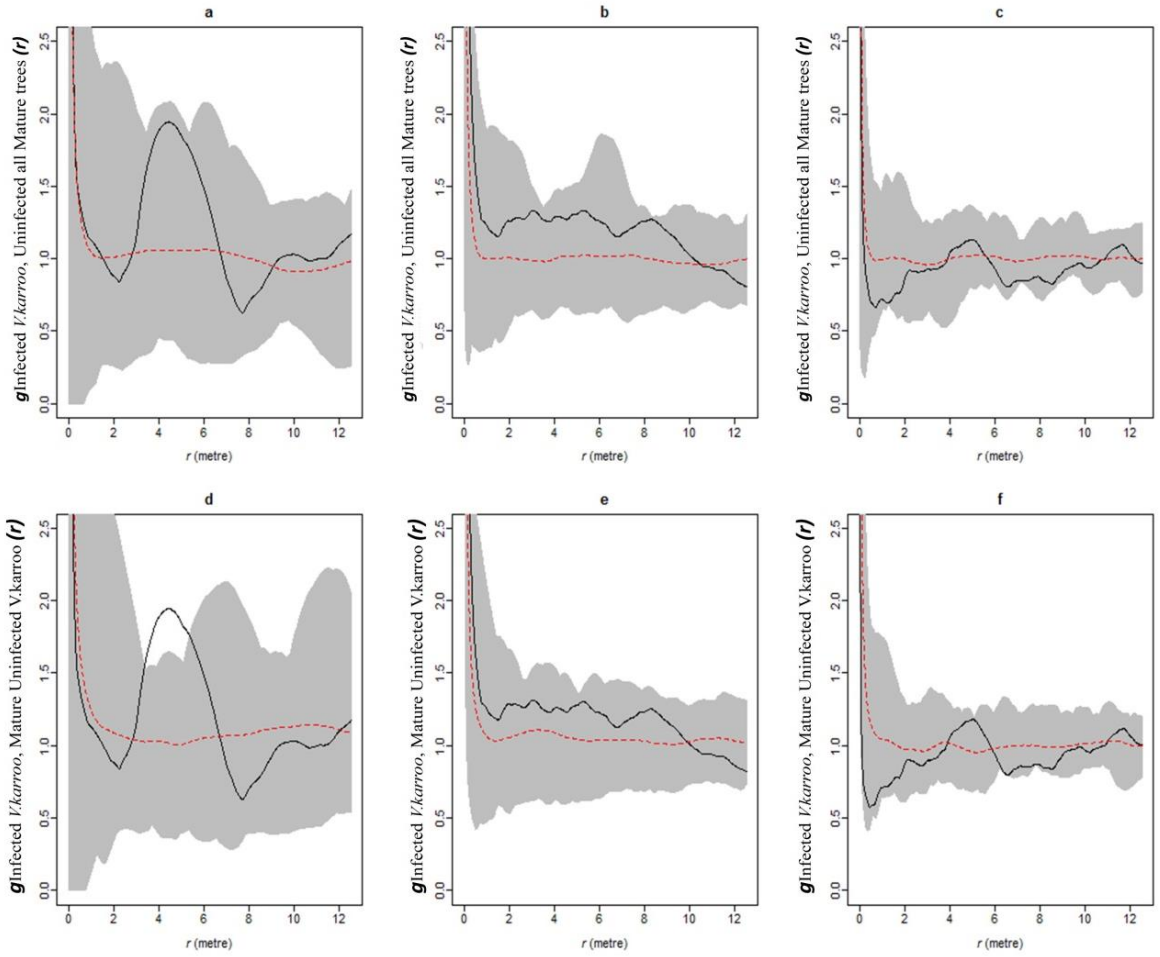


Fig. 9. Summary of the bivariate $g_{12}(r)$ relationship of mistletoe-infected trees with all uninfected mature trees (a-c), and with uninfected *Vachellia karroo* mature trees (d-f) for plots 1-3 respectively. The grey shaded areas show the upper and lower critical boundaries of the 95% point-wise envelopes i.e., the 5^{th} lowest and 5^{th} highest values of the pair correlation function estimated from 199 Monte Carlo simulations of the CSR null model (black lines). The position of the black line, determines the pattern, if it is either inside, below or above the simulation envelopes the pattern is regarded as uncorrelated, negatively, or positively correlated, respectively.

Mark correlation

The univariate mark correlation function in $plot_1$ showed that canopy areas of mistletoe-infected trees at shorter distances were larger than those further away from each other (Fig. 10a). However, overall mistletoe-infected tree canopy areas in $plot_1$ showed independence at all the distances. In contrast, in $plot_2$ and $plot_3$ the canopy areas of two nearby mistletoe-infected trees were smaller than pairs situated farther away from each other (Fig. 10b-c). Furthermore, there was a significant negative correlation at distances between 1-3m for both $plot_2$ and $plot_3$ signifying presence of competition at shorter distances (Fig. 10b-c, Appendix 11). Similarly, the number of mistletoes found on mistletoe-infected trees at shorter distances tended to be more than found at greater distances in $plot_1$ (Fig 10d). However, in $plot_2$ the number of mistletoes on two nearby trees was negatively correlated ($P < 0.05$) at distances between 1-2.8m (Fig. 10e). $Plot_3$ showed independence at distances from 0-6.8m before showing a marginal positive correlation at distances around 7m (Fig. 10f).

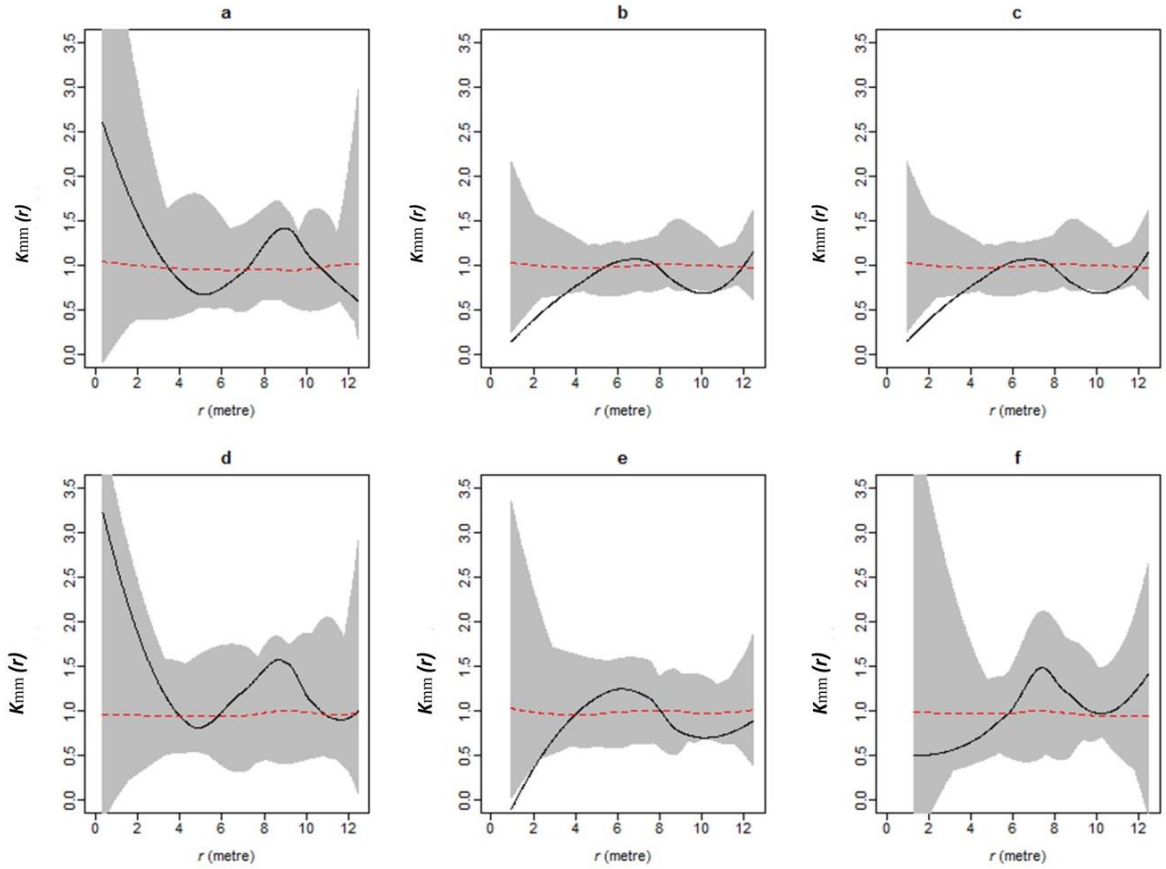


Fig. 10. Summary of the mark correlation function $k_{mm}(r)$ for canopy area (a-c), within three plots (a-c), and number of mistletoes on per tree. Grey-shaded areas show the envelopes (5th highest and 5th lowest value) constructed using 199 Monte Carlo simulations. The mistletoe-infected trees are either positively or negatively correlated if the black line is above or below the grey-shaded envelopes.

Discussion

A summary of six of the main results follows. (a) The univariate patterns for all the woody species and for all the *V. karroo* trees within each plot were consistent with aggregation. (b) In contrast, mistletoe-infected *V. karroo* trees and all the mature trees combined displayed a univariate random distribution. (c) Furthermore, smaller uninfected stage classes were aggregated around mistletoe-infected trees at smaller scales, particularly in $plot_1$. (d) However, the smaller size classes of *V. karroo* trees in $plot_2$ showed a negative association with mistletoe-infected trees at smaller scales. (e) Further, the bivariate relationship between *V. karroo* shrubs and mature uninfected trees around mistletoe-infected trees showed random patterns which are consistent with both competition and facilitation within the plots. (f) However, all the shrub species showed a positive association with mistletoe-infected trees at shorter distances.

Univariate analysis

The univariate analysis for all woody species and all *V. karroo* individuals showed significant clustering across the three plots. These results are similar to other studies that have also reported clustering as the overall univariate spatial pattern of *Vachellias* (formerly *Acacias*) and other woody species (Meyer *et al*., 2008; Pillay and Ward, 2012). The presence of both uninfected mature *V. karroo* trees and mistletoe-infected *V. karroo* trees results in high resource canopy patches, which could have promoted facilitation and recruitment of a variety of understory woody plants (Barot, 1999; Dale 2000; Meyer *et al*., 2008; Rayburn *et al*., 2011; Schleicher *et al*., 2011; Velázquez *et al*., 2014; Tamjidi and Lutz, 2020; Chapter 2, 3). In this study, saplings dominated all the plots and their high relative abundances could have influenced the overall aggregation pattern. Mistletoe-infected trees could be providing adequate moisture for the survival of seedlings to saplings (Wilson and Witkowski 1998; Chapter 2, 3). Furthermore, the high numbers of saplings could also explain the small-scale clustering (0-5m) observed in the bivariate analysis of mistletoe-infected trees with all the woody individuals and with all the other uninfected *V. karroo* individuals.

The univariate pattern of mistletoe-infected trees was consistent with a random pattern in all the three plots, and the nearest neighbour distances varied from 3.6-5m depending on the density of mistletoe-infected trees of the plots. This suggests that mistletoe-infected trees do not necessarily follow specific spatial patterns contrary to the hypothesis. The characteristics of mistletoe-infected trees also differed between plots and this could have further caused the disparities in the spatial patterns observed between plots. Regardless, these differences could indirectly be a baseline to predict potential scenarios that are most likely to occur at different mistletoe-infected tree densities. For instance, the number of mistletoe-infected trees with hexagonal tiles decreased with an increase in the density of mistletoe-infected trees ($plot_2$ and $plot_3$) suggesting weak competition for resources within these plots. Therefore, mistletoe-infected trees in $plot_2$ and $plot_3$ could have invested in their height rather than in extending their canopies a typical response to density dependent thinning thus the

overall pattern tends towards clustering. In contrast, mistletoe-infected trees in $plot_1$ had significantly larger canopy areas, which could result in stronger competition, thereby driving the pattern towards regularity, as shown by the higher number of hexagonal tiles.

The results also showed that mistletoe-infected trees could have as much as 25-31 mistletoes, yet this mistletoe intensity did not translate to mistletoe-infected trees exhibiting a clustered pattern. The random distribution of mistletoe-infected trees could be predominantly dependent on disperser preferences and on the interaction of mistletoes with the dispersers which may not necessarily be systematic (Kavanagh and Burns, 2012; Sayad *et al*., 2017). It could also relate to historical recruitment events of *V. karroo* prior to mistletoe infections. Some dispersers like birds can aggregate on one tree, and in such a scenario, dispersers can revisit and redeposit mistletoe seeds on their preferred trees, hence increasing mistletoe infections on individual trees (Aukema, 2004; Overton, 1996; Mora-Pinto 2005; Sayad *et al.*, 2017; Mellado and Zamora, 2017). Furthermore, bird dispersers prefer to perch, feed and to build nests on tall trees with large canopies and high food resources (Sayad *et al*., 2017). Likewise, mistletoes have been shown to survive at higher rates on older, taller, larger trees, because these hosts are a reliable source of nutrients and water, even throughout the dry season (Ndagurwa *et al*., 2012; Sayad *et al*., 2017).

This study shows that mistletoes were prevalent in tall hosts with larger stem diameters and canopy areas, thus showing the importance of host size and quality. Therefore, considering that both birds and mistletoes favour large trees, mistletoe-infected trees might be expected to exhibit a similar regular distribution pattern depicted by large trees, due to tree density dependent thinning. However, the results show that all the mature trees (inclusive of mistletoe-infected trees) were randomly distributed in the three plots. Furthermore, the mark correlation function results on canopy area and mistletoe counts for $plot_2$ and $plot_3$ showed a negative association of mistletoe-infected trees at distances between 1-3m and 1-2.8m, respectively. As distance increased the mark correlation function changed to a random pattern, similar to the univariate analysis. Therefore, this predominant random pattern exhibited by mistletoe-infected trees could be due to birds' preference for the larger and (or) the most competitive trees within the area. These preferences could have further accelerated thinning (due to mistletoe-induced tree mortality) of the already randomly distributed mature trees, thus possibly explaining the random pattern of mistletoe-infected trees.

Bivariate analysis

Canopy patches of mistletoe-infected trees were expected to facilitate positive plant interactions with understory woody seedlings and saplings. Subsequently, $plot_1$ showed significant clustering of woody seedlings and saplings and their conspecific seedlings and saplings at shorter distances around mistletoe-infected trees as did all the saplings in $plot_3$. This aggregation can be attributed to *V. karroo* trees being facultative sciophytes (heliophytes that can also grow under shade); hence, higher densities at short distances around the mistletoe-infected tree (O'Connor 1995; Chapter 3). In addition

to high seedling recruitment from accumulated seeds beneath mistletoe-infected trees (Flores and Jurado, 2003; Schleicher *et al*., 2011; Dohn *et al*., 2017; Mellado and Zamora, 2017, Chapter 3), vegetative reproduction, directed seed dispersal, and increased germination underneath the trees could also have contributed to the aggregation of saplings and seedlings (Philips and MacMahon, 1981; Barot, 1999; Witkowski and Garner, 2000; Walters and Milton, 2003; Getzin, 2006; Schleicher *et al*., 2011; Cheng *et al*., 2014; Chapter 4). Consequently, mistletoe-infected tree canopy patches supported nurse protégé interactions which resulted in coexistence and survival of heterospecific seedlings and their recruitment into saplings (Chapter 3). This positive spatial relationship between different species of plants growing in close proximity to large trees is common in semi-arid savannas, and may explain why there were more than 10 different species in each plot (Meyer *et al*., 2008; Schleicher *et al*., 2011; Pillay and Ward 2012; Dohn *et al*., 2017). However, it is expected that as the plants grow, competition for resources and space will increase, resulting in a negative spatial association in time, as observed with mature trees (Schleicher *et al*., 2011). Regardless, clustering of seedlings and saplings at shorter distances suggests their affinity to infected tree canopy patches i.e., mistletoephily, thus making mistletoe-infected trees 'safe sites', which facilitate successful germination and recruitment of seedlings and saplings (Chapter 3).

The difference in mistletoe prevalence and mistletoe-infection tree density in $plot_1$ compared to $plot_2$ and $plot_3$ could have been the source of heterogeneity in the patterns observed in the three plots. $Plot_2$ with an intermediate density of mistletoe-infected trees (relative to $plot_1$ and $plot_3$), showed competitive exclusion of seedlings, whilst $plot_3$ with higher densities of mistletoe-infected trees displayed a random distribution of seedlings around infected trees. Random distributions arise from both facilitation and competitive exclusion occurring at similar intensities, thus neutralising their associated effects (Meyer *et al*., 2008; Dohn *et al*., 2017). For instance, seedlings can benefit from augmented nutrients from high infected trees (Ndagurwa *et al*., 2013, 2014, 2015, 2020; Mellado *et al*., 2016; Mellado and Zamora, 2017), but also face competition from the infected tree and surrounding conspecific and heterospefic seedlings and saplings, thus masking the resource benefits (Barot, 1999; Ndagurwa *et al*., 2014b). Indeed, conspecific competition was evidenced by the repulsion of *V. karroo* seedlings and saplings by the mistletoe-infected trees.

Competitive exclusion can also be attributed to competition for water by the host with their understory plants (Ndagurwa *et al*., 2014) and from understory interspecific competition with other species including the abundant herbaceous species (Chapter 2, 3). Similarly, using a pot experiment, Bond *et al*. (2001) reported interspecific competition for light, water, and nutrients between *V. karroo* seedlings and grasses. Further, competition from shrubs which were also clustered with mistletoe-infected trees could have accelerated competitive exclusion of seedlings in $plot_2$. Regardless, species differ in the mechanisms in which they acquire resources and they can compete more with conspecifics rather than heterospecifics (Martinez *et al*., 2010). Repulsion at shorter scales in $plot_2$

could suggest poor access to resources by seedlings and saplings (O'Connor 1995; Cheng *et al*., 2014). However, because of root systems occurring at different depths (vertically and horizontally) (Getzin, 2011), larger trees utilised different resources to the other size-classes while seedlings and saplings possibly shared the same resource pools. Therefore, repulsion could have been mostly due to thinning, inter-stage competition, and possibly the intermediate density of mistletoe-infected trees within an area.

The negative association between mistletoe-infected trees and the *V. karroo* juveniles in $plot_2$ could also be a result of seeds being dispersed further away from the parent tree by wind or animals (Tamjidi and Lutz, 2020), or due to seedling and sapling herbivory, as observed in this study. Herbivore activity was pronounced underneath than beyond the large tree canopies which could have reduced the recruitment and establishment of *V. karroo* seedlings and saplings (Chapter 2). However, herbivory can reduce the grass sward which directly competes with tree seedlings and saplings thus enabling their establishment (O'Connor 1995; Schleicher *et al*., 2011). Nonetheless, unsystematic grazing/trampling could have influenced the random patterns of seedlings and saplings observed at longer distances.

Clustering of all shrubs around mistletoe-infected trees at short distances of less than 2m and then a random pattern at the rest of the distances can be attributed to weak competitive ability of the host (Press and Phoenix, 2004; Preston *et al*., 2010; Moghadam, 2012; Arruda *et al*., 2012; Mellado and Zamora, 2017) or increased nutrients enabling many smaller neighbours to thrive in the immediate vicinity of the infected tree despite the accelerated evapotranspiration and increased water demand within these patches (Sala *et al*., 2001; Ndagurwa *et al*., 2014b). However, it is also possible that the aggregation of shrubs around mistletoe-infected trees could have been influenced by other confounding factors that were not measured such as existing soil fertility, parent-daughter effects or nurse protégé interactions, which may not be directly influenced by mistletoes. Inter-tree distances are generally expected to increase with age or size due to space and resources limitations, leading to competitive exclusion and density dependent mortality (Philips and MacMahon, 1981; Barot, 1999; Meyer *et al*., 2008; Velázquez *et al*., 2014). However, in all the plots, all the mature trees were randomly distributed contrary to assertions that as trees mature, a regular pattern would become more pronounced (Pillay and Ward, 2012; Cheng *et al*., 2014; Pablo and Gusman, 2017; Muvengwi *et al*., 2018). The random patterns of mature trees could be due to the co-presence of aggregation and dispersion patterns within the same plots (Meyer *et al*., 2008; Dohn *et al*., 2017).

High mistletoe infections likely reduced host performance resulting in weak density dependent thinning among the mature trees (Pillay and Ward, 2012; Dohn *et al*., 2017) while the extra source of nutrients due to mistletoe facilitated aggregation of smaller mature trees around the infected trees. Consequently, even if competition was present, it could not induce mortality, but resulted in smaller

neighbours (Meyer *et al*., 2008; Pillay and Ward, 2012; Dohn *et al*., 2017). However, in the same spaces, some infected trees could withstand the negative impacts of the mistletoes, and managed to secure more resources, repelling smaller mature trees. Therefore, there was co-existence of aggregation and over-dispersion depending on the competitive strength of each infected tree indicating that the way in which each tree can influence spatial patterns is not universal but varies with each infected tree resulting in random patterns. Therefore, these findings provide strong evidence suggesting that the inconsistences in spatial pattern modification by mistletoes increase spatial heterogeneity in semi-arid savanna.

Conclusion

This study provides an insight on the distribution patterns influenced by mistletoe-infected *V. karroo* trees in a semi-arid savanna system. Mistletoe-infected trees were randomly distributed, possibly due to seed-disperser-bird preferences and already existing random patterns of large trees common in savanna. However, seedlings and saplings were clustered below mistletoe-infected trees due to facilitation via increased nutrients or the weak competitive ability of the disease-ridden host. Seedlings and saplings were also negatively associated with mistletoe-infected trees in the intermediate plot, suggesting inter- and intraspecific competition for nutrients and water. Further, the random pattern displayed by both shrubs and mature uninfected trees can be linked to the presence of both competition and promotion of growth from reduced competition. Together, these results strongly indicate that mistletoe infection plays an important role in shaping the woody plant spatial patterns of this savanna. In addition, given the distribution of mistletoes worldwide, these findings have important implication for spatial patterning and heterogeneity in many ecosystems. However, this study is really a snapshot, given that both *V. karroo* and mistletoes are long-lived species, thus long-term studies are required to better understand how mistletoe infection contributes towards the spatial patterning and overall ecosystem heterogeneity over longer time periods. Thus, these results show that mistletoes alter distribution patterns and spatial heterogeneity in semi-arid savannas.

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Appendices

Appendix 1. Composition (and relative percentages) of different stage classes of woody species and their sizes (stem diameter, height and canopy area) within three plots in *Vachellia karroo* dominated patches.

Size class	Attributes	Plot 1	Plot 2	Plot 3
Seedlings	Count	163 (14.8%)	294 (13.5%)	99 (7.8%)
	Stem diameter (cm)	0.10 ± 0.00	0.10 ± <0.01	0.1 ± <0.01
	Height (m)	0.11 ± 0.006	0.15 ± <0.01	0.12 ± 0.01
	Canopy area (cm^2)	37.81 ± 11.22	42.66 ± 6.77	61.74 ± 10.33
Saplings	Count	874 (79.5%)	1714 (78.8%)	941 (73.9%)
	Stem diameter (cm)	0.39 ± 0.01	0.37 ± <0.01	0.54 ± 0.01
	Height (m)	0.22 ± <0.01	0.23 ± <0.01	0.29 ± <0.01
	Canopy area (cm^2)	372.99 ± 21.30	250.01 ± 16.73	583.01 ± 25.15
Shrubs	Count	21 (1.9%)	89 (4.1%)	113 (8.9%)
	Stem diameter (cm)	2.95 ± 0.40	1.71 ± 0.07	1.49 ± 0.06
	Height (m)	1.03 ± 0.15	0.96 ± 0.07	0.92 ± 0.05
	Canopy area (cm^2)	5686 ± 1201	6043 ± 936	5361.28 ± 889
Mature trees	Count	42 (3.8%)	77 (3.5%)	120 (9.4%)
	Stem diameter (cm)	14. 27 ± 1.16	12.56 ± 0.69	10.00 ± 0.40
	Height (m)	5.19 ± 0.31	5.83 ± 0.18	5.38 ± 0.16
	Canopy area (cm^2)	273338 ± 34011	206453 ± 21863	137514 ± 10502
Total	Count	1100	2174	1273

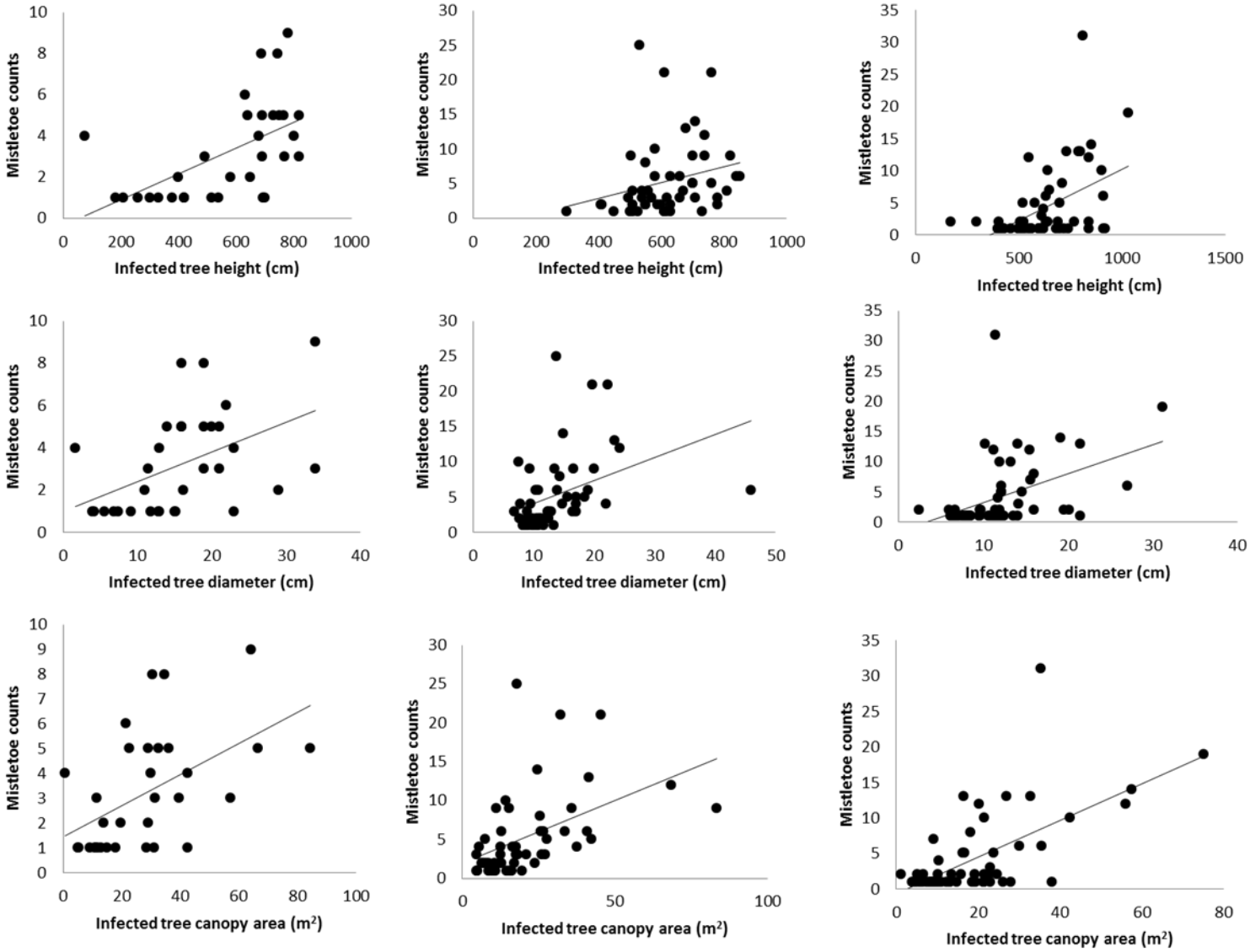
Appendix 2: Species composition based on number of individuals (with percentage relative abundance) in the three plots

Species Name	Plot 1	Plot 2	Plot 3
Vachellia karroo (Hayne) Banfi & Galasso	956 (86.9%)	1611 (74.1%)	296 (23.3%)
Ziziphus mucronata Willd.	109 (9.9%)	385 (17.7%)	625 (49.1%)
Flueggea virosa (Roxb.ex Willd.) Voigt	12 (1.1%)	17 (0.8%)	196 (15.4%)
Searsia pyroides (Burch.) Moffett	9 (0.8%)	76 (3.5%)	66 (5.2%)
Amehloenkomo	5 (0.5%)	62 (2.9%)	55 (4.3%)
Vachellia nilotica (L.) P.J.H.Hurter & Mabb. subsp. *kraussiana* (Benth.) *Kyal. & Boatwr.*	3 (0.3%)	8 (0.4%)	0
Grewia flavescens Juss.	2 (0.2%)	2 (0.1%)	19 (1.5%)
Senegalia galpinii (Burtt Davy) Seigler & Ebinger	2 (0.2%)	0	0
Combretum sp.	1 (0.1%)	0	5 (0.4%)
Euclea divinorum Hiern	1 (0.1%)	1 (0.05%)	2 (0.2%)
Dichrostachys cinerea (L.) Wight & Arn.	0	4 (0.2%)	7 (0.5%)
Grewia bicolor Juss.	0	2 (0.1%)	0
Combretum hereroense Schinz	0	1 (0.05%)	0
Combretum molle R.Br. ex G.Don	0	1 (0.05%)	0
Peltophorum africanum Sond.	0	1 (0.05%)	0
Vachellia gerrardii (Benth.) P.J.H.Hurter subsp. *gerrardii.*	0	0	1 (0.1%)
Total individuals	1100	2174	1273

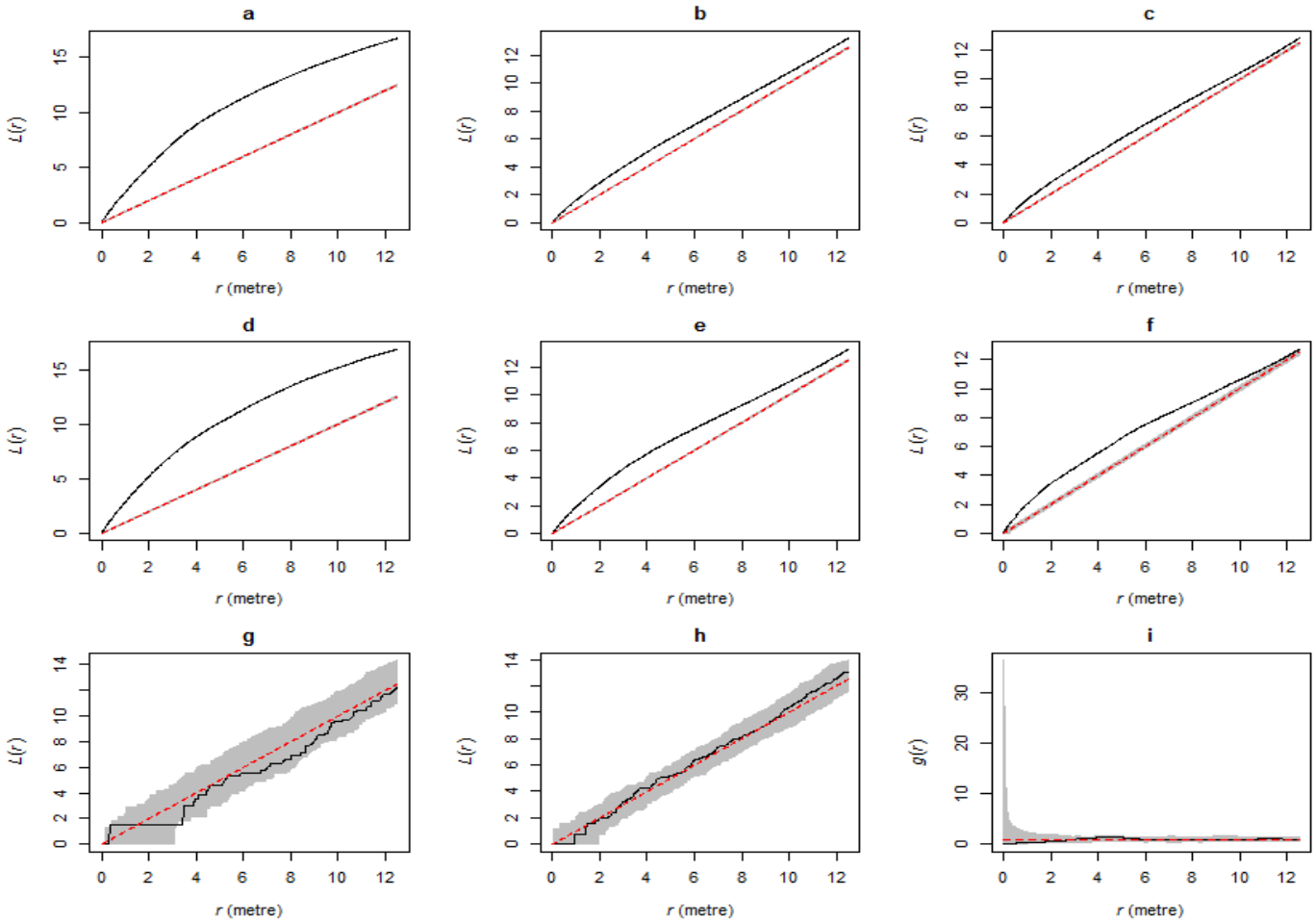
Appendix 3a: Spearman rank correlations tests of mistletoe counts with (i) height, (ii) stem diameter and (iii) canopy areas of infected trees. See Appendix 3b for x-y comparisons.

Interaction	S	*P*	Spearman's ρ
Height vs Mistletoes (Plot1)	2125.7	<0.001	0.61
Height vs Mistletoes (Plot2)	13254	0.003	0.40
Height vs Mistletoes (Plot3)	19410	<0.001	0.46
Stem diameter vs Mistletoes (Plot1)	2538	0.002	0.53
Stem diameter vs Mistletoes (Plot2)	9641.4	<0.001	0.56
Stem diameter vs Mistletoes (Plot3)	16432	<0.001	0.54
Canopy area vs Mistletoes (Plot 1)	2356.9	0.001	0.57
Canopy area vs Mistletoes (Plot 2)	8857.8	<0.001	0.60
Canopy area vs Mistletoes (Plot 3)	16842	<0.001	0.53

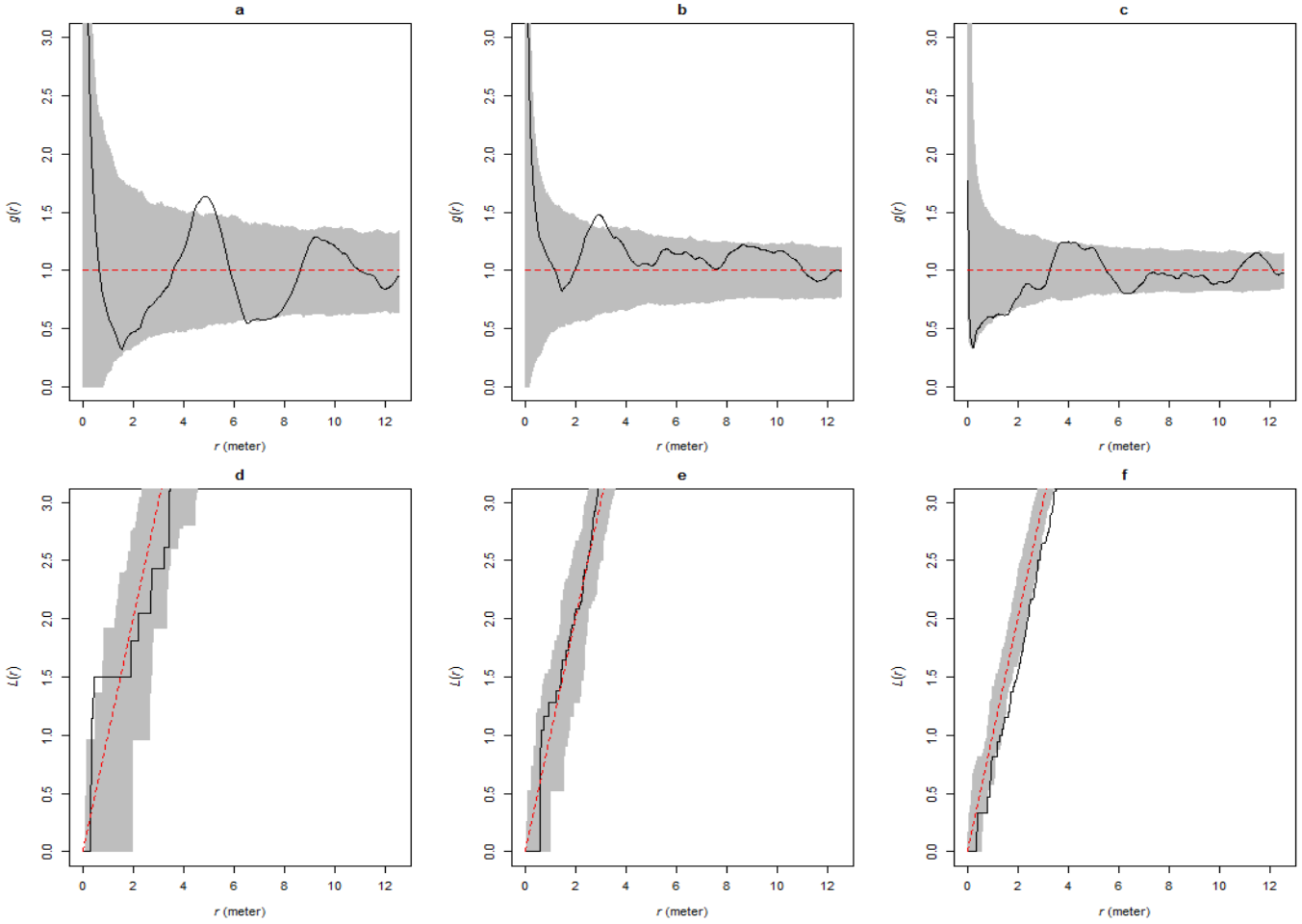
Appendix 3b: Regression analysis showing the relationship between mistletoe counts against height, diameter and canopy area within plot 1-3 respectively



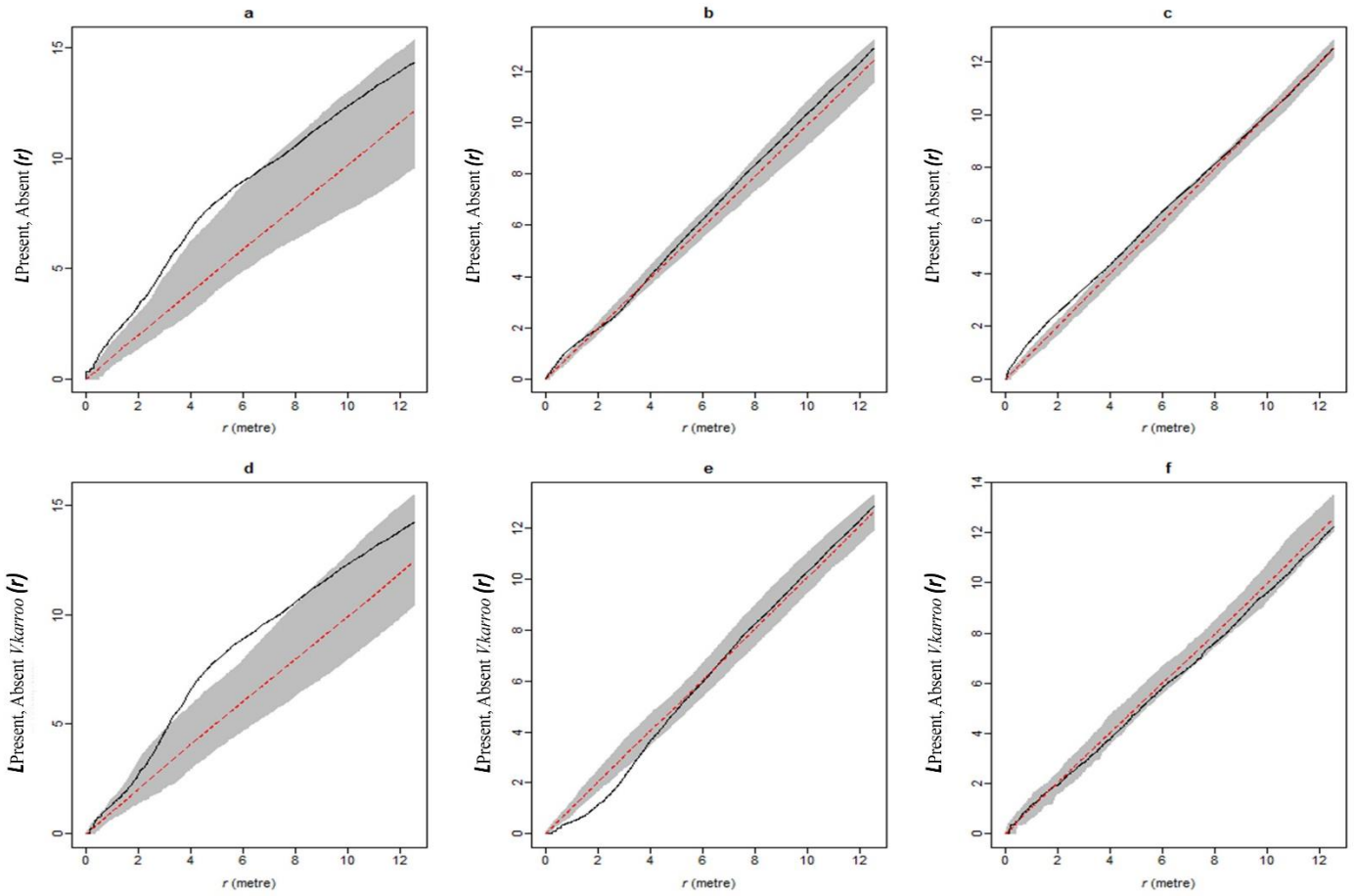
Appendix 4: Univariate analysis of all the woody species (a-c) for each plot 1-3 respectively, all *Vachellia karroo* trees (d-e) and mistletoe-infected trees only (g-i) using the $L(r)$ function. The grey shaded areas show the upper and lower critical boundaries of the 95% point-wise envelopes i.e. the 5th lowest and 5th highest values of the $L(r)$ function estimated from 199 Monte Carlo simulations of the CSR null model (black lines). The pattern is clustered or regular if the black line $L(r)$ is above or below the grey shaded envelopes respectively.



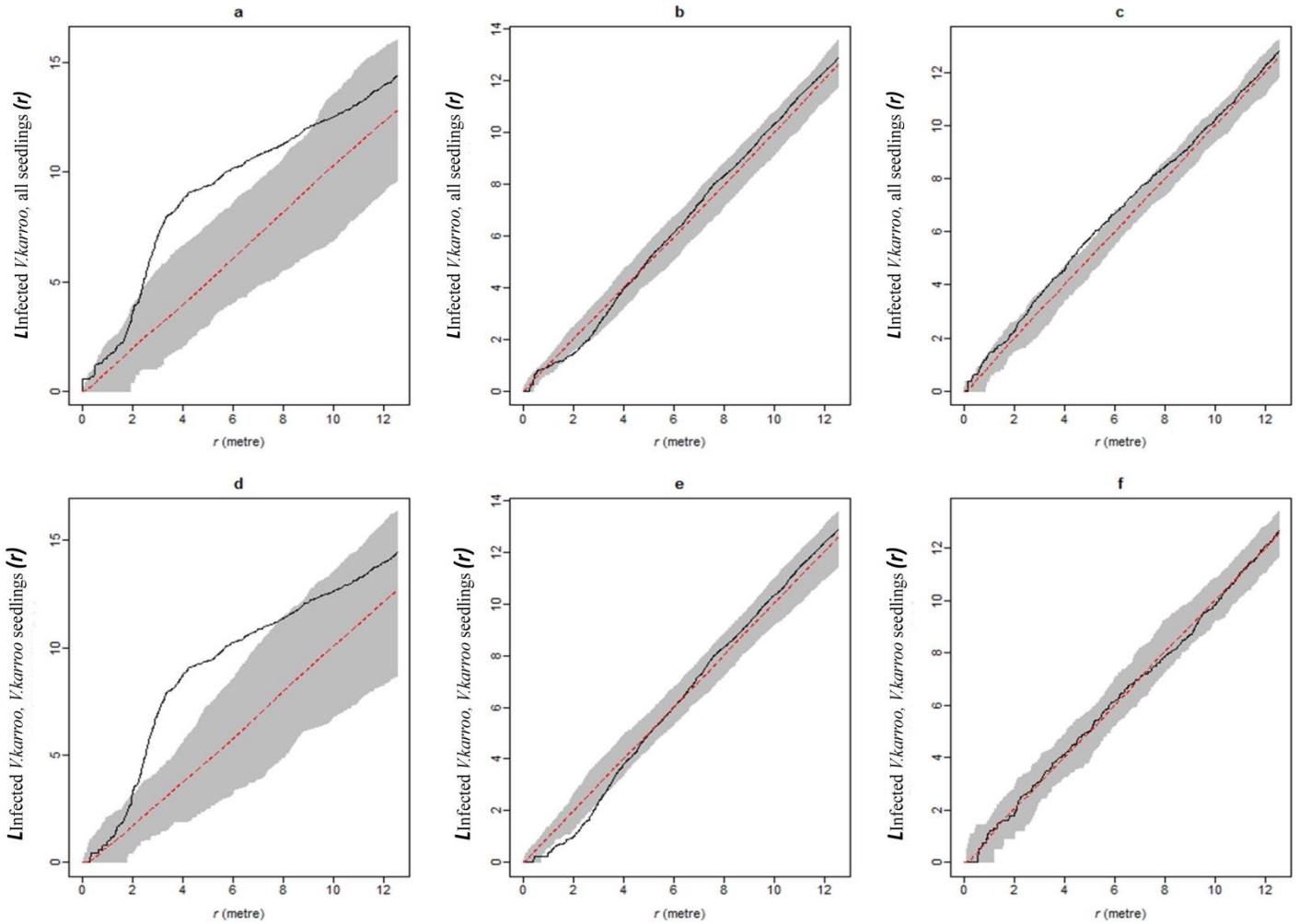
Appendix 5: Univariate analysis using the pair correlation univariate analysis (a-c) and the $L(r)$ function analysis for all the mature trees (d-f) for each plot 1-3 respectively. The grey shaded areas show the upper and lower critical boundaries of the 95% point-wise envelopes i.e. the 5^{th} lowest and 5^{th} highest values of the pair correlation function estimated from 199 Monte Carlo simulations of the CSR null model (black lines). The pattern is clustered or regular if the black line $L(r)$ is above or below the grey shaded envelopes respectively.



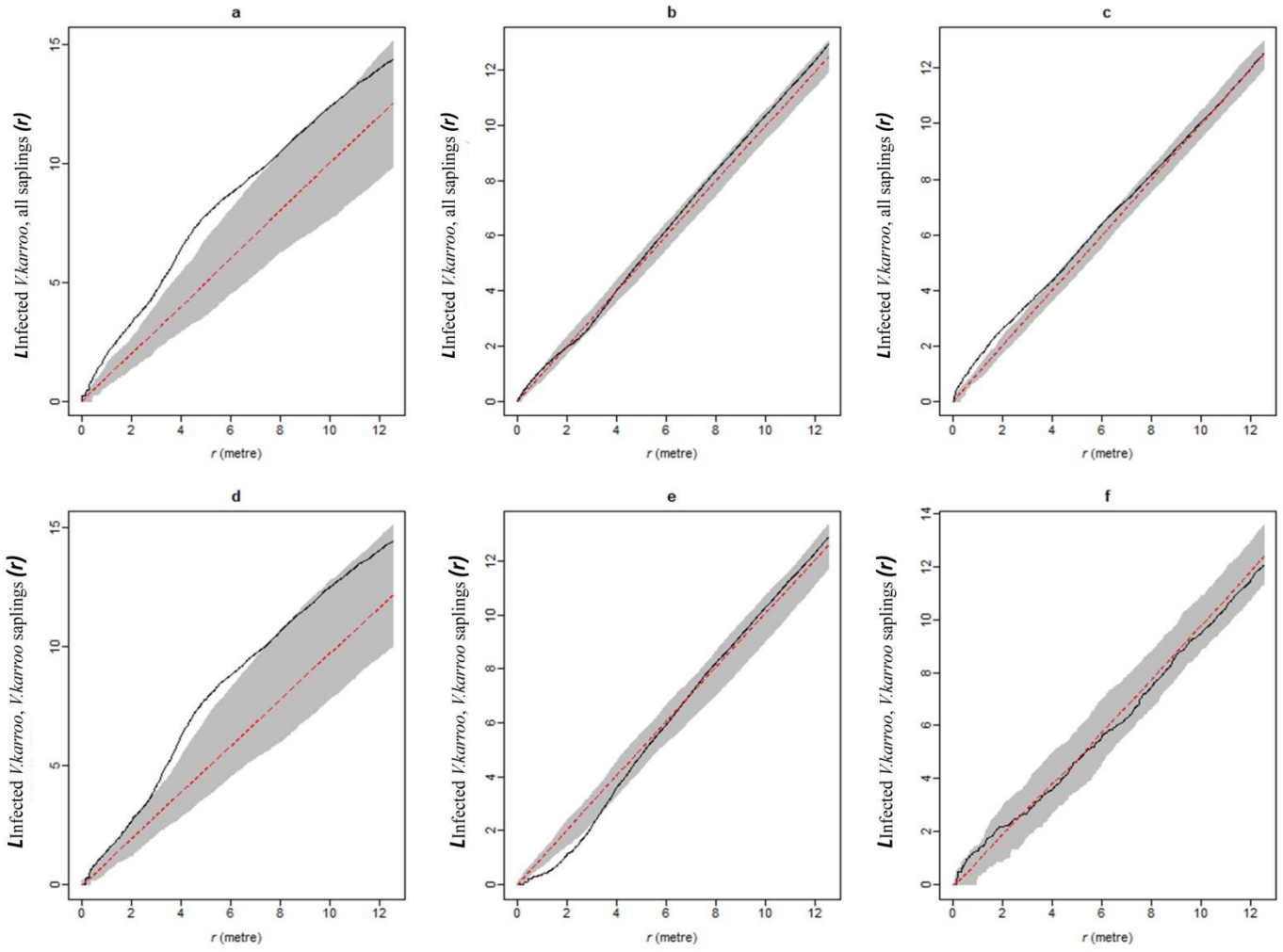
Appendix 6: Bivariate analysis using the $L_{12}(r)$ statistic between infected trees and all uninfected woody species (a-c); and infected trees with all uninfected *Vachellia karroo* trees (d-f) for plots 1-3 respectively. The grey shaded areas show the upper and lower critical boundaries of the 95% point-wise envelopes i.e. the 5th lowest and 5th highest values of the $L(r)$ function estimated from 199 Monte Carlo simulations of the CSR null model (black lines). The position of the black line across the scale, determines the pattern, if it is either inside, below or above the simulation envelopes the pattern is regarded as having no correlation, a negative correlation, or a positive correlation.



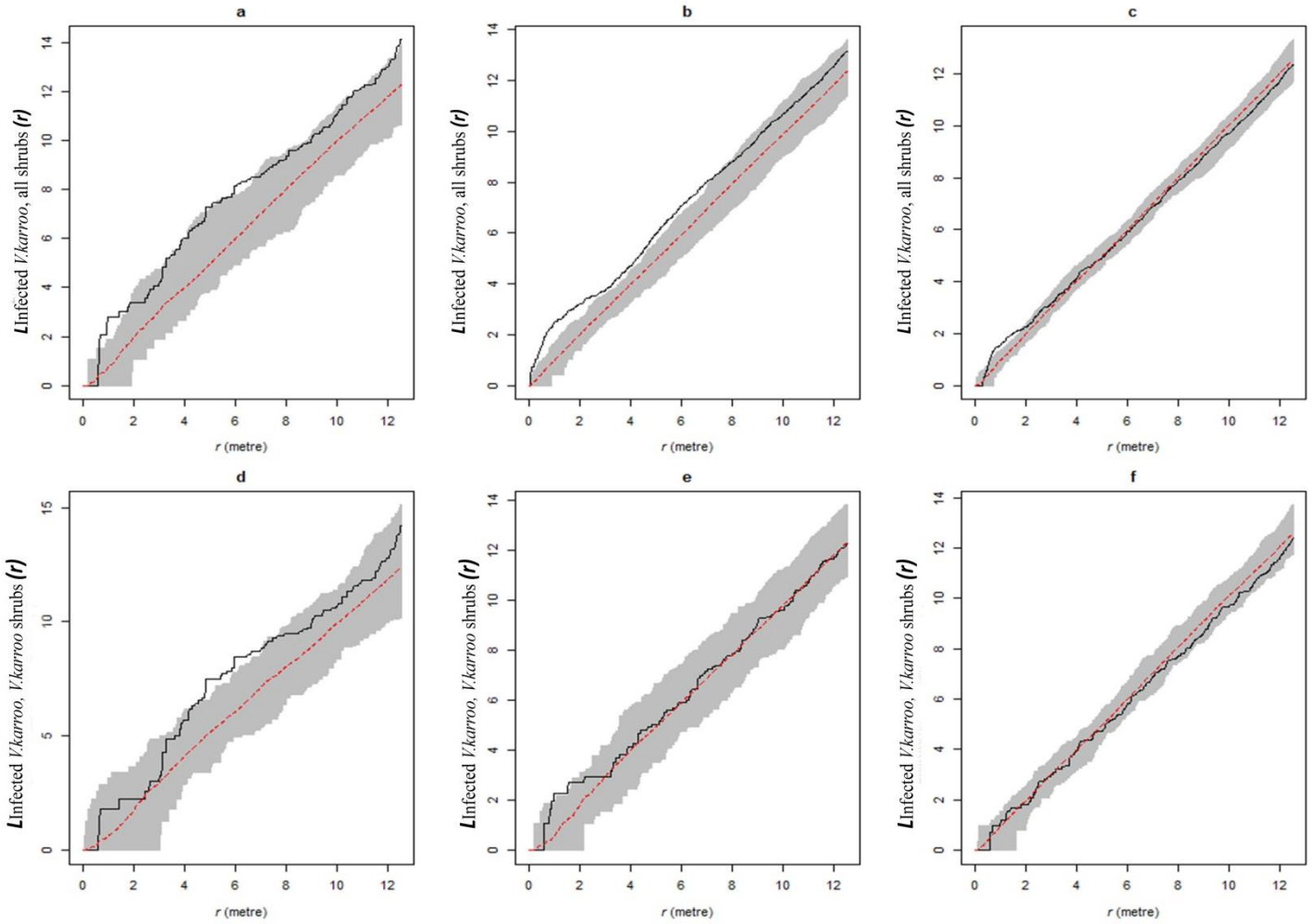
Appendix 7: Bivariate analysis using the $L_{12}(r)$ between infected trees and all seedlings (a-c) and between infected trees and *Vachellia karroo* seedlings (d-f) for plots 1-3. The position of the black line across the scale, determines the pattern, if it is either inside, below or above the simulation envelopes the pattern is regarded as having no correlation, a negative correlation, or a positive correlation.



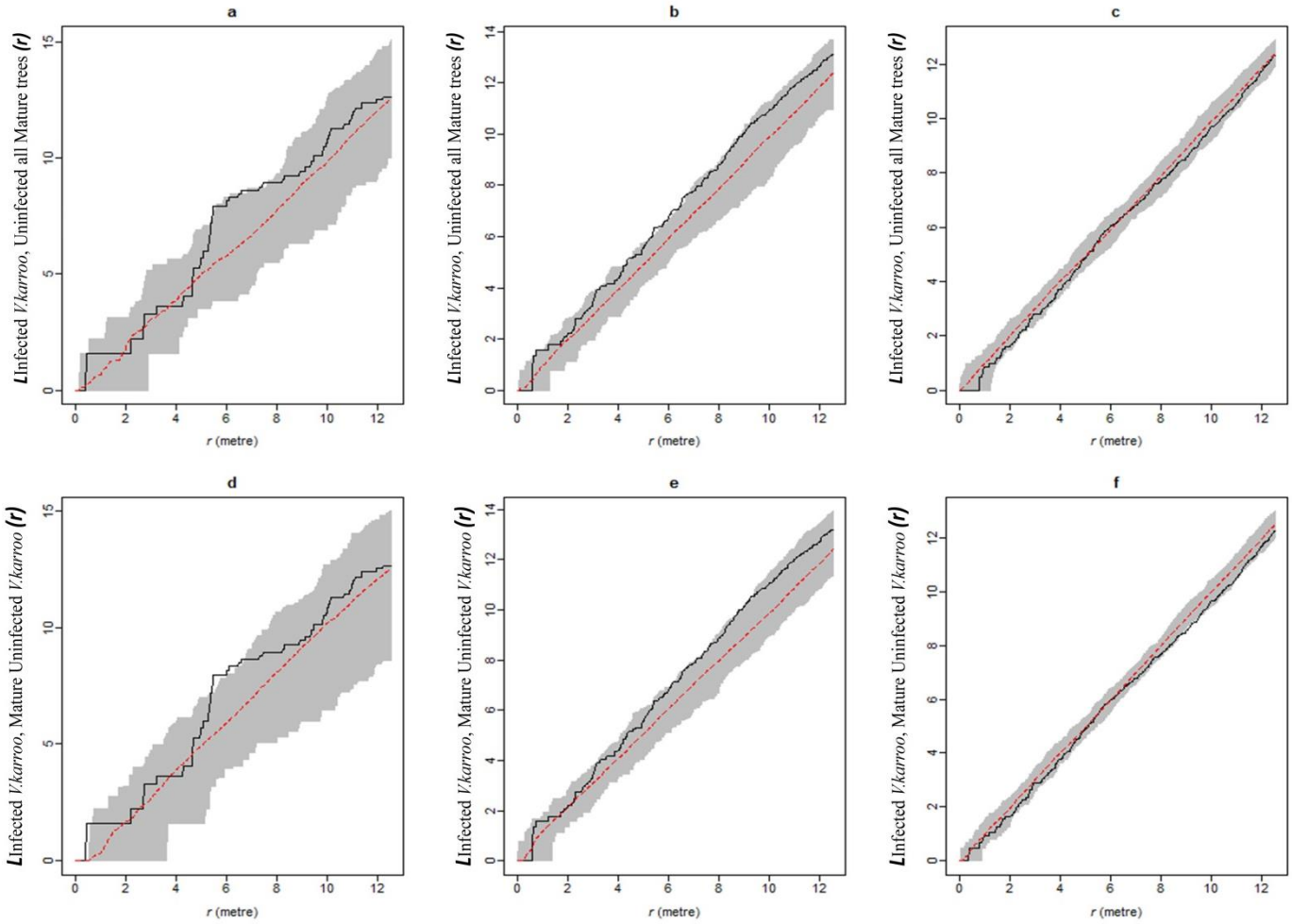
Appendix 8: Bivariate analysis using the $L_{12}(r)$ between infected trees and all saplings (a-c), and between infected trees and *Vachellia karroo* saplings (d-f) for plots 1-3 respectively. The position of the black line across the scale, determines the pattern, if it is either inside, below or above the simulation envelopes the pattern is regarded as having no correlation, a negative correlation, or a positive correlation.



Appendix 9: Bivariate analysis using $L_{12}(r)$ between infected trees and all shrubs (a-c) and between infected trees and *Vachellia karroo* shrubs (d-f) for plots 1-3 respectively. The position of the black line across the scale, determines the pattern, if it is either inside, below or above the simulation envelopes the pattern is regarded as having no correlation, a negative correlation, or a positive correlation.



Appendix 10: Bivariate analysis using the $L_{12}(r)$ between infected trees and all mature trees (a-c) and between infected trees and *Vachellia karroo* mature trees (d-f) for plots 1-3 respectively. The position of the black line across the scale, determines the pattern, if it is either inside, below or above the simulation envelopes the pattern is regarded as having no correlation, a negative correlation, or a positive correlation.



Appendix 11. Mark correlation results of canopy area and mistletoe counts as calculated using the Diggle-Cressie-Loosmore-Ford Test (DCLF) of CSR under Monte Carlo, based on 99 simulations with fixed number of points. The mistletoe-infected trees are either positively or negatively correlated if the P value is significant and this also dependent on the mark correlation value.

Canopy area	Plot number	u-value	*P*	rank
Canopy area	1	3.15	0.02	5
Canopy area	2	1.34	0.09	7
Canopy area	3	0.92	0.09	8
Mistletoe counts	1	7.68	0.02	3
Mistletoe counts	2	0.20	0.16	20
Mistletoe counts	3	2.39	0.25	22

CHAPTER 6

General discussion and synthesis

This study investigated how varying mistletoe infection degrees influence the regeneration capabilities of *Vachellia karroo* trees and how they affect the abiotic and biotic variables within and beyond mistletoe-infected canopy patches in a semi-arid savanna South-West, Zimbabwe. Firstly, this study explored how varying mistletoe infection degrees influenced the herbaceous biomass, grass height, litter content, temperature, relative humidity and soil moisture content within and beyond canopy patches (Chapter 2). In the third chapter the effects of varying mistletoe infection degrees on the species and functional diversity within *V. karroo* canopy patches and intercanopy spaces were analysed. Chapter 4 examined how varying mistletoe-infection degrees influence the regeneration capacity of *V. karroo* trees (Chapter 4). Lastly, Chapter 5 investigated how mistletoe-infected *V. karroo* trees influenced the spatial patterns of heterospecific and conspecific stage classes. Previous studies have shown how mistletoe-infected trees accelerate litter production and positively influence soil nutrient cycling (March and Watson, 2010; Ndagurwa *et al*., 2013, 2014a, 2014b, 2015, 2020; Muvengwi *et al*., 2015; Mellado *et al*., 2016; Al-Rowaily *et al*., 2020), plant species composition (Spasojevic and Suding, 2011; Ndagurwa *et al*., 2016, 2018; Hódar, *et al*., 2018), and infected hosts reproductive and regeneration capacities (Gomes and Fernandes, 1994; Silva and del Rio, 1996; Geils and Hawksworth, 2002; Press and Phoenix, 2004; Lamien *et al*., 2006; Mourão *et al*., 2009; Arruda *et al*. 2012; Daneshvar *et al*., 2014; Cruz Neto *et al*., 2017; Mellado and Zamora, 2020). In contrast, this study provides novel insight on how varying degrees of mistletoe-infection on *V. karroo* trees impact the understory species assemblages and the hosts' regeneration capabilities. Furthermore, this study integrated intercanopy spaces and animal disturbances which are mostly not included in many of the cited studies. However, intercanopy spaces are crucial in understanding whether mistletoe impacts extend beyond the putative trees' canopy patches.

Major findings

This study has successfully shown that varying degrees of mistletoe infection influence the abiotic and biotic micro-habitats within and beyond their canopy patches (Chapter 2). Canopy effects had more significant impacts on biotic variables compared to infection degree. This could be suggesting that trees without mistletoes are already improving nutrient cycling rates; however, presence of mistletoes further changes the abiotic and biotic localized canopy microhabitats (Muvengwi *et al*., 2015). Ndagurwa *et al*. (2016) and Hódar *et al*. (2018) reported higher understory grass biomass and herbaceous vegetation cover underneath mistletoe-infected trees compared to uninfected trees. Conversely, in this study, intercanopy spaces and low mistletoe-infection micro-habitats had between 1.09 to 1.53-fold higher herbaceous biomass and grass height compared to their respective canopy patches and high mistletoe-infection micro-habitats (Fig. 6.1).

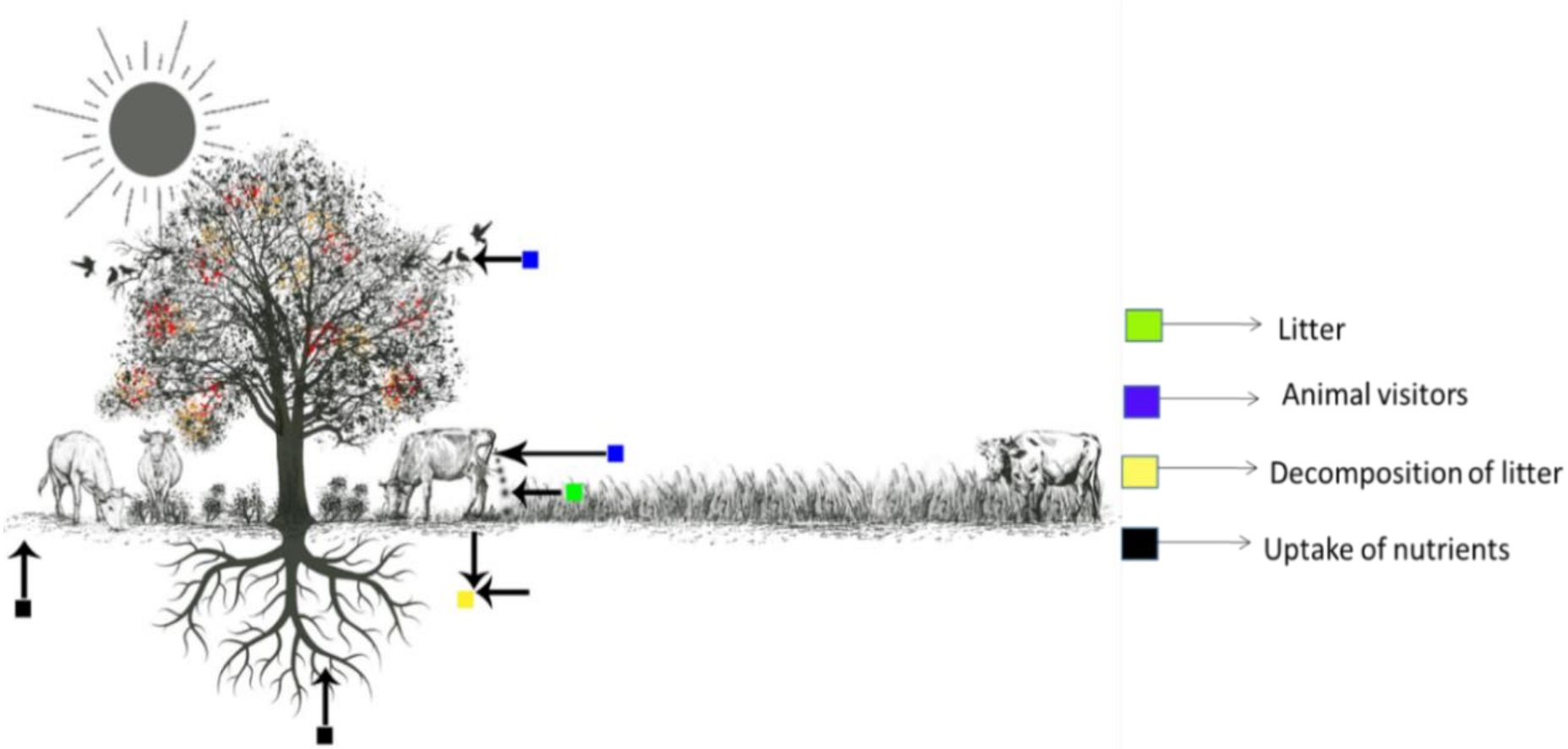


Fig. 6.1 Differences in the herbaceous biomass, grass heights, litter and animal visitations between mistletoe-infection canopy patches and intercanopy spaces (arrows point in the direction of greater amount/intensity of each variable).

Furthermore, grasses were shorter by 1.23 and 1.28-fold whilst herbaceous biomass was 1.03 and 1.21-fold lower within high- compared to low mistletoe-infection canopy patches. Lower herbaceous biomass and shorter grasses were attributed to greater grazing/trampling which was 1.24-fold higher in high compared to low mistletoe-infection canopy patch. Moreover, intense inter- and intra-specific competition within high mistletoe-infection canopy patches could have led to lower herbaceous biomass and grass heights (Fig 6.2). Consequently, lower herbaceous biomass within canopy patches and high mistletoe-infection microhabitats will most likely reduce the fuel load and incidence of fire; however, this can be transcended by the larger herbaceous biomass in the intercanopy spaces.

Soil relative humidity was 1.03-fold higher within high- compared to low mistletoe-infection canopy patches. It is possible that high mistletoe-infected trees are efficient in hydraulic lift and horizontal uptake of water from the intercanopy spaces. Moreover, higher litter quantities and organic matter content could have reduced mistletoe profligate transpiration rates and improved water holding capacity (Sala *et al*., 2001; Ludwig *et al*., 2003, 2004; Ndagurwa, 2015; Priyadarshini *et al*. 2016a, b). Additionally, higher litter composition and quantities within high- compared to low- mistletoe-infection canopy patches could have increased microbial activity, decomposition and soil respiration rates, thus resulting in the higher temperatures observed (0.5%). Furthermore, high mistletoe infection could have altered the physio-morphological structures of the host tree, thus increasing the light incidence and the amount of water that reached the soil surface of trees (Press and Phoenix, 2004; Mellado and Zamora, 2017). Therefore, it is expected that high mistletoe-infection canopy patches have higher nutrient cycling rates compared to low mistletoe-infection canopy patches due to higher temperatures, soil moisture and litter input (March and Watson, 2007, 2010; Ndagurwa *et al*., 2013, 2015, 2020; Muvengwi *et al*., 2015; Mellado *et al*., 2016; Al-Rowaily *et al*., 2020).

Compared to other studies (Ndagurwa, 2015; Ndagurwa *et al*., 2014, 2014b, 2016) this study found variations in relation to herbaceous biomass and soil moisture conditions. These differences could be caused by the equipment used, for example, Ndagurwa (2015) and Ndagurwa *et al*. (2014, 2014b, 2016) used oven-dried clipped herbaceous biomass samples from quadrats, and soil core samples to calculate the herbaceous biomass and soil moisture, respectively. In my study, a disc pasture meter (Zambatis *et al*., 2006), ibuttons and a hydrosense soil moisture sensor, which are more robust, were used to directly measure and calculate herbaceous biomass and soil moisture content, respectively. The former study provided snapshots, while in this study almost "continuous", and far more detailed results were obtained over 8 months using the ibuttons. Furthermore, compared to other studies, this study had more a detailed spatial comparison (i.e., canopy patch *vs* intercanopy spaces) and more replicates. For instance, soil moisture was measured using ibuttons which were planted over an 8month continuous period and the ibuttons recorded the data 8 times/day at 3-hour intervals. Therefore, this study could be showing that as spatial details and focus increase, there is a possibility that results are also improved and they provide a better representation of the overall mistletoe effects.

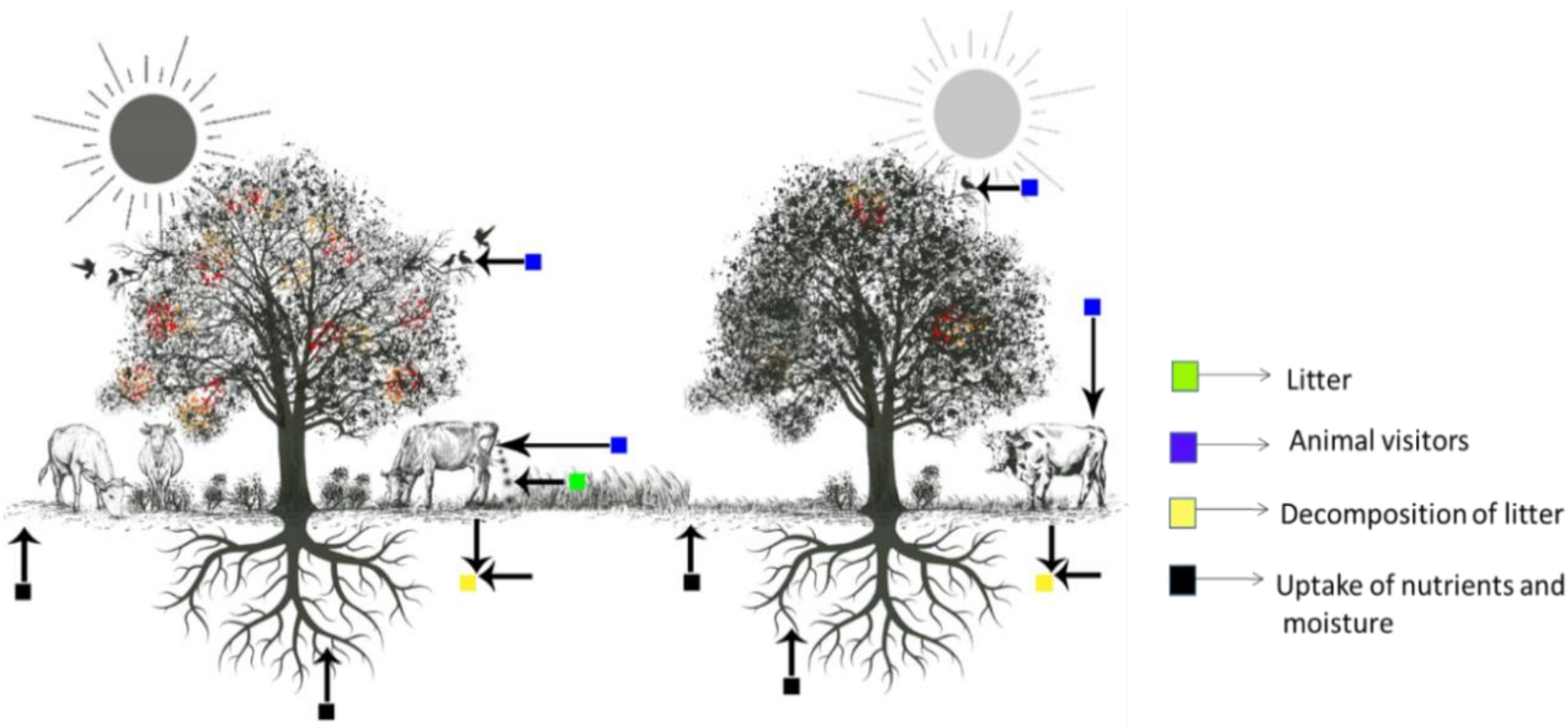


Fig. 6.2. Differences in the plant composition, productivity and animal visitations between high- and low mistletoe-infection canopy patches and intercanopy spaces (arrows from black and yellow boxes, point in the direction of greater amount/intensity of each variable, whilst the arrows from blue and green boxes are showing the animal visitors and their dung (litter) deposits).

Canopy patches had higher species richness, diversity and functional richness, dispersion and evenness compared to intercanopy spaces, and similarly these variables were higher in high mistletoe-infection micro-habitats than low infection micro-habitats (Chapter 3; Fig. 6.2). Intercanopy spaces had distinct plant assemblages dominated by graminoids in contrast to canopy patches which had a diverse composition of the three plant growth forms. Higher species diversity within canopy patches was attributed to intermediate to high shading and higher availability of nutrients and soil moisture (Chapter 2). Higher species diversity and functional attributes could further explain higher animal visitations to the canopy patches, particularly of high mistletoe-infection canopy patches compared to the intercanopy spaces (Fig. 6.2). However, intermediate grazing in low mistletoe-canopy patches could have increased the species diversity within these canopy patches.

These results provide a more detailed clarification on the plant species variations that can arise with different levels of mistletoe infection. High mistletoe-infection canopy patches had higher species richness, diversity and functional attributes despite higher grazing in contrast to low mistletoe-infection canopy patches. This is consistent with findings from other studies that reported higher species diversity and richness underneath mistletoe-infected trees compared to uninfected trees (Ndagurwa *et al*., 2016, 2018; Hódar, *et al*., 2018; Monteiro *et al*., 2020), showing the effects of mistletophilly as mistletoe-infection increases. High mistletoe-infection canopy patches are resource-rich and they could be facilitating the quick recovery of their understory plants despite heavy grazing (Janecke, 2020). This could be indicating that these canopy patches are nutrient and foraging hotspots that can support more diverse plant assemblages and herbivores. Grazing could also be maintaining the plants at a shorter height and prolonging immature stage classes in which their nutrient concentrations of N, P, K, Na and Mg would remain higher than in more mature stage classes, prolonging their palatability to grazers and browsers (Adler *et al*., 2001; Moe and Wegge, 2008). However, the presence of species like *Eragrostis curvula* is an indication of high disturbance (O'Connor *et al*., 2011; Van Oudtshoorn, 2014) within high mistletoe-infection canopy patches. Therefore, high species diversity does not necessarily translate to favourable species composition (O'Connor *et al*., 2011).

The study also shows that different degrees of mistletoe infection resulted in varying changes to the hosts physio-morphological structures (Chapter 2 and 3) and consequently dissimilar regeneration capacity of the hosts trees (Sala *et al.*, 2001; Press and Phoenix, 2004; Lamien *et al*., 2006; Mourão *et al*., 2009; Arruda *et al*. 2012; Chapter 4). In agreement with previous studies (Silva and del Rio, 1996; Daneshvar *et al*., 2014; Cruz Neto *et al*., 2017), high mistletoe-infected trees in this study had a lower number of flower buds and flowers by 40% to 68%, and the dimensions and/or mass of the pods and seeds were significantly lower by between 13% and 25% compared to low mistletoe-infected trees. This was attributed to high mistletoe-parasitism and low rainfall (Geils and Hawksworth, 2002;

Joubert *et al*., 2013; Daneshvar *et al*., 2014) at Matopos Research Station, the study site. Nevertheless, higher numbers of understory *V. karroo* juveniles and seedling and sapling density within high- compared to low mistletoe-infection canopy patches could be an indication of the weakening of the host tree could also be providing gaps for the subordinate seedlings and saplings to actively grow, thus making high mistletoe-infection canopy patches safe sites for *V. karroo* seedlings (Lamont *et al*. 1993; Mellado and Zamora, 2020). However, higher densities of saplings within high mistletoe-infection canopy patches could be due to low herbivory and *V. karroo* juveniles being successful heliophytes. As a result, high- compared to low mistletoe-infection canopy patches are safe sites for juvenile *V. karroo* trees particularly seedlings, but high mistletoe-infection intercanopy spaces are safer sites for saplings than high mistletoe-infection canopy patches, which might be due to lower competition and herbivory (Chapter 2 and 3).

Conversely, low-compared to high mistletoe-infection canopy patches were safer sites for seeds, as seed banks were 3.24-fold lower within high- (14.4 ± 5.8 seed/m^2) than low mistletoe-infection canopy patches (46.7 ± 10.7 seeds/m^2). This was attributed to higher temperatures and soil moisture within the top soils of high mistletoe-infection canopy patches, and higher animal visitors feeding on the seeds/pods, as well as bruchid beetle seed predation. Higher soil moisture contributes towards more rapid germination and/or decomposition, whilst higher temperatures may have led to desiccation of the smaller and softer-coated seeds from high mistletoe-infection *V. karroo* trees. Higher soil temperatures may also facilitate germination of many species in savanna ecosystems (e.g. van der Walt and Witkowski, 2017). Therefore, higher soil moisture may increase imbibition rate of *V. karroo* seeds (Wilson and Witkowski 1998), and hence may lead to greater levels of seedling establishment thereafter. However, high mistletoe-infection canopy patches had smaller-sized juveniles compared to low mistletoe-infection canopy patches, even though there was higher light incidence and soil resources (Chapter 2). This was probably due to greater levels of animal disturbances (Chapter 2) or accelerated inter- and intra-specific competition, attributed to relatively higher species diversity (Chapter 3 and 5). However, not all seedlings will survive the winter months, due to frost and lack of moisture and as a result, this will further decrease the number of seedlings that are recruited to saplings. Hence the possible net effect of a decline in *V. karroo* seed and germinable seed production/tree and lower sapling recruitment could lead to the dominance of other woody species, for example the high density of *Ziziphus mucronata* seedlings and saplings in the spatial study 50 x 50 m plot with the highest density of mistletoe-infected trees (see Chapter 5).

Seed production/tree and germinable seed production/tree of high mistletoe-infected trees were 32% and 20%, (respectively), that of low mistletoe-infected trees. Similarly, the total percentage germination of greenhouse seeds was 1.58-fold higher for low- (42%) than high mistletoe-infected tree seeds (26%). However, before scarification (≤ 30 days) percentage germination was 4-fold higher

for high infection seeds (12% *vs*. 3%), but after scarification (≥ 31 ≤ 73 days) low infection seeds had 2.9-fold higher percentage germination (34% *vs*. 12%). This was attributed to variations in seed size and the relatively different impacts of the seed scarification applied on the seeds from high versus low infection trees (Gomes and Fernandes, 1994; O'Connor *et al*., 2010; Daneshvar *et al*., 2014; Souza and Fagundes, 2014).

To date, no known study has focused on how mistletoe-infected trees influence other surrounding trees of different stage classes at smaller scales. Studies have mainly focused on the spatial patterns of trees without mistletoes and on the distribution of mistletoes on each tree. However, mistletoes have already been defined as keystone species (Watson, 2001) and ecosystem engineers (Ndagurwa, 2015). Therefore, besides investigating host-mistletoe interactions, it is crucial to understand the impacts of such interactions in semi-arid savanna plant ecosystems. The results show that the spatial distribution of mistletoe-infected trees can influence the spatial patterns of their conspecifics and heterospecifics at different stage classes (Chapter 5). Mistletoe-infected tree patterns were consistent with a random pattern, partly attributed to the random distribution of mature trees within the plots and also due to seed-disperser-bird preferences. The findings also show that mistletoe-infected trees exhibited both facilitation and/or competitive exclusion and this varied with stage classes and/or with species.

Facilitation resulted in aggregation being dominant amongst the juvenile stage classes. However, conspecific juveniles in some plots showed repulsion around infected trees. Aggregation was attributed to vegetative reproduction and targeted seed dispersal, which led to the accumulation of seeds and consequently high germination and seedling recruitment within the canopies' zone of influence (Witkowski and Garner, 2000; Walters and Milton, 2003; Flores and Jurado, 2003; Schleicher *et al*., 2011; Mellado *et al*., 2016; Dohn *et al*., 2017; Mellado and Zamora, 2017). Moreover, high availability of nutrients due to increased litter deposits and nutrient cycling rates (Ndagurwa *et al*., 2020; Al-Rowaily *et al*., 2020) could have made mistletoe-infection trees safe sites that support nurse protégé interactions of both conspecific and heterospecific juveniles and shrubs. Mistletoe-infected trees often lose their competitive edge against other subordinate plants, thus the weakening of the host trees could have also led to facilitation of conspecific juveniles and different species of shrubs. In the long run, the high diversity of trees (Chapter 3 and 5) could mean that the stands formerly dominated by *V. karroo* could change to mixed woodlands due to higher rates of mortality of heavily infected *V. karroo* trees. Mixed woodlands in semi-arid savannas result in higher ecological divergence and better niche occupancy, attributes which result in stable communities that are capable of surviving stochastic events and/or outcompeting invasive species (Chapter 3). However, repulsion of conspecific seedlings and saplings could have been due to competitive exclusion. *V. karroo* host trees could have outcompeted their juveniles especially in cases where they are using the same resource pools (O'Connor 1995; Cheng *et al*., 2014). Additionally, *V. karroo* juveniles could have also been competing for resources with herbaceous plants, particularly grasses

which can significantly reduce tree growth and recruitment within nutrient-rich patches (Bond *et al*., 2001; Porensky and Veblen, 2012; Chapter 2 and 3).

Mistletoe-infected trees exhibited a random pattern, with their heterospecific and/or conspecific uninfected mature trees due to co-existence of clustering and dispersion patterns (Meyer *et al*., 2008; Pillay and Ward, 2012; Dohn *et al*., 2017). The clustering of smaller but competitive subordinate mature trees around mistletoe-infected trees could be a result of weak density-dependent thinning due to the augmented nutrients. Meanwhile dispersion could be occurring around low mistletoe-infection trees as a result of high competition.

In summary, Figure 6.3 provides a conceptual framework of how canopy presence and physio-morphological changes due to varying mistletoe-infection degrees led to differences in the micro-climatic conditions amongst the four microhabitats (Fig 6.3a). These variations resulted in differences in species composition, diversity and functional attributes (Fig. 6.3b), which gave rise to differing rates of animal attraction and disturbances within the microhabitats (Fig. 6.3c).

Consequently, differing rates of animal disturbances and plant competition caused variations in the herbaceous biomass and grass heights among the four microhabitats (Fig. 6.3d). Moreover, the physio-morphological changes to the canopy led to a considerable reduction of regeneration capacity of high- compared to low-mistletoe-infection trees (Fig. 6.3e). In turn, all these factors contributed to the different spatial patterns that were exhibited by heterospecific and conspecific woody plants at different growth stages within this semi-arid ecosystem (Fig. 6.3f).

Implications

Mistletoes at different infection degrees can indirectly alter the spatial redistribution of soil nutrients by having a positive and negative relationship with animal visitors. This can result in a scenario wherein as mistletoe infection increases, the number of animals attracted to the canopy and canopy patches increases due to abundance of food both in the canopy and within the understory patches as shown in Chapter 2 and 3 (Figure 6.4a). Animals that visit these trees directly deposit excreta (i.e., nutrients) within these canopy patches (Chapter 2) thus providing positive feedback by increasing nutrient cycling rates (Ndagurwa *et al*., 2013; Mellado *et al*., 2016; Watson, 2016). The combination of high litter from mistletoes, birds and that of livestock (Chapter 2) can augment soil nutrients within canopy patches thus increasing many factors including plant productivity and diversity to more mistletophillous plant species (Chapter 3).

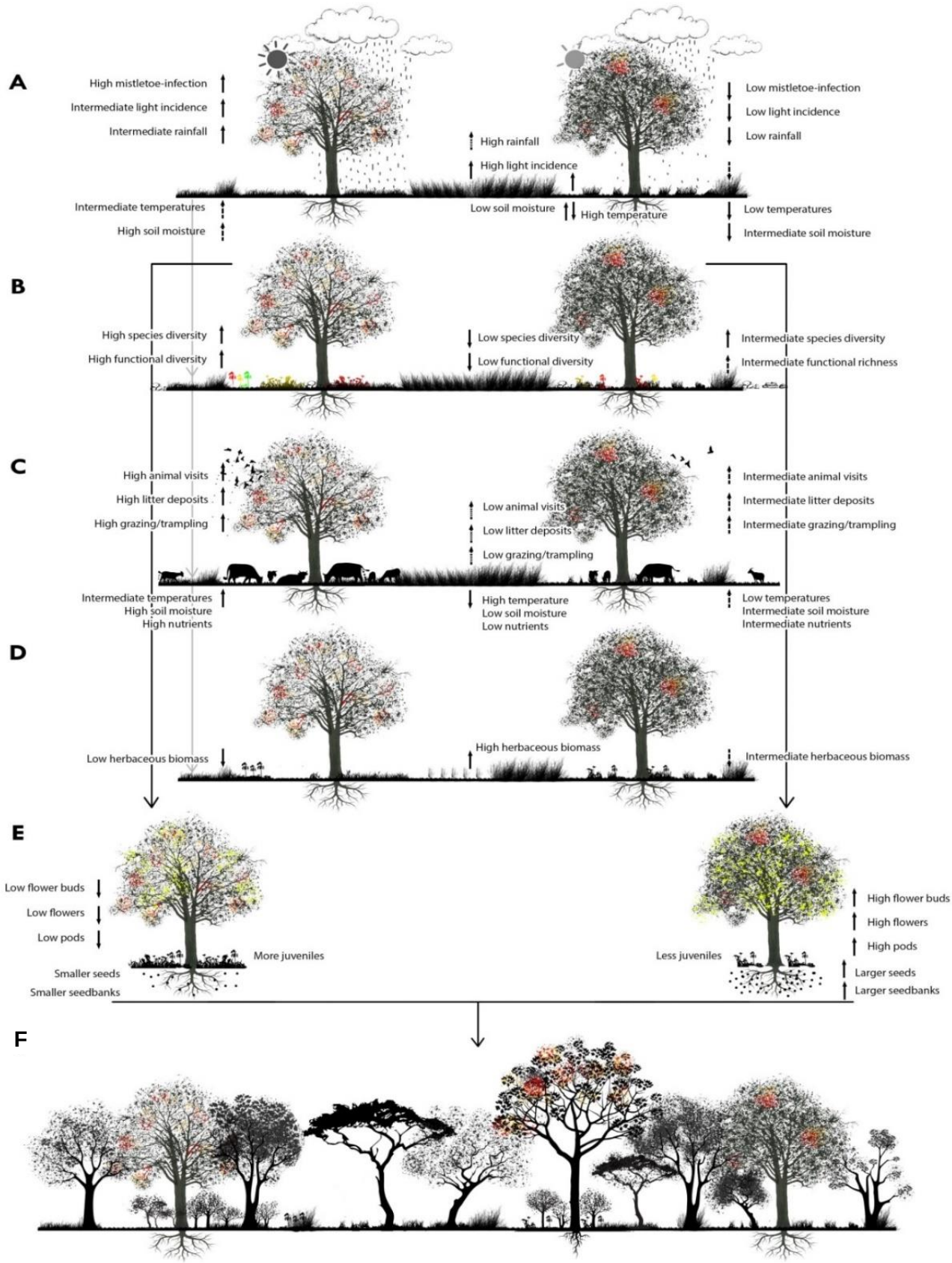


Fig. 6.3. A synthesis diagram of the results on how mistletoes affect plant growth and reproduction as well as regeneration ecology.

As mistletoe infection intensifies, the physio-morphological structures of the hosts' are reduced (Cullings *et al.,* 2005; Press and Phoenix, 2005; Manthiasen *et al.*, 2008; Mellado and Zamora, 2017; Chapter 2 and 4). Therefore, bird visitors that are normally attracted to closed canopies and those that prefer to shelter and/or feed on mistletoes and on invertebrates found in and on the litter bed are expected to be reduced due to declining canopy area and litter quantities (Watson and Herring, 2012; Watson, 2015; Mellado *et al*., 2019).

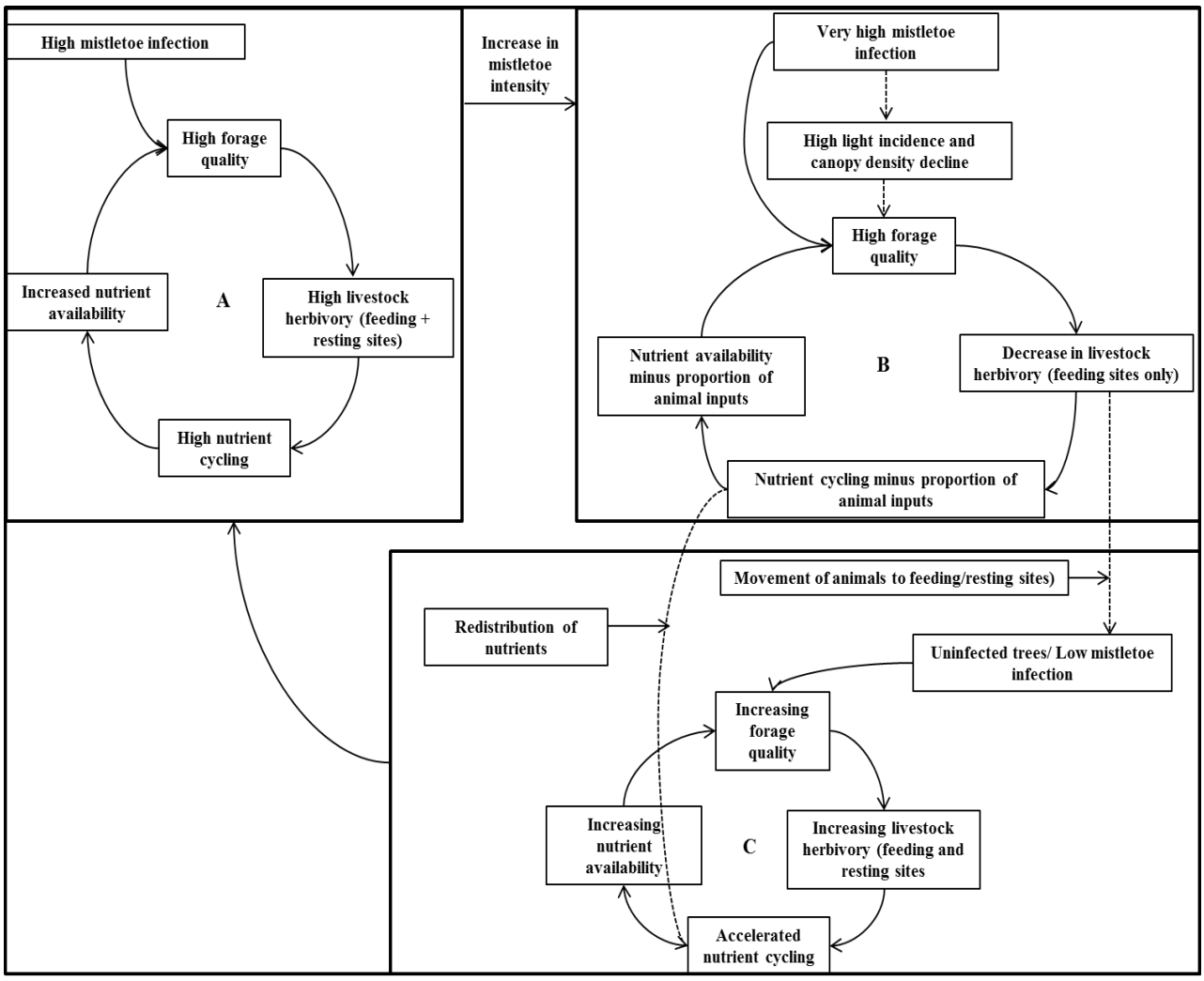


Fig. 6.4. A hypothetical diagram detailing the positive feedback between high mistletoe infection canopy patches and animals. A) the ideal system where birds, livestock and insects deposit excreta and increase nutrient cycling and nutrient use by plants, thus providing nutrient-rich forage. B) a scenario where light incidence increases with mistletoe intensity due to physio-morphological changes thus reducing shade. This leads to a reduction in the number of birds, livestock and insects visiting, foraging and resting within high mistletoe-infection tree canopies and canopy patches. The animals shift to C) represented by uninfected and low mistletoe-infection trees where they both feed and rest. This accelerates nutrient cycling and increases the nutrient availability in these patches. Solid lines are

showing a feedback loop, whilst dashed lines are showing factors causing negative changes to the feedback loop.

Smaller animals such as rodents may also limit their feeding underneath the open canopies of high mistletoe-infection trees due to increased susceptibility to raptors. This may decrease animal diversity in these patches. Simultaneously, a decline in the physio-morphological attributes is likely to reduce *V. karroo* litter inputs within the canopy patches but still result in more litter from the mistletoes and animals (Mellado *et al*., 2016; Chapter 2). Moreover, as mistletoe-infection continues to intensify to greater levels, herbivores that were previously attracted to the shade and the palatable understory plants (Chapter 2 and 3) are likely to be diverted in numbers (due to higher light incidence; Figure 6.4b) to low mistletoe-infection trees or uninfected trees with more complete shade (Figure 6.4c).

Uninfected and low mistletoe-infected tree canopy patches can benefit from positive feedback as they have intact canopies that provide fewer within canopy gaps (complete shade) and palatable food sources, thus they benefit from animals feeding and redepositing (redistributing) nutrients within their canopy patches (Augustine *et al*., 2003). Furthermore, animals can still feed on the understory plants within high mistletoe-infection canopy patches and then spatially redistribute and export nutrients to low mistletoe-infection and uninfected tree canopy patches where they rest and/or sleep (Augustine, 2003, 2004; Augustine *et al*., 2003; Singer and Schoenecker, 2003; Moe and Wegge, 2008; van der Waal *et al*., 2011) (Figure 6.4c). However, high mistletoe-infection canopy patches can still be favoured as feeding sites, since cows are also known to avoid the risk of parasite infection by avoiding patches contaminated with fresh excreta (Moe and Wegge, 2008). Regardless, a more open canopy shade can still reduce the number and intensity of animals visiting the high mistletoe-infection hosts' canopies and canopy patches. Further, it is likely that a reduction in animal litter can also reduce insect numbers and/or species and functional diversity, especially Coleoptera, hence leading to a reduction in insectivores. Consequently, the combination of mistletoes and herbivores can maintain and/or create soil nutrient and vegetation structural heterogeneity in semi-arid ecosystems.

Nonetheless, animals tend to prefer habitats that increase their fitness, survival and improve their dietary requirements especially if animal competition is not too high (Bell, 1971; Muposhi *et al*., 2016). Therefore, despite, the low grazing value of some of the species within high mistletoe-infection canopy patches, they could have higher leaf nutrient rewards resulting in higher visitation rates. It is also possible that eventually, cows (largely grazers) will become the most dominant visitors within high mistletoe-infection canopy patches, rather than goats (largely browsers), due to the mixed grazing value plants. Regardless, if this is the case, eventually this will reduce overgrazing of the most palatable species due to absence of selective feeding thus possibly reverting to the original species composition (Bowman *et al*., 2016). Smaller bodied animals will eventually prefer and select low mistletoe-infection canopy patches with more digestible and palatable grasses (Bell, 1971; Muposhi *et*

al., 2016; Chapter 3). This is speculative and more studies should be done to test the nutrient levels of the understory plants within the micro-habitats and their interactions with existing grazers/browsers.

An increase in heterogeneity results in more varied niches that promote coexistence of species with different functional attributes, within a wide range of trophic levels within a given ecosystem (Tews *et al*., 2004; Janecke, 2020; Leitner *et al*., 2020). Vegetation spatial heterogeneity ensures that herbivores have diverse feeding patches and this increases food resource stability and nutritional balance (De Beer and Van Aarde, 2008). However, homogeneous or selective grazing can result in a decline in the spatial heterogeneity of vegetation whilst heterogeneous grazing can increase vegetation spatial heterogeneity (Adler *et al*., 2001). Therefore, variations in grazing intensities among microhabitats can also contribute towards spatial heterogeneity of vegetation characteristics and traits (Janecke, 2020; Chapter 3) and this can probably result in permanent changes to the plant community. For instance, over time, the overall vegetation structure of high mistletoe-infection canopy patches can be changed to unpalatable increaser I and II grasses and forbs that are fast-growing and short-lived species. These plants can rapidly replace any foliage that is lost due to continuous herbivory (Adler *et al*., 2001; Porensky and Veblen, 2012; Guo *et al*., 2016). There is also a chance that key species (especially grasses) that are not adaptable to continuous or heavy grazing will be lost throughout this process, thus changing the species composition towards forbs and juvenile woody plants underneath these trees. Moreover, these patches are most likely to be colonized by plant species that are not favoured by grazers (Adler *et al*., 2001).

However, due to these canopy patches having higher soil resources and plant functional diversity, it is not anticipated that these changes will be 'drastic' or that these patches will be vulnerable in the face of perturbations or stochastic events. The plants may remain palatable due to high soil nutrient concentrations. Nevertheless, there is still a need to compare the nutrient values of understory plants within the different microhabitats in areas with and without livestock. Over time, animals will shift to low mistletoe-infection trees or to uninfected large trees that have nutrient-enriched and high grazing value plants (Chapter 3). This pattern may vary depending on the season, for instance in the dry season when constraints are more pronounced, high mistletoe-infection canopy patches are highly utilised as shown by grazing lawns during the dry season as they are higher moisture patches (Chapter 2), whilst in the wet season, animals could be more selective and also feed in low mistletoe-infection and uninfected tree canopy patches. However, this also needs further investigation.

The above differences in the vegetation structure are expected to be influenced by the variations in the topsoil characteristics across the four microhabitats. Differences in litter quality and quantity (Chapter 2), for instance, can result in heterogeneous soil conditions, and arthropods and microbial activity which often lead to diverse plant communities with varying functional traits (Ndagurwa *et al*., 2016; Watson, 2016; Guo *et al*., 2018; Mellado *et al*., 2019; Al-Rowaily *et al*., 2020). Dominance of

mistletoe leaf litter can lead to changes to arthropods, microbial and fungal communities (March, 2007; Těšitel *et al*., 2020), thus resulting in greater heterogeneity beneath uninfected, low- and high mistletoe infected, dead trees and intercanopy spaces. Further, an increase in the spatial heterogeneity of nutrients and water availability diversifies habitats and consumer (vertebrates and invertebrates) patterns of use in semi-arid savannas.

The variations in grazing in Fig. 6.4, attributed to mistletoe-infection intensity, will also reduce the fuel load and the impacts of fire in this area. Generally, fires are abiotic consumers that can restructure vegetation especially in savanna ecosystems and they behave in a similar way to generalist herbivores (Archibald *et al*., 2005; Bond, 2005; Bond and Keeley, 2005; Davies *et al*., 2012, 2013; Bowman *et al*., 2016). At MRS there have been no instances of fire for over 10 years (pers. comm. Resident Ecologists 2017), and the area is predominantly "brown" and "green", i.e., it is regulated by herbivores and influenced by resource availability, respectively (Bond, 2005; Bowman *et al*., 2016). However, more frequent fires are a possibility in future, due to increased likely anthropogenic pressures, thus, it could be beneficial to "carefully", look into the 'pyrodiversity begets biodiversity' hypothesis, i.e., on how manipulation of different fire intensities and frequency can further increase species and functional diversity of the area (Davies *et al*., 2012).

The effects of fire are dependent on intensity, severity, frequency and the season (Bond *et al*., 2005; Bond and Keeley, 2005). For instance, Davies *et al*. (2013) reported that decomposition rates in semi-arid to intermediate areas was accelerated by frequent fires and this was attributed to an increase in fungus-growing termites that are responsible for decomposition. Therefore, if both fire and fungus-growing termites are coupled with the overall predicted systematic grazing as shown in Fig. 6.4, it is likely that nutrient cycling rates will increase alongside the species and functional diversity at different trophic levels (Archibald *et al*., 2005; Davies *et al*., 2012, 2013; Bowman *et al*., 2016).

However, selective grazing within high mistletoe-infection canopy patches (Fig. 6.4a) can result in very tall grasses and shrubs that are highly combustable in intercanopy spaces and low mistletoe-infection subcanopies. This can lead to very intense and severe fires that can have serious deleterous effects, which may alter the nutrient cycling and decomposition processes (Archibald *et al*., 2005; Davies *et al*., 2013). Furthermore, high intensity fires can also destroy the grazing lawns within high mistletoe-infection subcanopies, thus reducing the number of animals (especially birds) that visit the large trees and mistletoes in this semi-arid savanna, hence distorting the overall predicted systematic grazing patterns in Fig. 6.4 (Archibald *et al*., 2005; Davies *et al*., 2013; Bowman *et al*., 2016).

Nevertheless, most semi-arid savanna fires are surface fires which are fueled by materials that are near the ground, and they may not have significant impacts on the overstory and its functioning (Bond and Keeley, 2005). In such cases, surface fires can give seeds (e.g. *Ziziphus mucronta* that are deposited by animals visiting the tree and mistletoes) and seedlings a competitive advantage against

grasses. As a result, it is possible that the combination of fire and overgrazing underneath high mistletoe-infected canopies, may further accelerate the invasion of numerous woody plant species leading to bush encroachment and low species richness (Bowman *et al*., 2016), and also invasion of invasive alien species.

There is also a possibility that due to their low fuel load, high mistletoe-infection subcanopies could act as fire breaks that prevent/ slow down/ reduce intensity and the spread of the fire at MRS (e.g. Archibald *et al*., 2005). This may result in the migration of grazers from the unburnt (or poorly burnt) high mistletoe-infection subcanopies to completely burnt areas (intercanopy spaces and low mistletoe-infection subcanopies) that have palatable regrowth (Archibald *et al*., 2005). Consequently, this can give high mistletoe-infection subcanopies time to fully recover, albeit this is dependent on the recovery rate of understory plants, thus reducing the undesired impacts of overgrazing within these patches (Archibald *et al*., 2005). However, issues relating to fire are complex and multi-factorial and hence a need for further investigation.

Overall the results from this study show that indeed the ability of mistletoes to shape or alter their immediate environments increases with mistletoe infection intensity (number of mistletoes/tree). Moreover, parasitism can reduce the hosts' competitive edge thus a diversity of understory woody plants have the ability to become more competitive. Consequently, if high mistletoe-infected trees eventually die, there will be an increase in space and reduced competition for resources. In the long run this may promote bush encroachment of the already established, diverse understory woody plants. Additionally, the decomposition of mistletoes and host litter would favour the recruitment of diverse species of woody plant seedlings and saplings, despite competition from other growth forms. Unfortunately, if this is coupled with a reduction in the regeneration capacity of high mistletoe-infected trees, particularly if mistletoe-infection is recurrent in the area, a decrease in dominance of the *V. karroo* trees at Matopos Research Station is expected (Watson, 2016). In Chapter 5 one of the study plots was already dominated by immature stage classes of *Ziziphus mucronata*, and this is showing that mistsletoes are now contributing to a shift towards mixed woodlands. In this regard mistletoes could be a keystone species that are restructuring the *V. karroo* monospecific stands in semi-arid savannas, similar to Mediterranean pinelands (Mellado and Zamora, 2017). Furthermore, high mistletoe-parasitism will reduce the life span of the reproductive *V. karroo* trees. However, death or a reduction in host performance can also open up gaps for invasive species to occupy, thus reducing the functional richness in that area.

Lastly, the findings show that mistletoe effects can be masked by other factors that can also generate heterogeneity such as herbivory, rainfall, fire, soil conditions and catenal effects (which do not apply to any extent in this site due to the very flat terrain). Regardless, this study has shown that mistletoes are important in maintaining heterogeneity not only for nutrients, but their impacts cascade and

increase heterogeneity in other trophic levels, such as plant and herbivore (vertebrate and invertebrate) communities in semi-arid savannas. Micro-habitat variations will probably stabilize and increase the resilience of these semi-arid savannas to perturbations and stochastic events. In conclusion, the results demonstrate that mistletoes are 'keystone structures' (Tews *et al.*, 2004) and important drivers of semi-arid savanna ecosystem processes. However, the extent to which mistletoes are to be conserved still needs further investigation.

Recommendations for future studies

There is need for a study that will investigate how absence of, and increasing intensity of mistletoes can influence both biotic and abiotic factors within and beyond subcanopies. In this study trees without mistletoes were present but they were significantly smaller than both high- and low mistletoe-infection trees. In such instances, it would be ideal to remove mistletoes from some of the large trees in order to make comparisons between trees of much the same sizes.

Grazing played an integral part in shaping the herbaceous biomass quantities and grass heights and most certainly could have been one of the drivers of plant species composition. Therefore, it is suggested that studies investigate how different grazing intensities coupled with varying degrees of mistletoe infection influences the species composition, diversity and spatial patterns of understory plants. Furthermore, it is recommended that comparative studies be done in protected areas in semi-arid savannas to fully ascertain how varying mistletoe infection degrees influence their understory plants within and beyond their canopies. This could be achieved by using exclusion plots. There is also need to investigate the understory species dynamics from the first mistletoe infection up to the death of the host due to mistletoe infection. No study in African semi-arid savannas has studied the progression of mistletoe-infection on the host over an extended period of time, and how this is linked to the structure of understory and surrounding plants.

Furthermore, the reduction of the physio-morphological attributes of the hosts also negatively affected the regeneration of high mistletoe-infected trees. Therefore, more studies should investigate the extent in which mistletoe infection impacts pollination (pollen viability, pollen grain abundance and ovule sizes) of *V. karroo* trees and on the behaviour of pollinators visiting both the parasites and the hosts. More studies should be done on other host species on different soil types in semi-arid savannas to determine whether mistletoe-impacts on the regeneration capacities are similar. Moreover, studies should investigate whether there is a variation in the allocation of nutrients (N and P) to different reproductive traits at varying mistletoe infection intensities to fully understand the regeneration effort of each category of trees (Witkowski and Lamont, 1996). Studies can be done to examine whether there is a link between the allocations of nutrients to reproductive traits to the available soil nutrients.

There is also a need to investigate how mistletoes affect the canopy structure i.e., host specific leaf traits, branch sizes, photosynthesis, respiration, transpiration and nutrient contents using comparing between nitrogen fixing and non-nitrogen fixing plants, from inception of first infection up to the death of the host tree.

This study indirectly provided a baseline on the possible spatial patterns that can arise in *V. karroo* dominated plots with different densities of mistletoe-infected trees. Therefore, spatial patterns for plots with similar mistletoe-infected tree densities can be investigated to predict the patterns that are most likely to develop in such scenarios. This will entail comparing within and between sparse, medium-density, and closed density plots. Furthermore, these spatial patterns can be compared across different species (e.g., using non-nitrogen fixing trees) infected with mistletoes and in areas with different rainfall and temperature patterns, as well as geology and topography. Since livestock can also influence the distribution patterns of the juvenile trees that are mostly found within the canopy patches, mistletoe infected tree spatial pattern can be investigated in pristine areas with no livestock disturbance or by using exclosures. It is important to emphasize the need for permanent plots from which spatial patterns can be measured over long periods. This will contribute towards the understanding of how mistletoe-infected trees influence plant spatial patterns over time.

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Appendices

Appendix A: Termitaria *vs*. mistletoe: Effects on soil properties and plant structure in a semi-arid savanna.

Appendix B: Mistletoe litter accelerates the decomposition of recalcitrant host litter in a semi-arid savanna, south-west Zimbabwe.

Appendix A:

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Termitaria *vs*. mistletoe: Effects on soil properties and plant structure in a semi-arid savanna

Hilton G.T. Ndagurwa[a,b,*], Tsitsi S. Maponga[a,c], Beauty Dube[b], Tendai M. Nzuma[d], Justice Muvengwi[a,e]

[a] School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, Wits, 2050, South Africa
[b] Department of Forest Resources and Wildlife Management/Forest Ecology Laboratory, Faculty of Applied Sciences, National University of Science & Technology, P.O. Box AC 939 Ascot, Bulawayo, Zimbabwe
[c] Department of Mammalogy, Natural History Museum of Zimbabwe, P.O. Box 240, Bulawayo, Zimbabwe
[d] Department of Agriculture and Natural Resources Sciences, Faculty of Natural Resources and Spatial Sciences, Namibia University of Science & Technology, Private Bag 13388, Windhoek, Namibia
[e] Department of Natural Resources, Bindura University of Science Education, Private Bag 1020, Bindura, Zimbabwe

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ABSTRACT

Termite mounds by creating patches of increased resource availability (e.g. water and nutrients) are a major source of spatial heterogeneity in savannas. Likewise, mistletoes via input of nutrient-rich litter alter nutrient and water availability increasing environmental heterogeneity in semi-arid savanna. Despite this recognition, the influence of termitaria and mistletoe on soil properties and plant community have not been investigated together. We established eight 100 m^2 plots each on termitaria, under mistletoe-infected trees and in the surrounding savanna and examined the soil properties and the structure of *Securinega virosa* (Euphorbiaceae) and *Euclea divinorum* (Ebenaceae) in semi-arid savanna, southwest Zimbabwe. Soil properties significantly differed among the sampling sites ($p = 0.001$) with soils of increasing clay, soil moisture, pH and phosphorus, calcium and ammonium concentrations occurring on termite mounds. Soils under mistletoe-infected trees were associated with silt, organic matter, sodium, potassium, magnesium and nitrate and the surrounding savanna was associated with soils of increasing sand content. Plant structure also differed significantly between sites with greater basal area of both *S. virosa* and *E. divinorum* on termitaria relative to mistletoe-infected trees and the surrounding savanna. However, the stem density of *S. virosa* was greater under mistletoe-infected trees than on termitaria and in the surrounding savanna. Plant structural variables of individuals of the same species were affected by different soil properties across treatments. The major patterns showed that plant structure was influenced positively by soil moisture and nitrate and negatively by phosphorus on termitaria; positively by clay, soil moisture and ammonium and negatively by potassium under mistletoe-infected trees; and by phosphorus and calcium in the surrounding savanna. These findings show that soil properties, plant structure and their relationships differ between termitaria, mistletoe-infected trees and surrounding savanna, and these differences are suggested to increase heterogeneity in soil resources availability and vegetation structure in semi-arid savanna.

1. Introduction

Ecosystem spatial heterogeneity is a key characteristic of African savanna landscapes (Scholes, 1990). One of the major sources of spatial heterogeneity in savannas are patches of increased resource availability (e.g. nutrients) such as those created by termites (Sileshi et al., 2010) and mistletoes (Ndagurwa, 2015). Termites through mound building activities redistribute and concentrate nutrients on termite mounds relative to the surrounding areas (Muvengwi et al., 2016). Likewise, mistletoes by depositing nutrient-rich litter over extended periods beneath parasitized trees also concentrate nutrients within their immediate vicinity (March and Watson, 2007, 2010; Ndagurwa et al., 2013, 2014a). Thus, the activity of termites and mistletoes could modify resource availability and spatial heterogeneity in savanna ecosystems, and to our knowledge, despite this recognition, the influence of these factors on soil properties and plant assemblages are yet to be investigated together. Here, we compare how termites and mistletoes affect soil properties and plant structure in a semi-arid savanna.

* Corresponding author. School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, Wits, 2050, South Africa.
E-mail address: hilton.ndagurwa@nust.ac.zw (H.G.T. Ndagurwa).

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Termite species of the Macrotermitinae family such as *Macrotermes falciger* Gerstacker construct the most conspicuous and largest mounds in African savannas. These termites process and redistribute soil particles, organic matter, and plant materials to construct their mounds. Using saliva secretions and excreta, the plant material and organic matter are incorporated intimately with mineral constituents, particularly clay in nest construction (Lee and Wood, 1971). As a result, termite nest structures have more clay and silt, organic matter and mineral elements such as carbon, nitrogen, phosphorus and exchangeable cations than in the surrounding soils (Holdo and McDowell, 2004; Konaté et al., 1999; Muvengwi et al., 2016; Seymour et al., 2014; Sileshi et al., 2010). Along with a high clay content, the nest architecture improves soil structure and porosity which increases infiltration, and thus soil water availability (Konaté et al., 1999). As a result, by modulating the availability of resources to other organisms, termites are regarded as ecosystem engineers (Dangerfield et al., 1998).

Likewise, mistletoes can impact soil nutrients and water availability via their effects on litter quality and quantity as well as host tree water use. Mistletoes are aerial hemiparasites which obtain water and nutrients from their host plants via a specialized vascular attachment called a haustorium (Kuijt, 1969; Ehleringer and Marshall, 1995). Although mistletoes may obtain up to 60% of their carbohydrates from the host, most mistletoe species photosynthesize hence they are termed hemiparasites (Hull and Leonard, 1964). Because of their growth form they are buffered against large-scale fluctuations in resource availability by the host plant (Ehleringer and Marshall, 1995). Mistletoes maintain high transpiration rates to enable the movement of solutes from the host xylem to the parasite (Ehleringer and Marshall, 1995), and in the process, they increase whole tree water use (Sala et al., 2001). Consequently, parasitized trees are associated with low soil water availability (Ndagurwa et al., 2014b, 2015). As the flow of nutrients is unidirectional i.e. from host to parasite, mistletoes accumulate mineral elements to much higher concentrations than their hosts (Ehleringer and Marshall, 1995). In addition, the litter remains enriched due to minimal withdrawal of elements prior to leaf abscission (Leonard and Hull, 1965), and such litter decomposes faster and may also stimulate the decomposition of recalcitrant litter of co-occurring species (Quested et al., 2002). Furthermore, due to elevated and more extended periods of litterfall than their hosts, mistletoes contribute large volumes of nutrient-rich litter beneath infected trees enhancing nutrient returns, decomposition and nutrient cycling (March and Watson, 2007; Ndagurwa et al., 2015). Indeed, numerous studies have demonstrated changes in soil properties after mistletoe establishment (e.g., March 2007; Mellado et al., 2016; Muvengwi et al., 2015; Ndagurwa, 2015; Ndagurwa et al., 2013, 2016; Watson, 2016).

Given the ability of both termites and mistletoes to modify soil properties, there is a strong potential for these savanna landscape features to alter the structure and function of the local plant community. Previous research work has shown termitaria to contain plant assemblages that differ from the surrounding areas, mainly enriched in woody plants (Muvengwi et al., 2016; Sileshi et al., 2010; Van der Plas et al., 2013). Similarly, mistletoe infection has also been associated with increase in local plant species composition and productivity relative to areas without mistletoe (March, 2007; March and Watson, 2007; Ndagurwa et al., 2016). However, it is unclear how nutrient enrichment and plant community structure may differ between termite mounds and mistletoe-infected trees. Termites and mistletoes markedly differ in the mechanisms with which they modify soil properties, and therefore they are also likely to assemble plant communities of varying composition and structure, which, to our knowledge, is yet to be examined. Moreover, the soil properties which structure plant communities on termitaria or under mistletoe-infected trees remains to be addressed.

Here, we focus on the soil physical and chemical properties and vegetation structure on termitaria, beneath mistletoe-infected trees and in the surrounding savanna. In this ecosystem, termite mounds and mistletoes are dominant features of the landscape. Therefore, the objectives of this study were to determine whether (a) mistletoes influence soil nutrient concentrations and plant structure like *Macrotermes falciger* mounds and (b) differences in soil physical and chemical properties are reflected in the structure of individuals of the same species growing on termitaria, beneath mistletoe-infected trees and in the surrounding savanna.

2. Materials and methods

2.1. Study area description

We studied soil nutrient concentrations and vegetation structure on termitaria, beneath mistletoe-infected trees and in the savanna matrix in 2016 at the 160 ha National University of Science and Technology Field Laboratory (20°08′S, 28°36′E, 1341 m a.s.l.) in the southern highveld of Zimbabwe. The study site experiences three climatic seasons: a hot wet period (November to April), a cool dry period (May to July) and a hot dry period (August to October). The mean annual rainfall ranges between 325 and 915 mm, with a mean annual rainfall of 600 mm. Mean annual temperatures is 23.7 °C (Mlambo et al., 2005). The study site is a semi-arid deciduous open woodland savanna with a tree crown cover of 30–40%. The overstory is dominated by *Colophospermum mopane* (Kirk ex Benth.) Kirk ex J. Leonard, *Acacia karroo* Hayne, *Acacia gerrardii* Benth., *Acacia nilotica* (L.) Willd. Ex Delile and *Dichrostachys cinerea* (L.) Wight & Arn. and a diverse herbaceous layer dominated by perennial grasses such as *Panicum maximum* Jacq., *Schmidtia pappophoroides* Steud., *Heteropogon contortus* L., *Bothriochloa radicans* (Letim.) A. Camus and *Enneapogon scoparius* Stapf and such as *Tragus berteronianus* Schult., *Aristida adscensionis* L., *Chloris virgata* Swartz, and *Dactyloctenium aegyptium* (L.) Willd. (Mlambo et al., 2007).

Mounds of *Macrotermes falciger* occur randomly across the reddish-brown chromic luvisols (FAO, 1988) of the study site. These termites construct subterranean nests essentially for shelter and cultivation of exosymbiotic *Termitomyces* fungi, which alters soil physical and chemical properties (Muvengwi et al., 2016). *Macrotermes* mounds have higher soil moisture, clay content and soil nutrient concentrations than the surrounding soils, with approximately twice the concentrations of soil nutrients (e.g., Muvengwi et al., 2014, 2016; Sileshi et al., 2010).

Aerial hemiparasites (hereafter mistletoes) occur in the study site, mainly on *Vachellia* spp. (formerly *Acacia*). The most common mistletoes in the study site are *Erianthemum ngamicum* (Sprague) Danser (Loranthaceae), *Plicosepalus kalachariensis* (Schinz) Danser (Loranthaceae) and *Viscum verrucosum* Harv. (Viscaceae) (Mapaura and Timberlake, 2004). Previous research work demonstrated, for four *Vachellia* spp. studied, that mistletoe infections were higher in large than small trees (Ndagurwa et al., 2012). Mistletoe-infected trees were also associated with higher litterfall, soil nutrient concentrations, soil microbial biomass and lower soil moisture than uninfected trees (Ndagurwa et al., 2013, 2014a,b, 2016). The consequence of these changes to the soil layer include shifts in the composition, abundance and productivity of understorey plant and animal communities (e.g. litter-dwelling arthropods Ndagurwa et al., 2014b; grasses, Ndagurwa et al., 2016).

2.2. Sampling design and data collection

For this study, we selected termite mounds (*Macrotermes falciger*), mistletoe-infected trees (*Vachellia karroo* Hayne.) and savanna woodland plots/control plots (hereafter surrounding savanna) ($n = 8$ for each category) (Fig. 1). Termite mounds with surface area of at least 100 m^2 and height ≥1 m were selected because they have a stable soil chemistry and contain a variety of plant assemblages (Joseph et al., 2012; Seymour et al., 2014). We measured the long and short diameter of each termite mound at right angles using a tape measure and calculated area approximating the mound as an ellipse (Muvengwi, 2017). The mean ± SE termite mound surface area was 112.4 ± 9.54 m^2 and

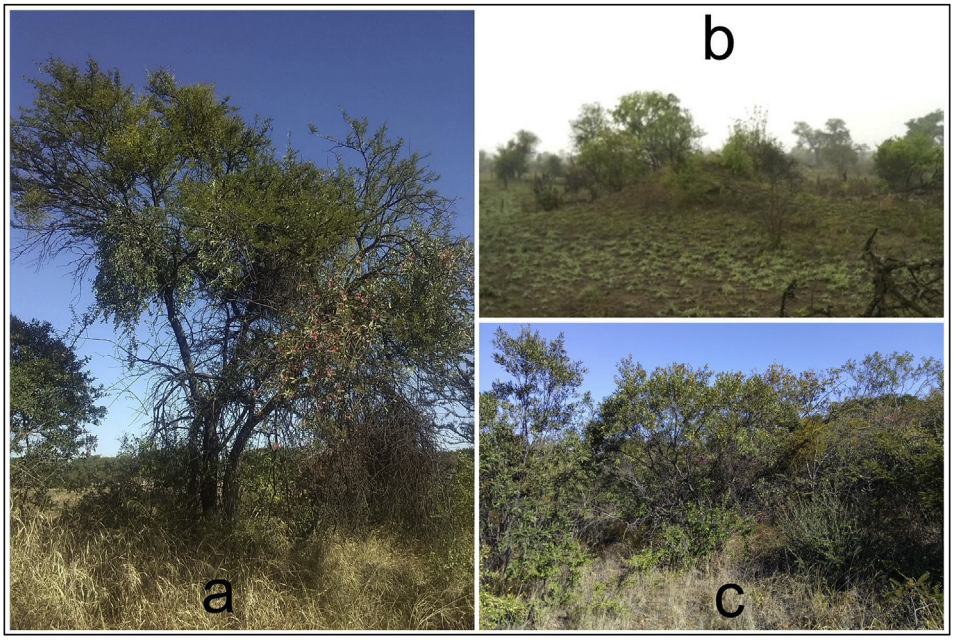


Fig. 1. Photographs of the sampling sites (a) mistletoe-infected *Vachellia karroo* tree, (b) termite mound and (c) open savanna vegetation in a semi-arid savanna, southwest Zimbabwe.

height was 1.98 ± 0.44 m. Considering that mounds of *Macrotermes* spp. grow to a height of 1 m in 2–3 years (Lepage, 1984), we also selected parasitized trees with mistletoes of a similar age. The age of the mistletoe plants was determined using the branch structure, by counting the shoot segments of the plant. This morphological feature has been shown to be highly correlated to age determined by anatomical methods (Dawson et al., 1990), and allows rapid determination of age in the field. Only trees parasitized by at least ten large (diameter > 30 cm) *Plicosepalus kalachariensis* (Schinz) Danser (Loranthaceae) mistletoes were selected, and therefore all references to mistletoes hereafter refer to this species. Circular savanna woodland plots with surface area of 100 m^2 were selected 30 m away from termitaria or mistletoe-infected trees. A new sampling site was only chosen when there was another sampling site within 30 m.

Considering that plant communities differ noticeably among the different treatment areas, we only sampled *Securinega virosa* (Roxb. ex Willd.) Baill. and *Euclea divinorum* Hiern because they are common throughout the treatment areas. However, control plots with *S. virosa* and *E. divinorum* were less numerous and difficult to find at the study site, and thus restricted our sample size (n = 8 for each category). We measured height, basal diameter, and canopy long and short diameters of *S. virosa* and *E. divinorum* on termite mounds, beneath mistletoe-infected trees and in the surrounding savanna. Basal diameter was measured using a forest calliper at just above the buttress swelling. Since the focal plants are multi-stemmed woody plants, basal diameters were measured for each individual stem, and basal area was calculated separately and summed up to obtain the total for that individual plant. Canopy long and short diameters and tree height were measured using a tape measure and a 10-m graduated pole, respectively. Stem density was estimated as the number of stems divided by plot area. Tree canopy cover (C) was calculated using the following equation:

$$C = \pi \left(\frac{d1}{2}\right)\left(\frac{d2}{2}\right)$$

where $d1$ is the longer diameter of the tree and $d2$ is the perpendicular short diameter measured using a tape measure. To minimize measurement error, all measurements were done by the first author.

2.3. Soil characteristics

To examine differences in soil physical and chemical properties among the treatment areas, we collected and analysed soil samples from termitaria, mistletoe-infected trees and the surrounding savanna. We extracted soil cores from four random points midway between the centre and edge of the termite mound, tree canopy and control plot in the top 10 cm using a soil auger (Seymour et al., 2014; Ndagurwa et al., 2013). Soil cores within each sampling site were then bulked, mixed thoroughly and a composite sample was stored in zip-lock bags and transported to the laboratory for analysis. Soils were tested for moisture content, organic matter content, texture (clay: < 0.002 mm, silt: 0.02–0.002 mm, sand: 0.02–2 mm), pH, total nitrogen (N), resin extractable phosphorus (P) and exchangeable calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K). The soil moisture content was determined gravimetrically by weighing after oven drying soil samples at 105 °C for 48 h while soil texture and pH were obtained using a hydrometer and the $CaCl_2$ method, respectively (Okalebo et al., 2002). Soil organic carbon (SOC) was determined by the Walkley–Black partial oxidation method (Walkley, 1947). Total N was estimated following semi-micro Kjeldahl procedure by acid-digestion, distillation, and titration whereas the available P was estimated by colorimetry using the ascorbic acid–molybdate method. Exchangeable bases were determined by dissolving soil using aqua regia and the mixture dried under ultraviolet light (Anderson and Ingram, 1993). The resulting compound was dissolved in concentrated HCl, filtered and diluted with distilled water. Then using a spectrophotometer total Ca and Mg were read at 0.460 nm and 0.595 nm, respectively, and flame emission was used for K and Na. All chemical analyses were done at the Department of Research and Specialist Services, Chemistry and Soil Research Institute in Harare, Zimbabwe.

2.4. Statistical analysis

Prior to analysis, all data was tested for normality using the Shapiro-Wilk test and homogeneity of variances using the Levene statistic. Some variables e.g. diameter, height and density data deviated from normality. As a result, we used statistical tests that account for distributions which lack normality and homogeneity of variances. Plant

structural variables were compared across the three sampling sites using the Kruskal Wallis test, followed by a post hoc analysis using the *pairwise multiple comparison of mean ranks* package (PMCMR) (Pohlert, 2014) in R Core Team (2018). Permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) was used to test the differences in soil physical and chemical properties among the sampled sites in Primer 7. Where Pseudo-F tests showed significant differences, PERMANOVA pairwise tests were carried out to determine which sites significantly differed from the other. Principal component analysis (PCA) was used to test the relationship between soil nutrients and the sampling sites. Principal component analysis (PCA) ordination was performed using CANOCO version 4.5 (TerBraak and Smilauer, 2002). We used a general linear model (GLM) to determine the effect of soil physical and chemical properties on the plant structural variables, using plant structural variables (height, diameter, canopy cover, stem density and basal area) as response variables and soil physical and chemical variables as explanatory variables. Multicollinearity was tested for all variables using the variance inflation factor (VIF) prior to model selection. Problems due to collinearity and associated convergence failures were minimized by standardizing the variables. Variables were standardized by dividing the difference between each raw value and the mean by the standard deviation for each variable (Logan, 2011). All the possible model candidates were generated in XLSTAT (Addinsoft, 2014; Germany). Considering our sample size (n) divided by the number of parameters (K) was less than 40 (Burnham and Anderson, 2002), we selected the best (most parsimonious) model using the corrected Akaike Information Criterion (AICc).

3. Results

3.1. Soil physical and chemical properties

PERMANOVA tests revealed that soil physical and chemical properties were significantly different among the sampling sites (Pseudo-F = 116.63, $p = 0.001$). Further, PERMANOVA pairwise tests showed that the sampling sites were significantly different from each other in terms of soil physical and chemical properties (mistletoe-infected *vs.* termite mound: t = 9.67, $p = 0.001$; mistletoe-infected *vs.* savanna: t = 8.57, $p = 0.001$; termite mound *vs.* savanna: t = 13.26, $p = 0.001$). Indeed, these findings were also confirmed by the PCA ordination as indicated by complete separation of the sites (Fig. 2). Savanna sites, mistletoe-infected trees and termitaria were associated with soils of increasing sand, silt and clay content, respectively (Fig. 2). Termite mounds were associated with soils of increasing soil moisture, pH and concentrations of P, Ca and NH_4^+ (Fig. 2). In contrast, mistletoe-infected trees were associated with soils of increasing SOC and concentrations of Na, K, Mg and NO_3^- (Fig. 2).

3.2. Vegetation attributes

Of the measured variables, the basal area of *S. virosa* was greater on termitaria, followed by mistletoe-infected trees and least in the surrounding savanna (Kruskal-Wallis $\chi^2 = 6.84$, $p = 0.033$; Table 1). Similarly, the basal area of *E. divinorum* was significantly greater (Kruskal-Wallis $\chi^2 = 7.51$, $p = 0.023$) on termitaria compared with mistletoe-infected trees and savanna (Table 1). Stem density of *S. virosa* was significantly greater (Kruskal-Wallis $\chi^2 = 11.5$, $p = 0.003$) under mistletoe-infected trees than on termitaria and savanna, but did not differ between termitaria and savanna (Table 1). However, for *S. virosa* and *E. divinorum* we found no significant differences in diameter (Kruskal-Wallis $\chi^2 = 0.39$, $p = 0.822$; Kruskal-Wallis $\chi^2 = 2.74$, $p = 0.253$, respectively), height (Kruskal-Wallis $\chi^2 = 2.71$, $p = 0.258$; Kruskal-Wallis $\chi^2 = 2.47$, $p = 0.291$, respectively) and canopy cover (Kruskal-Wallis $\chi^2 = 3.38$, $p = 0.184$; Kruskal-Wallis $\chi^2 = 0.87$, $p = 0.647$, respectively) between treatments (Table 1).

3.3. Relationship between soil and plant variables

Model selection criteria based on AICc minimization was used to determine the best predictors of the structural variables of *S. virosa* and *E. divinorum* plants occurring on termitaria, mistletoe-infected trees and in the savanna. The model selection results showed that relationships between plant structural variables and soil physical and chemical properties differed with plant species and sampling site (Tables 2 and 3). On termitaria, the best model for the diameter of *S. virosa* plants included SOC and NO_3^- (Table 2) while that for *E. divinorum* included sand (Table 3). The height of *S. virosa* plants was best predicted by silt and NO_3^- (Table 2) and clay content was the best predictor of the height of *E. divinorum* plants (Table 3). The canopy cover of *S. virosa* was negatively affected by K (Table 2) and positively by clay and negatively by P for *E. divinorum* plants (Table 3). Soil moisture positively while P negatively influenced the stem density of *S. virosa* plants (Table 2). In contrast, the stem density of *E. divinorum* plants was best predicted by clay content and soil Ca concentration (Table 3). The basal area of *E. divinorum* was negatively affected by silt and positively by NO_3^- (Table 3) while soil moisture and Mg were best predictors of the basal area of *S. virosa* plants (Table 2).

Under mistletoe-infected trees, the diameter of *S. virosa* was best predicted by soil moisture and NH_4^+ (Table 2) and by sand and Ca for *E. divinorum* (Table 3). The height of *S. virosa* was positively influenced by NH_4^+ and negatively by K (Table 2). In contrast, the height of *E. divinorum* was positively influenced by clay and NH_4^+ (Table 3). The canopy cover of *S. virosa* was negatively affected by K (Table 2) and positively by clay and negatively by K for *E. divinorum* (Table 3). Clay and Mg positively affected the stem density of *S. virosa* (Table 2) whereas the stem density of *E. divinorum* was influenced positively by P and negatively by K (Table 3). The basal area of *S. virosa* was positively influenced by NH_4^+ and NO_3^- (Table 2) while that of *E. divinorum* by soil moisture and pH (Table 3).

In the savanna, NH_4^+ was the best predictor of the diameter of *S. virosa* plants (Table 2). In contrast, the diameter of *E. divinorum* was positively affected by silt and Ca (Table 3). The height of *E. divinorum* was best predicted by NO_3^- and P (Table 3). Silt and pH positively influenced the canopy cover of *S. virosa* (Table 2). The stem density of *S. virosa* was positively predicted by P and negatively by Ca (Table 2). In contrast, the stem density of *E. divinorum* was negatively affected by sand (Table 3). Soil NO_3^- and Mg concentration positively affected the basal area of *E. divinorum* plants (Table 3).

4. Discussion

In this study, we show that soil properties and plant structure of *S. virosa* and *E. divinorum* differ between termitaria, mistletoe-infected trees and in the surrounding savanna. Using model selection based on AICc minimization, we demonstrate that shifts in soil resources due to mistletoe and termitaria benefit individuals of the same species differently resulting in corresponding differences in plant structure with important implications for savanna heterogeneity.

Our results showed that soil physical and chemical properties significantly differed between the sampling sites, with complete separation of sites in PCA ordination. Termite mounds had more clay content than soils under mistletoe-infected trees and in the savanna. During mound construction, termites select and transport large quantities of fine soil particles such as clay and silt from deep to upper soil layers (Lee and Wood, 1971) and, over time, mounds become enriched in these fine soil particles (Sileshi et al., 2010; Muvengwi et al., 2016). In contrast, mistletoe-infected trees were associated with soil of increasing silt content possibly due to accumulation below canopy of atmospheric dust trapped by the tree canopy and input to the soil through rainfall and litterfall (Bernhard-Reversat, 1982). Consistent with previous research (Konate et al., 1999; Muvengwi et al., 2016; Van der Plas et al., 2013) we found that termitaria were associated with soils of increasing

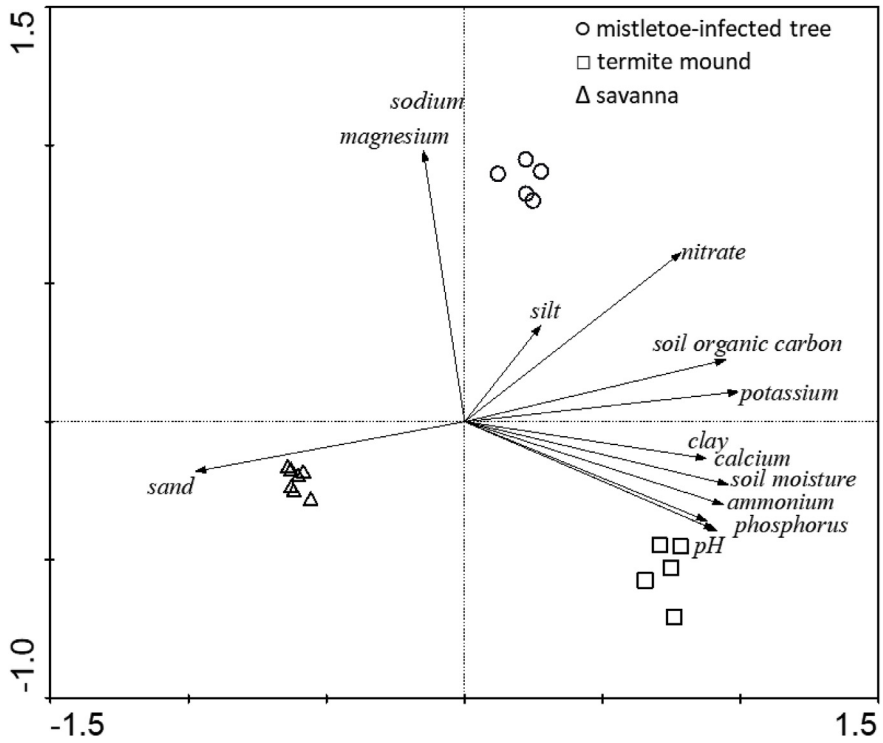


Fig. 2. Biplot based on a PCA (first two axes explaining 91.7% of total variation) illustrating the relationships between the soil physical and chemical properties and the sampling sites (mistletoe-infected trees, termite mound and surrounding savanna) in a semi-arid savanna, southwest Zimbabwe.

Table 1
Mean (± SE) structural variables of *Securinega virosa* and *Euclea divinorum* on termite mounds, under mistletoe-infected trees and in the surrounding savanna ($n = 8$ in each category). Means *not sharing a common letter* within each row differ significantly ($p < 0.05$).

Parameter	Termite mound	Mistletoe-infected tree	Savanna
Securinega virosa			
Diameter (cm)	1.93 ± 0.39	1.81 ± 0.46	1.73 ± 0.43
Height (m)	1.59 ± 0.21	2.11 ± 0.24	1.65 ± 0.16
Canopy cover (m^2 ha^{-1})	182.6 ± 42.3	296.6 ± 70.5	140.7 ± 45.7
Basal area (m^2 ha^{-1})	0.47 ± 0.03^a	0.28 ± 0.09^b	0.13 ± 0.01^c
Stem density (stems m^{-2})	4.75 ± 2.25^a	9.40 ± 2.42^b	4.71 ± 1.04^a
Euclea divinorum			
Diameter (cm)	4.55 ± 0.02	3.03 ± 0.67	5.20 ± 0.94
Height (m)	2.99 ± 0.66	2.71 ± 0.59	2.41 ± 0.26
Canopy cover (m^2 ha^{-1})	508.8 ± 24.3	660.7 ± 42.1	185.5 ± 43.2
Basal area (m^2 ha^{-1})	1.78 ± 0.73^a	0.50 ± 0.18^b	0.25 ± 0.07^c
Stem density (stems m^{-2})	2.80 ± 0.98	3.50 ± 1.02	1.86 ± 0.74

soil moisture compared to the other treatments. Termitaria have high soil moisture because of the high organic and clay content in mound soil which improve water retention (Konate et al., 1999). However, mistletoes are profligate water users which increases whole tree water use (Sala et al., 2001) resulting in depletion of soil water resources beneath mistletoe-infected trees (Ndagurwa et al., 2014b, 2015).

Although mound soils accumulate high organic matter due to successive building by generations of termites (Lee and Wood, 1971; Sileshi et al., 2010), we found that mistletoe-infected trees were associated with soils of increasing organic matter. Ndagurwa et al. (2013, 2014b, 2016) demonstrated that mistletoe-infected trees had up to 3 times more litter returns than uninfected trees resulting in deeper and more persistent litter beds. Over time, the litter is incorporated into the soil hence the high SOC beneath mistletoe-infected trees. The greater litter returns lead to increase in nutrient returns to the soil by a factor of 2.5 for Mg, 2.8 for N up to 5.3 for K (Ndagurwa et al., 2014a). Further, mistletoe-infected trees are associated with high N mineralization and nitrification rates (Ndagurwa et al., 2013) leading to elevations in soil nutrient concentrations (Mellado et al., 2016; Muvengwi et al., 2015; Ndagurwa et al., 2016). Indeed, mistletoe-infected trees were associated with soils of increasing concentrations of Na, K, Mg and NO_3^-. Consistent with previous studies (Holdo and McDowell, 2004; Muvengwi et al., 2016; Sileshi et al., 2010), termite mounds were associated with soils of increasing pH and concentrations of P, Ca and NH_4^+. The greater proportion of clay increase soil water retention reducing the leaching of exchangeable bases such as Ca (Dangerfield et al., 1998; Konate et al., 1999). In addition, the increased Ca must be derived from ingested plant material particularly wood typically high in Ca content (Lee and Wood, 1971). The high P and NH_4^+ concentrations on mounds can be attributed to soil mineralization by termites (Konate et al., 1999; Muvengwi et al., 2016). However, soils in the surrounding savanna were nutrient-poor possibly because of a low soil moisture and SOC which likely negatively affected mineralization rates. Alternatively, the high sand content may have increased the leaching of nutrients from the surface soil.

The basal area of both *S. virosa* and *E. divinorum* was greater on termitaria than under mistletoe-infected trees and in the surrounding savanna. These results are consistent with previous research showing greater woody basal area on termite mounds than in surrounding vegetation (Muvengwi et al., 2014, 2016). However, the stem density of *S. virosa* was greater under mistletoe-infected trees than on termitaria and in the surrounding savanna consistent with increased plant growth reported beneath mistletoe-infected trees in other studies (March and Watson, 2007; Ndagurwa et al., 2016). Increased plant growth beneath mistletoe-infected trees is attributed to increased litterfall, nutrient returns and soil fertility due to mistletoe (March, 2007; Ndagurwa, 2015). In addition, the impact of hemiparasites on host performance shifts the competitive balance from host species (usually competitive dominants) to non-host plants (competitive subordinates) (Press, 1998). Therefore,

Table 2
Standardized coefficients and *p* values of the best model parameters selected based on Akaike Information Criteria (AICc) minimization of the relationship between soil nutrient concentrations and vegetation structural variables of *Securinega virosa* on termitaria, under mistletoe-infected trees and in surrounding savanna in a semi-arid savanna, southwest Zimbabwe. Significant *p* values appear in bold. D, diameter; H, height; CC, canopy cover; BA, basal area; SD, stem density.

Parameter	Termite mound					Mistletoe-infected trees					Savanna				
	D	H	CC	SD	BA	D	H	CC	SD	BA	D	H	CC	SD	BA
clay									0.64						
									0.021						
silt		0.61													
		0.0004													
sand															
soil moisture				0.937	0.912	0.578									
				0.0001	**0.001**	**0.049**									
SOC	0.511												0.475		5.01
	0.018												0.078		0.075
NH_4^+						0.748	1.138			0.674	−0.747				
						0.020	**0.0004**			**0.006**	**0.038**				
NO_3^-	0.675	0.853								0.933					
	0.006	**0.0001**								**0.002**					
pH															
P				−0.231										0.663	
				0.045										0.018	
K			−0.708				−0.399	−1.099							
			0.021				**0.035**	**0.005**							
Ca														0.863	
														0.006	
Mg					0.645				0.614						
					0.006				**0.024**						
AICc	93.6	43.5	59.4	−9.72	21.9	−83.5	31.4	68.4	12.2	42.4	−76.2	−16.3	73.5	37.3	55.1
R^2	0.89	0.97	0.76	0.96	0.90	0.76	0.93	0.82	0.81	0.89	0.64	0.53	0.61	0.83	0.60
F	22.5	89.1	8.32	63.6	24.2	7.74	35.3	11.1	10.8	22.2	4.55	2.88	3.92	12.4	3.86
p	**0.003**	**0.0001**	**0.025**	**0.0002**	**0.0026**	**0.029**	**0.001**	**0.014**	**0.015**	**0.003**	0.074	0.146	0.094	**0.011**	0.096

Table 3
Standardized coefficients and *p* values of the best model parameters selected based on Akaike Information Criteria (AICc) minimization of the relationship between soil nutrient concentrations and vegetation structural variables of *Euclea divinorum* on termitaria, under mistletoe-infected trees and in surrounding savanna in a semi-arid savanna, southwest Zimbabwe. Significant *p* values appear in bold. D, diameter; H, height; CC, canopy cover; BA, basal area; SD, stem density.

Parameter	Termite mound					Mistletoe-infected tree					Savanna				
	D	H	CC	SD	BA	D	H	CC	SD	BA	D	H	CC	SD	BA
clay		0.958	0.717	1.248			0.766	0.788							
		0.002	**0.003**	**0.001**			**0.002**	**0.001**							
silt					−0.694						0.816		0.771		
					0.007						**0.002**		**0.02**		
sand	0.847					0.371								0.559	
	0.013					**0.02**								**0.04**	
soil moisture										0.622					
										0.033					
SOC															
NH_4^+							0.752								
							0.002								
NO_3^-					0.642							1.10			0.487
					0.01							**0.003**			**0.03**
pH										0.539			0.683		
										0.03			**0.03**		
P			−0.484						1.01			0.869			
			0.006						**0.0001**			**0.02**			
K								−0.758	−0.541						
								0.002	**0.003**						
Ca				0.938		1.021					0.802				
				0.001		**0.002**					**0.002**				
Mg															0.657
															0.01
AICc	64.1	15.9	78.2	−2.57	8.07	82.1	18.0	76.7	−7.37	29.3	79.8	25.8	77.9	36.6	42.0
R^2	0.76	0.92	0.95	0.89	0.87	0.95	0.92	0.97	0.97	0.78	0.84	0.91	0.70	0.65	0.83
F	7.85	30.8	45.9	21.1	17.2	45.7	27.1	97.1	70.7	8.80	15.95	29.30	6.91	5.55	14.3
p	**0.03**	**0.002**	**0.001**	**0.002**	**0.01**	**0.002**	**0.002**	**0.0001**	**0.001**	**0.02**	**0.003**	**0.002**	**0.03**	**0.04**	**0.01**

S. virosa plants beneath mistletoe-infected trees had a high stem density because of the reduced competition with the weakened host tree. Stem densities on termitaria were lower than those under mistletoe-infected trees possibly because of greater competition for resources with other vegetation on termitaria (Muvengwi et al., 2016; Van der Plas et al., 2013). However, we found no significant differences in diameter, height and canopy cover between treatments for both *S. virosa* and *E. divinorum* suggesting that plastic allocation responses for these plants may be similar between sites.

Model selection showed that the plant structural variables of individuals of the same species were affected by different soil physical and chemical variables across treatments. The major pattern which emerged showed that plant structure was consistently influenced positively by soil moisture and NO_3^- and negatively by P on termitaria, positively by soil moisture, clay and NH_4^+ and negatively by K under mistletoe-infected trees and by P and Ca in the savanna. In addition to increasing soil moisture, the high SOC and clay content in mounds increase mineralization rates and consequently soil nutrient concentrations (Konate et al., 1999; Lee and Wood, 1971), which enhance the growth of mound vegetation. On the other hand, although mistletoes have been shown to reduce soil water availability below host canopies (Ndagurwa et al., 2014b, 2015; Sala et al., 2001), the deep and persistent litter beds resulting from increased litterfall protect the soil from air temperature. As a result, soil temperatures and evaporation are reduced thereby enhancing soil water availability and holding capacity (Facelli and Pickett, 1991) hence the positive effect of soil moisture on plant structure beneath mistletoe-infected trees. Further, the elevated litter and nutrient returns increase the concentration of soil nutrients such as NH_4^+ below host canopies (Ndagurwa et al., 2013) with significant effects on plant growth as N is often limiting in the savanna (Scholes, 1990). Similarly, plant growth in savannas is also limited by P (Scholes, 1990). Thus, P had a significant influence on the plant structure of both *E. divinorum* and *S. virosa* in the savanna. We also found that plant structure was significantly influenced by Ca in the savanna because of the positive effects of Ca on woody plants (Lautner and Fromm, 2010; Muvengwi et al., 2016). Calcium plays a significant role in many growth and stress response processes in trees (Lautner and Fromm, 2010). However, the influence of Ca was not limited to the savanna sites only, plants on termitaria and under mistletoe-infected trees were also positively influenced by Ca. Indeed, termite mounds and mistletoe-infected trees are associated with high soil Ca concentrations from termite activity (Muvengwi et al., 2016; Sileshi et al., 2010) and increased mistletoe litterfall and nutrient returns, respectively (March and Watson, 2010; Ndagurwa et al., 2014a).

Previous research work has shown that mistletoe infection lead to elevations in soil nutrient concentrations by up to 39× for K in semi-arid savanna (Muvengwi et al., 2015). Similarly, mistletoe-infected trees in this study were associated with soils of increasing K concentrations, an element which competes for soil exchange surfaces with NH_4^+ (Ndagurwa et al., 2013). Hence the negative effects of K on plant structure beneath infected trees. Further, the structural variables of plants on termitaria were negatively influenced by P possibly due to low P availability which can impose nutritional constraints to plant growth (Bates and Lynch, 2000). Surprisingly, despite the negative effects of soil salinity on plant survival, growth and water use in semi-arid savannas (Belsky, 1990), we found no relationship between plant variables and Na suggesting that concentrations may be within the tolerance range of the focal plants. It is therefore possible that other factors such as shading may be more important in determining differences in plant structure between termitaria and under mistletoe-infected trees, an area open to future research. Regardless, these results show that termite mounds and mistletoe-infected trees had varying effects on the structure of *S. virosa* and *E. divinorum*, which suggest that resources on termite mounds and under mistletoe-infected trees benefit individual plant species differently resulting in corresponding differences in vegetation structure. Further, given plant traits respond differently to different abiotic factors (Van der Plas et al., 2013), changes in resource availability due to termitaria and mistletoe can also increase the productivity, species composition and functional diversity of plant communities in this very savanna. The ramifications of increase in vegetation structure and heterogeneity include changes in the landscape choices of insects, birds and mammals, and consequently their distribution (Levick et al., 2010; Muvengwi et al., 2014); increase in ecosystem resilience (*sensu* Holling, 1973) and better nutrient use and retention (Hooper and Vitousek, 1997).

In this study, we have shown that effects on soil nutrient parameters and plant structure by mistletoes may be as significant as termitaria – findings which suggest that mistletoe infection may have wider consequences for the structure and functioning of savanna systems. For example, changes in soil physical and chemical properties due to termite activity is known to affect plant species composition (Muvengwi et al., 2016; Sileshi et al., 2010), nutrient status (Holdo and McDowell, 2004), plant functional traits (Joseph et al., 2012; Van der Plas et al., 2013), and animal landscape choices (Muvengwi et al., 2014). Similarly, changes in soil properties and vegetation structure with mistletoe infection as demonstrated here and in other studies (March, 2007; Ndagurwa, 2015) show that mistletoes have important implications for community structure in savannas. In addition, mistletoes have a clumped distribution (Aukema, 2003; Ndagurwa et al., 2012) which alters the spatial distribution of soil resources, and consequently increase environmental heterogeneity (March and Watson, 2010). However, these effects will depend on many factors including longevity, abundance and species of the mistletoes as well as other factors such as herbivory or fire. Thus, further research work is required to examine the broader ecological role of mistletoes in savanna systems. Nonetheless, the significant shifts in soil nutrient concentrations and plant structure due to mistletoe infection lend support to the hypothesis that mistletoes function as keystone resources particularly in nutrient-poor environments (Watson, 2001).

5. Conclusions

In conclusion, termite mounds were associated with soils of increasing moisture content, clay content and concentrations of P, Ca and NH_4^+ with greater plant basal area while mistletoe-infected trees had a significant effect on soil organic matter, K, Na, Mg and NO_3^- concentration and greater stem density of *S. virosa* plants relative to the other treatments. Collectively, our findings show that effects on soil nutrient concentrations and plant structure as well as the relationships between soil nutrients and plant structural variables differ between termitaria, mistletoe-infected trees and the surrounding savanna, and we suggest that these differences increase heterogeneity in resource availability and plant structure in semi-arid savanna. Further, our work adds to the growing body of knowledge of how termitaria (Muvengwi et al., 2013, 2014; 2016; Seymour et al., 2014; Sileshi et al., 2010; Van der Plas et al., 2013) and mistletoes (Muvengwi et al., 2015; Ndagurwa et al., 2013, 2014a,b, 2015, 2016) alter soil properties and vegetation structure with important implications for savanna heterogeneity.

Author contributions

HGTN developed the conceptual framework and designed the sampling protocols of this study; BD, TM, JM and TSM participated in data collection, processing and interpretation; and HGTN wrote the paper with contributions from all authors.

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Appendix B:

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Mistletoe litter accelerates the decomposition of recalcitrant host litter in a semi-arid savanna, south-west Zimbabwe

HILTON G. T. NDAGURWA,*[1,2] TSITSI S. MAPONGA[1,3] AND JUSTICE MUVENGWI[1,4]

[1]*School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, Wits, 2050, South Africa (E-mail: hilton.ndagurwa@nust.ac.zw);* [2]*Department of Forest Resources & Wildlife Management, Faculty of Applied Science, National University of Science and Technology;*

[3]*Natural History Museum of Zimbabwe, Bulawayo, Zimbabwe; and* [4]*Sustainability Research Unit, Nelson Mandela University, George, South Africa*

Abstract Litter mixing plays an important role in enhancing carbon and nutrient cycling, but little is known about the effects of nutrient-rich mistletoe litter on the decomposition of slow-decaying litter in nutrient-poor environments. We investigated the effects of mistletoe litter on the decomposition and nutrient release of host *Vachellia karroo* litter in semi-arid savanna, south-west Zimbabwe. Mass loss and nutrient release was quantified in litter of each single species, two-species, three-species and four-species mixtures of mistletoe, *Erianthemum ngamicum*, *Plicosepalus kalachariensis* and *Viscum verrucosum*, and the host *V. karroo* in 30 × 30 cm nylon-mesh litterbags under field conditions at 2-month intervals for one year. Repeated-measures analysis of variance was used to test the effects of litter type, incubation time and their interaction on mass loss and nutrient release. The effects of initial litter quality, incubation time and litter mixture on decomposition rate were also tested. Litter mixtures of mistletoes and *V. karroo* decomposed three times faster than *V. karroo* decomposing alone, and decomposition of litter mixtures was influenced by initial litter quality and incubation time. Further, non-additive effects are reported, with synergistic interactions being greater after 12 months and common regarding mass loss, phosphorus and carbon, whereas antagonistic interactions were common in nitrogen release. These effects varied both in magnitude and direction between litter-mixing treatments and with incubation time ($P < 0.05$). Our findings show that mistletoe litter enhance the decomposition of recalcitrant host litter consistent with findings in other ecosystems that contain hemiparasites, suggesting that hemiparasite litter plays an important role in carbon and nutrient flux in this system. Further, by enhancing the decomposition and nutrient release rate of recalcitrant host litter, mistletoes increase nutrient availability to other organisms within the ecosystem lending support to the premise that parasitic plants function as keystone species in environments where they occur.

Key words: decomposition, ecosystem engineer, hemiparasite, litter mixtures, nonadditive effects, plant-soil interactions, savanna, soil organic carbon.

INTRODUCTION

Mistletoes have a major influence on community composition and ecosystem processes in many woodland and forest ecosystems worldwide. Indeed, recent research work has demonstrated that effects of mistletoes extend beyond their hosts to higher trophic levels altering the species richness, abundance and distribution of plants and animals in their immediate vicinity and beyond (March & Watson 2007, 2010; Watson 2009; Fisher *et al.* 2013; Ndagurwa *et al.* 2014a, 2016, 2018; Mellado *et al.* 2016). These effects are greater than expected given the biomass of mistletoes and have resulted in the formal proposal that mistletoes and generally parasitic plants act as keystone species and ecosystem engineers (Press 1998; Watson 2001; Press & Phoenix 2005). In this regard, understanding the role of parasites in their natural communities and how they positively or negatively interact with surrounding communities becomes increasingly important.

One major mechanism with which mistletoes influence natural communities is through effects on belowground processes such as decomposition. In addition to producing their own photoassimilates, mistletoes obtain water and mineral resources from host plants (Kuijt 1969). The flow of nutrients is unidirectional, that is from the host to the parasite, enabling mistletoe leaf tissue to accumulate mineral elements such as nitrogen (N) to much higher concentrations than observed within host tissues (Ehleringer and Marshall 1995; Marshall *et al.* 1994). For example, up to 20 times more potassium concentration has been recorded in mistletoe relative to host foliage (Lamont 1983). Upon senescence, the leaves

*Corresponding author.
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of mistletoes remain enriched due to the lack of any translocation of minerals from the parasite back to its host (Leonard & Hull 1965), thereby producing nutrient-rich litter. Consequently, mistletoes have important implications for decomposition processes since initial litter quality is a good predictor of decomposition rate (Hättenschwiler & Vitousek 2000). Mistletoe litter has been shown to decompose faster than litter of co-occurring species, when decomposing alone (Ndagurwa *et al.* 2015). However, in natural systems, mistletoe and host litter as well as litter from co-occurring plant species are often mixed and decompose together. Thus, by interacting with the litter of other species, nutrient-rich mistletoe litter may alter decomposition and nutrient loss in litter mixtures.

Litter mixtures have decomposition rates, total nutrient loss and nutrient release patterns that differ from the same litters decomposing alone due to either synergistic or antagonistic interactions of the different litters during decomposition (Chapman *et al.* 1988; Wardle *et al.* 1997; Liu *et al.* 2007; Lecerf *et al.* 2011). Synergistic interactions enhance while antagonistic interactions retard decomposition, and these non-additive interactions can be substantial with up to 65% deviation from expected mass loss (Gartner & Cardon 2004). Non-additive interactions have been recorded in many studies examining litter-mixing effects on decomposition and nutrient loss, with synergistic effects appearing to be more common (Gartner & Cardon 2004; Hättenschwiler & Gasser 2005; Lecerf *et al.* 2007) especially when a single component of the mixture has high initial litter quality (Wardle *et al.* 1997). Although the direction and magnitude of litter-mixing effects on mass loss varied strongly between methods, that is microcosm, field litterbag and litterbed, nutrient-rich parasite litter has been shown to enhance decomposition and nutrient release of litter of co-occurring species in subarctic heaths (Quested *et al.* 2002, 2005). However, information on the effects of mistletoe litter on decomposition of other litters and the contributing factors is lacking for this semi-arid savanna ecosystem, which limits our understanding of local and regional carbon and nutrient fluxes.

In a previous study, mistletoe species were found to decompose faster releasing up to three times more nitrogen than litter of the host species, *V. karroo* (Ndagurwa *et al.* 2015). However, Ndagurwa *et al.* (2015) study examined the decomposition of single species litter, yet in natural systems litter of different species is often mixed. In this regard, we examined the effects of mixing three mistletoe species, *Erianthemum ngamicum* (Sprague) Danser (Loranthaceae), *Plicosepalus kalachariensis* (Schinz) Danser (Loranthaceae) and *Viscum verrucosum* Harv. (Viscaceae), and *Vachellia karroo* Hayne (Fabaceae) litter on decomposition and nutrient loss in a semi-arid savanna, south-west Zimbabwe. Specifically, we hypothesised that mixtures of mistletoe and host litter would decompose and release nutrients faster than the individual species decomposing alone. Incubation time determines niche complementarity effects and detritivore colonisation in litter mixtures and consequently the magnitude of non-additive mixing effects (Lecerf *et al.* 2011). Therefore, we test the influence of incubation time on the decomposition and nutrient release of litter mixtures. Since previous studies revealed marked differences in litter chemical composition between these species (Ndagurwa & Dube 2013; Ndagurwa *et al.* 2013; Ndagurwa 2015), we also tested the possible role of initial litter chemistry (nitrogen: N, phosphorus: P, carbon: C and carbon/nitrogen ratio: C/N) on the decomposition and nutrient loss of the litter mixtures.

METHODS

Study site

The study was performed from May 2015 to May 2016 in the Matopos Research Station (20°23′S, 28°28′E; 1340 m asl), a savanna woodland located 30 km south-west of Bulawayo, Zimbabwe. Mean annual rainfall and temperature averages 600 mm and 23.6°C, respectively (Dye & Walker 1987). Frost occurs during the dry season (May–September). Soils are medium textured schists-derived siallitic red clay soils of moderately high fertility (Ward *et al.* 1979). The dominant tree vegetation has been described as a *Vachellia* (formerly *Acacia*) tree–bush savanna dominated by *V. karroo* Hayne, *Vachellia nilotica* (L.) Willd. ex Delile, *Vachellia gerrardii* Benth., *Vachellia rehmanniana* Schinz., *Senegalia nigrescens* Oliv. and *Maytenus senegalensis* (Lam.). *Heteropogon contortus* L., *Themeda triandra* Forssk. and *Cymbopogon plurinodis* Stapf ex Burtt Davy with *Setaria incrassata* (Hochst.) Hack. Abh. dominate the understorey vegetation. The most common mistletoes in the study site include *E. ngamicum*, *P. kalachariensis* and *V. verrucosum* (Mapaura & Timberlake 2004). However, *Agelanthus subulatus* (Engl.) Polhill & Wiens and *Erianthemum dregei* (Eckl. & Zeyh.) also occur in the study area.

Plant material collection

Litter samples of three mistletoe species, *E. ngamicum*, *P. kalachariensis* and *V. verrucosum*, and that of *V. karroo* were obtained from five randomly selected and tagged representative plants of each species at the peak of leaf litterfall in August 2014. Fully senescent leaves (yellow or brown leaves that could be removed from a stem with only slight pressure) with a well-developed abscission layer were collected by hand at the study site (after Quested *et al.* 2005; March 2007; Ndagurwa *et al.* 2015). However, *V. verrucosum* does not have leaves (Pope *et al.* 2006); twigs <2 mm

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in diameter were collected. In the laboratory, the litter was pooled and mixed thoroughly to homogenise the sample, air-dried for 5 days to constant weight and stored in polyethylene bags at 20–25°C until sample preparation (Ndagurwa *et al.* 2015). The litter samples were collected at the same time of the year to minimise seasonal changes within the plants. Further, since the chemical composition of mistletoes is dependent on the host species (Madibela *et al.* 2004; Umucalılar *et al.* 2007), litter samples of all the mistletoe species were collected from adult mistletoes in *V. karroo* trees to avoid intraspecific variation in chemical composition (Ndagurwa *et al.* 2015).

Litter decomposition experiment

Litter mass loss and nutrient release patterns were determined using the standard litterbag technique (Falconer *et al.* 1933) in a randomised block design. Prior to litterbag placing, we removed all the litter underneath mistletoe-infected trees and installed litter traps above the litterbags. Then, litterbags were placed mid-way between the tree bole and canopy edge of the selected mistletoe-infected trees. We used mistletoe-infected trees with litter removed beneath the canopy for several reasons. First, if we had placed litterbags under uninfected trees or away from tree cover, the effect of mistletoe on decomposition could have been underestimated since any organisms that favour mistletoe litter may not have been present in the soil. Second, litterbags were placed beneath mistletoe-infected trees so that bags would be exposed to decomposer communities associated with tree and mistletoe litterfall in the study area. Third, we removed all the litter beneath infected trees so that no litter would be available to detritivores outside of the litterbags. Therefore, the effects reported here can be attributed to the mixture contained within the bag. However, since the host is a legume, there are issues of N availability in the soil from N-fixing bacteria, which can generally influence decomposition, and thus, mistletoe litter effects may represent an additional effect on carbon and nutrient fluxes in this system.

Litterbags (30 × 30 cm) constructed from nylon material (2 mm mesh size) were used. The mesh size was small enough to avoid excessive loss of leaf fragments but large enough to allow access to mesofauna and most macrofauna. In each litter bag, 25 g of litter was enclosed, and the litter bags were tagged for identification purposes and secured to the ground using wire pins to prevent movement. We prepared 11 types of litterbags containing: (i) litter of each single species (four types), (ii) two-species mixtures of *V. karroo* and each mistletoe species (three different combinations with a loading ratio 50:50), (iii) three-species mixtures of *V. karroo* and any two different mistletoe species (three different combinations with a loading ratio 33.3:33.3:33.3) and (iv) one four-species mixture (25:25:25:25). As a result, a total of 396 litterbags (11 types × 6 sampling dates × 6 replicates) were used. Litter mixtures used here do not reflect litter fractions found beneath mistletoe-infected trees because of limited studies (Ndagurwa 2015) on mistletoe and host litterfall in this savanna system. In this regard, we used equal proportions in all litter mixtures. Further, we only used *V. karroo* because it is the dominant host in the study area.

Litterbags were harvested at 2-month intervals, starting in mid-July 2015 until mid-May 2016. The materials recovered from the litterbags were air-dried and carefully brushed to remove attached soil particles and finally were oven-dried at 60°C to constant weight. Then, the dry weight of the clean sample was recorded after correcting for any increase in weight caused by soil contamination. Differences in decomposition and nutrient loss from contrasting litter types are more apparent in the early stages of litter decomposition (Jensen 1974; Moore *et al.* 2006). Thus, the 12-month period was appropriate for comparisons between litter types, although effects of litter mixing occurring beyond 12 months are missed.

Chemical analysis

For each species, three ($n = 3$) subsamples of leaf litter were retained for the determination of initial nitrogen (N), phosphorus (P) and carbon (C). Litter C was determined by the Walkley–Black method (Walkley 1947), N by the semi-micro Kjeldahl method, and available P was estimated by colorimetry using the ascorbic acid–molybdate method (Anderson & Ingram 1993).

Statistical analysis

Mixed litter decomposition experimental data were analysed using the traditional method to determine synergism and antagonism of non-additive effects. In this regard, litter mass loss and litter nutrient contents observed in mixtures were compared with the values expected from the component species decomposing alone (Wardle *et al.* 1997). We calculated the expected litter mass loss and nutrient content for all multiple species mixtures as described by Bonanomi *et al.* (2010):

$$E_{\mathrm{ML}} = \sum_{i=1}^{s} O_{\mathrm{ML}_i} \times P_{\mathrm{IM}_i} \quad (1)$$

where E_{ML} is the expected mass loss (%), O_{ML_i} is the observed mass loss (%) in monoculture of species i, and P_{IM_i} is the initial proportion of species i in the mixture. Similarly, the expected N, P and C contents were calculated using the above formula with the appropriate substitution made.

The difference between O_{ML} and E_{ML} indicates deviation from additivity. Following Wardle *et al.* (1997), we calculated a signed mixing effects term (ME, or the interaction effect between litters):

$$\text{Mixing effects} = O_{\mathrm{ML}} - E_{\mathrm{ML}} \quad (2)$$

where negative deviations indicate synergistic responses (acceleration of litter breakdown), and positive deviations indicate antagonistic responses (deceleration of litter breakdown) of litter mixtures (Lecerf *et al.* 2011). O_{ML} and E_{ML} are as defined above.

The decomposition rate (k year^{-1}) was calculated following Olson (1963). Mass loss and nutrient release data deviated from normality and were arcsine square root transformed to homogenise variances (Zar 1999). A

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repeated-measures analysis of variance was used to test the effects of litter type, harvest time and their interaction on mass loss, N, P, C and C/N ratio. The initial time step (time = 0) was excluded from the analysis of mass loss data. Where the assumption of sphericity was not met, the Greenhouse–Geisser correction was applied. The difference in the decomposition rate constant (k year^{-1}) and the initial litter quality (N, P, C, C/N) between the studied species was tested using a one-way analysis of variance, with a subsequent multiple comparison test (Tukey's HSD). The differences between observed and expected values were tested using two-sample t-tests. Bivariate correlations were performed to determine the relationship between mass loss and nutrients remaining and the initial litter N, P, C and C/N concentrations in the single-type litterbags. We conducted statistical tests using SPSS 21 for Windows (SPSS Inc., 2012, Chicago, IL, USA), with an accepted significance of $P < 0.05$ in all tests. The mass and element content remaining are expressed as a per cent of their initial quantity and content, respectively.

RESULTS

Litter chemistry

The litter samples differed in their initial concentration of N, P, C and C/N ratio (Table 1). The litter N of the mistletoes, *E. ngamicum*, *P. kalachariensis* and *V. verrucosum*, was significantly greater than that of *V. karroo*, but no differences were detected between the mistletoe species (Table 1). Likewise, the concentrations of C and C/N ratio were higher in the mistletoes than in *V. karroo* (Table 1). Mass loss, after 6 months, was greater in *P. kalachariensis* than in the other species litter (Table 1). After 12 months, mass loss was significantly greater in mistletoe litter than in *V. karroo* (Table 1). Correspondingly, the rate of litter decomposition was greater in mistletoe litter relative to *V. karroo* ($F_{3,92} = 3.52$; $P = 0.025$; Fig. 1).

Mass loss and decomposition

The repeated-measures ANOVA showed significant effects of litter type, harvest and their interaction on mass loss (Table 2), suggesting that litter mass loss varied not only with litter type but also that species specific decomposition also varied with time (Table 3; Fig. 2). Litter mass declined slowly in *V. karroo*/*V. verrucosum*, whereas the other litter mixtures showed a constant rapid decline in litter mass throughout the study period (Fig. 2). In contrast, litter mass loss in *V. karroo*/*P. kalachariensis*/*V. verrucosum* was slow in the first 6 months but declined rapidly thereafter (Fig. 1). After 12 months, the proportion of dry weight lost was greatest in *V. karroo*/*P. kalachariensis* (86%) followed by *V. karroo*/*P. kalachariensis*/*V. verrucosum* (78%) and least in *V. karroo*/*V. verrucosum* (38%), and except for *V. karroo*/*V. verrucosum*, all the litter mixtures had lost more than 50% of their initial litter mass (Table 3; Fig. 2).

The mixed litter samples decomposed faster than the expected values, particularly after 12 months of decomposition (Table 3). Indeed, except for *V. karroo*/*V. verrucosum*, the decomposition rate k of mixed litters was consistently and significantly higher than the expected k (Fig. 3), indicating that litter mixing had a positive effect on litter decomposition. The enhanced or synergistic (non-additive) interactions were observed in 71% and 86% of the litter mixture samples after 6 and 12 months, respectively, and the per cent synergism was particularly greater after 12 months of decomposition (Table 3). In contrast, we found negative or inhibitory interactions only in *V. karroo*/*V. verrucosum* and *V. karroo*/*P. kalachariensis*/*V. verrucosum* over the course of the experiment (Table 3). However, in *V. karroo*/*P. kalachariensis*/*V. verrucosum* litter mixture samples, interactions were antagonistic in early phases of decomposition, but progressively became synergistic after 12 months of decomposition (Table 3). As a result of the differences in litter mass loss, the litter mixtures

Table 1. The initial nitrogen (N), carbon (C), phosphorus (P) and potassium (K) content, C: N ratio, decomposition rate (k year^{-1}) and mass remaining after 6 and 12 months for the study species, *Vachellia karroo*, *Erianthemum ngamicum*, *Plicosepalus kalachariensis* and *Viscum verrucosum*

					Mass remaining	
Litter type	N (%)	P (%)	C (%)	C/N	6 months	12 months
V. karroo	1.32 ± 0.21^{a}	0.07 ± 0.01^{a}	3.71 ± 0.34^{a}	2.81 ± 0.05^{a}	86.1 ± 4.92^{a}	65.1 ± 3.12^{a}
E. ngamicum	2.16 ± 0.23^{b}	0.13 ± 0.01^{b}	7.12 ± 0.21^{b}	3.28 ± 0.08^{b}	73.1 ± 1.48^{b}	52.2 ± 1.88^{b}
P. kalachariensis	1.74 ± 0.43^{b}	0.16 ± 0.02^{b}	7.26 ± 0.86^{b}	4.17 ± 0.42^{b}	60.4 ± 2.43^{c}	49.3 ± 1.96^{b}
V. verrucosum	1.96 ± 0.06^{b}	0.09 ± 0.01^{a}	6.88 ± 0.52^{b}	3.51 ± 0.13^{b}	65.0 ± 2.10^{c}	45.3 ± 2.64^{b}
	$F_{3,8} = 3.52$	$F_{3,8} = 4.36$	$F_{3,8} = 4.32$	$F_{3,8} = 19.5$	$F_{3,115} = 3.58$	$F_{3,115} = 8.15$
	$P = 0.025$	$P = 0.003$	$P = 0.011$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Results are presented of one-way analysis of variance (F-values, degrees of freedom and P-value). Means *not sharing a common letter* within each column differ significantly (Tukey HSD, $P < 0.05$). All values are mean ± standard error.

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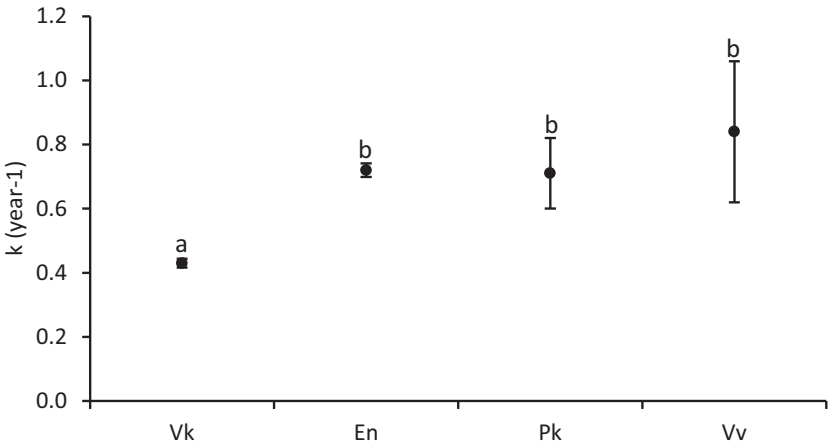


Fig. 1. Decomposition rate constant (k year^{-1}) of the single species litter samples after 12 months of decomposition. *Vachellia karroo* (Vk); *Erianthemum ngamicum* (En); *Plicosepalus kalachariensis* (Pk); *Viscum verrucosum* (Vv). All values are mean ± standard error. Means *not sharing a common letter* differ significantly ($P < 0.05$). Symbols with no apparent error bars have error estimates too small to see or hidden by the symbol.

Table 2. *F*-ratio and level of significance of repeated-measures ANOVA testing the effect of litter type, harvest and their interaction on mass loss (%) and the nitrogen (N), phosphorus (P), carbon (C) and C/N ratio of litter samples

		Element concentration (%)			
Effects	Mass loss	N	P	C	C/N
Litter type	56.6 (**0.001**)	5.12 (**0.02**)	1.14 (0.421)	321.1 (**0.0001**)	20.3 (**0.0001**)
Harvest	887.3 (**0.002**)	34.4 (**0.0001**)	1.84 (0.205)	4.93 (**0.001**)	18.6 (**0.001**)
Litter type × Harvest	10.6 (**0.001**)	1.78 (0.125)	1.12 (0.426)	10.2 (**0.002**)	3.31 (**0.001**)

Significant values ($P < 0.05$) appear in bold.

significantly differed in decomposition rate ($F_{6,161} = 66.2$; $P = 0.025$). The decomposition rate was highest in *V. karroo*/*P. kalachariensis* followed by *V. karroo*/*P. kalachariensis*/*V. verrucosum* and least in *V. karroo*/*V. verrucosum* (Fig. 2). Overall, decomposition rate was significantly ($t = 4.16$, d.f. = 42, $P < 0.05$) higher in mixed litters (mean $k = 1.32 \pm 0.22$ year^{-1}) than in single species litters (mean $k = 0.68 \pm 0.13$ year^{-1}) over the course of the experiment.

There were significant differences between observed and calculated expected N, P, C and C/N contents (Table 4), and this difference varied with litter type, harvest and litter type × harvest interaction except for P (Table 2). Like mass loss, we found both synergistic and antagonistic interactions in nutrient release in litter mixtures (Table 4). Antagonistic effects were more common regarding N, with five cases compared with the two of synergistic interactions (Table 4). Examination of N loss showed that the initial phase was characterised by N accumulation persisting for up to 6 months in *V. karroo*/*V. verrucosum* (Fig. 4a). In contrast, *V. karroo*/*E. ngamicum* had no period of N accumulation losing N rapidly in the first 2 months before levelling off thereafter till the end of the study period (Fig. 4a). After 12 months, N loss was greatest in *V. karroo*/*P. kalachariensis* followed by *V. karroo*/*P. kalachariensis*/*V. verrucosum*, litter mixtures that had significant synergistic interactions (Table 4). In contrast, the least N loss was recorded in *V. karroo*/*E. ngamicum*/*V. verrucosum* and *V. karroo*/*V. verrucosum* litter mixtures in which antagonistic interactions were observed (Table 4). Except for *V. karroo*/*P. kalachariensis* which had lost 57% of its initial N content after 12 months, all the other litter mixtures lost <50% of the initial N content, despite the loss of leaf mass (Fig. 4a). The loss of P was not influenced by litter type, harvest and their interaction (Table 2). Nevertheless, except for *V. karroo*/*E. ngamicum*, there was an increase in P in litter mixtures in the first 2 months persisting up to the 6th month in *V. karroo*/*V. verrucosum* and *V. karroo*/*E. ngamicum*/*V. verrucosum* (Fig. 4b). Although enhanced or synergistic interactions were common regarding P, the differences were not statistically significant ($P > 0.05$; Tables 2,4).

Litter mixture, time and their interaction had a significant influence on the concentration of C and C/N

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Table 3. Observed and expected mass remaining and per cent synergism of the litter mixture samples after 6 and 12 months of decomposition

	6 months			12 months		
Litter type	Observed	Expected	ME	Observed	Expected	ME
Vk (En)	60.7 ± 3.67	79.6 ± 3.08**	−18.9	26.2 ± 3.15	58.7 ± 4.03***	−32.5
Vk (Pk)	49.0 ± 4.58	73.3 ± 2.12**	−24.3	14.3 ± 4.20	57.2 ± 3.06**	−42.9
Vk (Vv)	82.3 ± 5.20	75.6 ± 2.86*	6.70	62.3 ± 3.21	55.2 ± 2.70*	7.10
Vk (En & Pk)	64.6 ± 2.41	73.2 ± 1.68*	−8.60	22.0 ± 3.55	55.5 ± 1.98***	−33.5
Vk (En & Vv)	60.1 ± 3.83	74.7 ± 3.11*	−14.6	38.2 ± 5.19	54.2 ± 3.28**	−16.0
Vk (Pk & Vv)	78.6 ± 5.30	70.5 ± 1.64*	8.10	18.1 ± 3.97	53.2 ± 2.06***	−35.1
Vk (En & Pk &Vv)	64.3 ± 4.31	71.2 ± 1.02*	-6.90	28.4 ± 3.31	52.9 ± 3.87*	−24.5

Vachellia karroo (Vk); *Erianthemum ngamicum* (En); *Plicosepalus kalachariensis* (Pk); *Viscum verrucosum* (Vv). ME: mixing effects or the interaction effect between litters. Asterisks indicate levels of significance for differences between observed and expected mass remaining of the same retrieval point.

All values are mean ± standard error.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

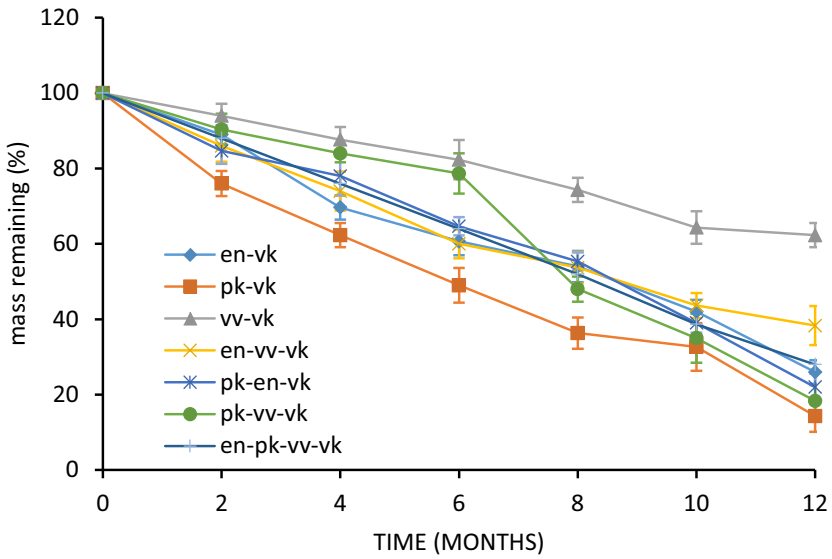


Fig. 2. Leaf litter mass remaining (%) over time of litter mixtures. *Vachellia karroo* (Vk); *Erianthemum ngamicum* (En); *Plicosepalus kalachariensis* (Pk); *Viscum verrucosum* (Vv). Error bars show the standard error of the mean. Symbols with no apparent error bars have error estimates too small to see or hidden by the symbol.

ratio of the decomposing litter (Table 2). Synergistic interactions were common, with four cases compared to three of synergistic interactions (Table 4). Carbon loss was rapid in *V. karroo*/*V. verrucosum* and *V. karroo*/*P. kalachariensis*/*V. verrucosum* which lost >50% after 4 and 6 months, respectively (Fig. 4c). However, *V. karroo*/*E. ngamicum*/*V. verrucosum* and *V. karroo*/*E. ngamicum*/*P. kalachariensis* had the slowest and lowest C loss than other litter mixtures (Fig. 4c), losing <35% of the initial C content after 12 months possibly due to the observed antagonistic interactions in these litter samples (Table 4). After 12 months, C loss was greatest in *V. karroo*/*V. verrucosum* (67%) and *V. karroo*/*E. ngamicum* (63%), litter samples associated with synergistic interactions in C release (Table 4). As the rate of litter N release was mostly lower than the C release rate, there was an initial reduction in the C/N ratio of all litters followed by an increase in C/N ratio in *V. karroo*/*P. kalachariensis*, *V. karroo*/*P. kalachariensis*/*V. verrucosum* and *V. karroo*/*E. ngamicum*/*P. kalachariensis* (Fig. 4d). The C/N ratios were between 1.4 and 4.6 throughout the study period. After 12 months of decomposition, the highest C/N ratio was recorded in *V. karroo*/*P. kalachariensis* and the lowest in *V. karroo*/*V. verrucosum* (Fig. 4d).

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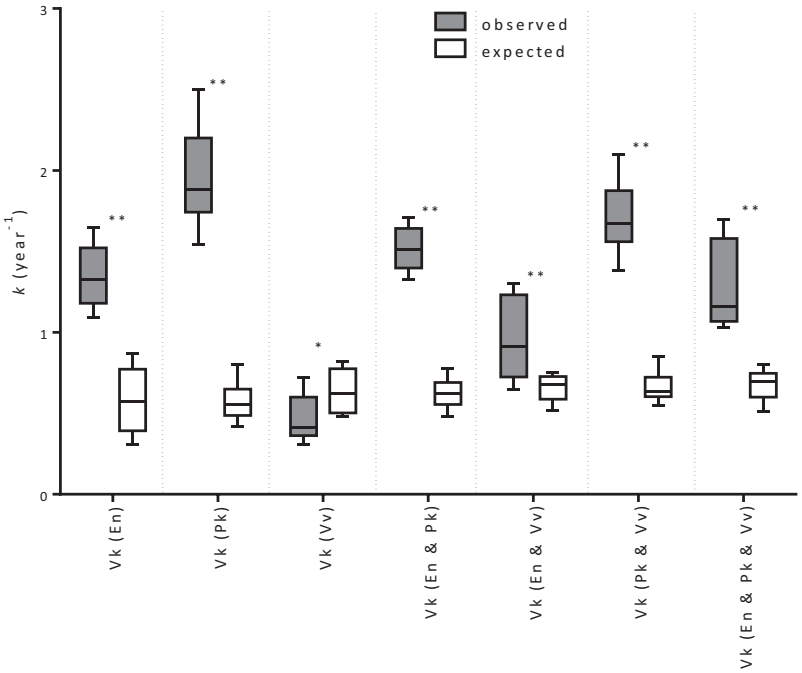


Fig. 3. Observed and expected decomposition rate constant (k year^{-1}) of the litter mixture samples after 12 months of decomposition. *Vachellia karroo* (Vk); *Erianthemum ngamicum* (En); *Plicosepalus kalachariensis* (Pk); *Viscum verrucosum* (Vv). $^{\star}P < 0.05$, $^{\star\star}P < 0.01$.

Table 4. Observed and expected nutrient remaining and per cent synergism of the litter mixture samples after 12 months of decomposition

	Nitrogen			Phosphorus			Carbon		
Litter type	Observed	Expected	ME	Observed	Expected	ME	Observed	Expected	ME
Vk (En)	68.2 ± 5.03	61.2 ± 2.35⋆	7.00	50.3 ± 3.25	77.5 ± 2.34	−27.2	37.1 ± 1.73	57.2 ± 2.18⋆⋆	−20.1
Vk (Pk)	43.4 ± 4.21	65.5 ± 1.96⋆	−22.1	67.2 ± 3.81	65.0 ± 3.34	2.20	48.5 ± 2.01	47.8 ± 1.10	0.70
Vk (Vv)	80.1 ± 3.98	75.0 ± 2.94	5.10	70.5 ± 3.17	53.5 ± 1.06	17.0	33.2 ± 1.65	55.2 ± 1.98⋆	−22.0
Vk (En & Pk)	68.0 ± 4.10	62.3 ± 1.84⋆	5.70	54.1 ± 6.52	69.3 ± 2.42	−15.2	63.3 ± 2.69	49.2 ± 1.33⋆	14.1
Vk (En & Vv)	84.3 ± 3.88	68.7 ± 2.09⋆⋆	15.6	58.5 ± 2.50	61.7 ± 2.35	−3.20	66.0 ± 3.07	54.1 ± 2.43⋆	11.9
Vk (Pk & Vv)	59.5 ± 2.50	71.7 ± 2.22⋆	−12.2	79.4 ± 3.76	53.3 ± 1.46	26.1	44.2 ± 1.84	47.8 ± 1.36	−3.60
Vk (En & Pk &Vv)	69.3 ± 7.86	67.8 ± 2.64	1.50	58.7 ± 3.11	59.5 ± 1.66	−0.80	44.1 ± 2.00	48.9 ± 2.10⋆	−4.80

Vachellia karroo (Vk); *Erianthemum ngamicum* (En); *Plicosepalus kalachariensis* (Pk); *Viscum verrucosum* (Vv). ME: mixing effects or the interaction effect between litters. Asterisks indicate levels of significance for differences between observed and expected nutrient remaining of the same retrieval point.

All values are mean ± standard error.

$^{\star}P < 0.05$.

$^{\star\star}P < 0.01$.

$^{\star\star\star}p < 0.001$.

Initial litter chemistry and mass loss correlations

Mass loss was positively correlated with initial litter N concentration in both single species and mixed litterbags, but negatively correlated to C and C/N ratio in single species litterbags (Table 5). Similarly, the N remaining content was positively correlated with initial N in single species litterbags but negatively correlated to initial P and C/N ratio (Table 5). The C remaining content was negatively correlated to initial N in mixed litters

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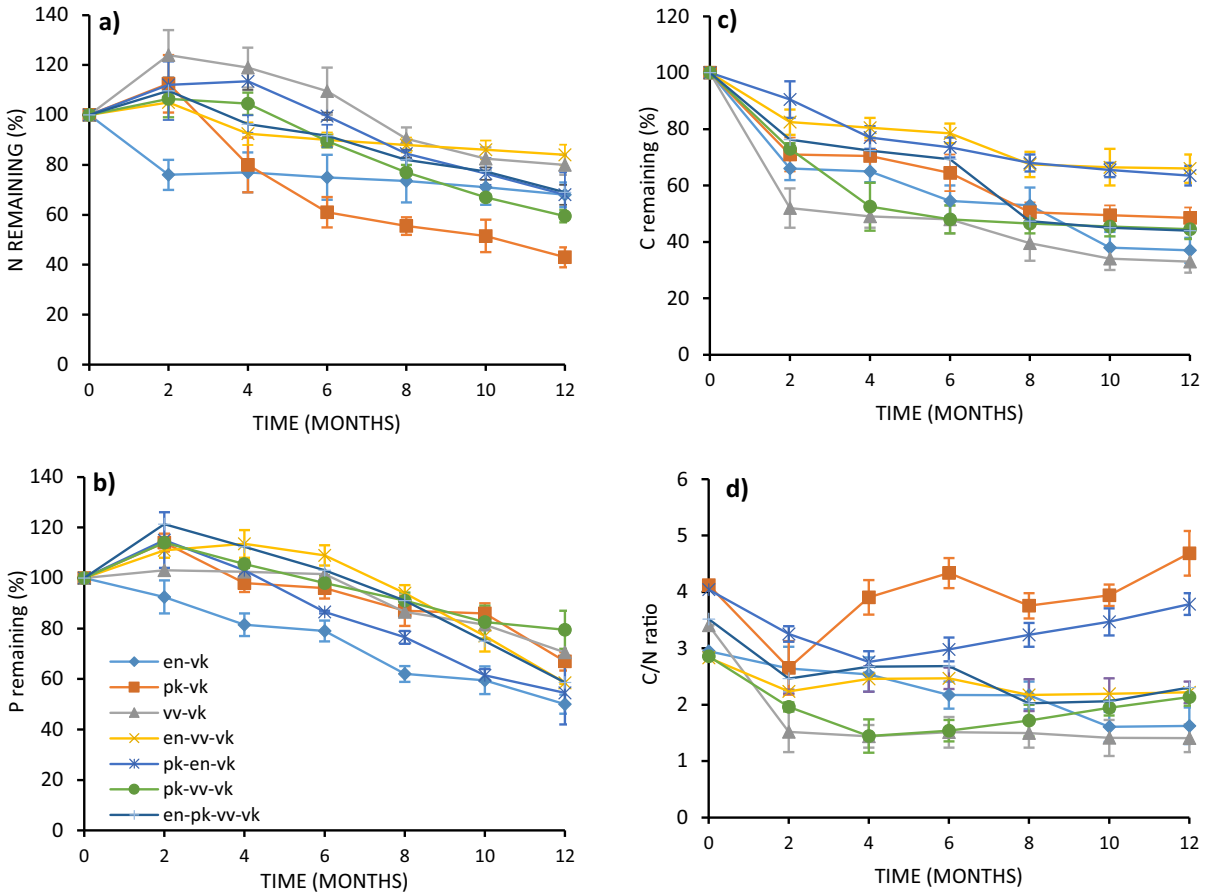


Fig. 4. Dynamics of (a) nitrogen, (b) phosphorus, (c) carbon and (d) C/N ratio observed during the decomposition of litter mixture samples. *Vachellia karroo* (Vk); *Erianthemum ngamicum* (En); *Plicosepalus kalachariensis* (Pk); *Viscum verrucosum* (Vv). Error bars show the standard error of the mean. Symbols with no apparent error bars have error estimates too small to see or hidden by the symbol.

and to C in both single species and mixed litterbags (Table 5).

DISCUSSION

In this study, we found significant non-additive effects of litter mixing on decomposition and litter N, P and C dynamics, and these effects varied both in magnitude and direction between litter-mixing treatments and with incubation time. Although litter species richness affected two response variables, that is mass loss and P, significant non-additive effects were different even among litter mixtures with the same number of litter species. Thus, our findings suggest that mixing multiple species litter produces idiosyncratic effects on decomposition that are better explained by the length of litter incubation and species composition (Quested *et al.* 2005; Lecerf *et al.* 2011).

Mixing species of different litter qualities enhances resource heterogeneity resulting in variable effects on decomposition of recalcitrant litters (Quested *et al.* 2005). March (2007) found that the presence of *Amyema miquelii* (Miq.) Tiegh mistletoe litter led to reduced decomposition of its host *Eucalyptus* species litter. In litter mixtures of the hemiparasite *Bartsia alpina* L. and co-occurring dwarf shrub species, Quested *et al.* (2005) found that interactions were absent or negative in litterbed and non-significant in field litterbag experiments. However, in a laboratory microcosm experiment, mixtures of *B. alpina* and dwarf shrub litter significantly lost more mass and released more CO_2 than expected (Quested *et al.* 2002). Likewise, in this study, we found that *V. karroo* litter quality, mass loss and nutrient release were affected by high-quality mistletoe litter present in mixtures. Litter mixtures of *V. karroo* and mistletoe (mean k-value = 1.32 year^{-1}; Table 3) decomposed three times faster than *V. karroo* decomposing alone

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Table 5. Pearson's correlation coefficients between initial litter quality variables and mass loss, N, P and C remaining contents of litter in single species and mixtures after 12 months of decomposition

		Initial litter chemistry			
Litter type	Variable	N	P	C	C/N
Single	Mass loss	0.84**	−0.24	−0.54*	−0.33*
	N remaining	0.60*	0.18	−0.04	0.05
	P remaining	0.08	−0.26	−0.31	−0.24
	C remaining	−0.16	−0.28	−0.60*	−0.37
Mixture	Mass loss	0.46*	−0.06	0.26	−0.34
	N remaining	0.28	−0.48*	−0.23	−0.49*
	P remaining	0.02	0.12	0.08	−0.17
	C remaining	−0.53*	0.27	−0.63***	0.24

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

here and in a previous study (0.63 $year^{-1}$: Ndagurwa *et al.* 2015). In addition, decomposition rates reported here for mistletoe–host litter mixtures are also greater than those reported for *Eucalyptus* species decomposing in the presence of *A. miquelii* in Australian woodlands (March 2007). Unlike the March (2007) study where litter mixing was achieved by placing litterbags with only red gum litter in an environment where mistletoe litter was predominant, we placed litterbags with a mixture of mistletoe and host litter beneath mistletoe-infected trees. Thus, we believe our decomposition environment would have better simulated the natural decomposition conditions, exposing the litter to mistletoe-induced physical, chemical and biological environment than the March (2007) study. Among other factors discussed therein, it is also possible the decomposition environment in the March (2007) study may have limited litter decomposition. This finding not only underscore the need to consider the decomposition environment in cross-study comparisons, but also suggests that standardisation of decomposition experiments in future studies is necessary to enable better between-study comparisons and quantification of mistletoe litter effects.

Initial litter quality parameters such as N and P have been suggested as good indicators of the decomposition rate (Hättenschwiler & Vitousek 2000). Here, initial N, C and C/N contents of leaf litter were major influences on decomposition rate. Mass loss was positively correlated with initial litter N concentration in both single species and mixed litterbags but negatively correlated with C and C/N ratio in single species litterbags. Litters of high initial N provide N for active heterotrophs and may also induce a priming effect on nutrient-poor litter facilitating faster decomposition (Chapman *et al.* 1988; Wardle *et al.* 1997; Liu *et al.* 2007). High initial C and C/N ratios may retard litter decomposition and nutrient release rates. However, the C/N ratios were between 1.4 and 4.6 over the course of the experiment, which is much lower than the 25:1 threshold at which N becomes a limiting factor for microbial growth (Swift *et al.* 1979). Therefore, other litter chemical components such as secondary compounds may have inhibited decomposition in some of the litters. For example, *V. karroo*/*V. verrucosum* probably had the least decomposition rate (0.47 $year^{-1}$) because of the high condensed tannin content associated with these species (Ndagurwa & Dube 2013; Ndagurwa *et al.* 2013; Ndagurwa 2015). These inhibitory polyphenols are known to form complexes with proteins that are highly resistant to decomposition (Hättenschwiler & Vitousek, 2000). However, effects of secondary compounds such as condensed tannins on decomposition were not measured in this study, an area open for future research.

Non-additive interactions (synergistic and antagonistic) are common in many studies examining the effect of litter mixing on mass loss and nutrient release, with most synergistic effects (Hättenschwiler & Gasser 2005; Lecerf *et al.* 2007). Enhanced or synergistic (non-additive) interactions were observed in five out of seven litter mixture samples with per cent synergism being particularly greater after 12 months of decomposition. The high-quality mistletoe litter, due to increased resource competition (*sensu.* Tilman 1982), may have increased the development, composition and activities of decomposer organisms in litter mixtures (Chapman *et al.* 2013), and consequently litter processing rates. Also, mixing litters of different qualities probably increased diversity of niches and substrates allowing more functionally even (bacteria and fungi) microbial communities to exist on mixed litter partly contributing to the non-additive effects of litter mixtures (Chapman *et al.* 2013). Indeed, other studies have also demonstrated greater numbers of invertebrates, nematodes, bacterial numbers and

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higher heterotrophic activity in litter mixtures than in single species litter (Chapman *et al.* 1988; Blair *et al.* 1990). Ndagurwa *et al.* (2014a) found that the litter layer of mistletoe-infected trees was associated with a high diversity of litter-feeding arthropods, suggesting that mistletoe litter enhanced the decomposition of *V. karroo* litter by altering the abundance and diversity of decomposers on litter mixtures, which confirms the positive effects of nutrient-rich parasitic plant litter on mass loss. However, the development of decomposer communities on the litter mixtures was not assessed, which suggest future research directions.

In contrast, we found negative or inhibitory interactions on mass loss only in *V. karroo*/*V. verrucosum* and *V. karroo*/*P. kalachariensis*/*V. verrucosum*. It is possible that the high initial N concentration in mixed litter combined with greater concentrations of lignin associated with *V. karroo* and *V. verrucosum* litter (Ndagurwa & Dube 2013; Ndagurwa *et al.* 2013) may have led to the formation of highly stable lignin–protein complexes, which are resistant to decomposition. Thus, exchange of nutrients between mistletoe and *V. karroo* litter may have influenced the chemical characteristics of the litter mixture and therefore decomposition rates (March 2007). Alternatively, decomposer communities (e.g. fungi, bacteria or invertebrates) may be different in the mistletoe environment leading to slower decomposition (March 2007). Further, we found a shift in interactions in *V. karroo*/*V. verrucosum* and *V. karroo*/*P. kalachariensis*/*V. verrucosum*, which could be related to the progressive development of the decomposer community. Antagonistic interactions in *V. karroo*/*P. kalachariensis*/*V. verrucosum* mass loss were recorded in the early phase of decomposition, progressively becoming synergistic after 12 months of decomposition, suggesting a successional change in dominant litter consumers such as fungi, detritivores or bacteria involved in litter decomposition (Hättenschwiler *et al.* 2005; Lecerf *et al.* 2011). Therefore, the length of incubation period is important for the detection of additive and non-additive effects in litter mixtures (Lecerf *et al.* 2011), and we recommend that future studies should be carried out over longer time frames to establish the temporal variation in synergistic and inhibitory interactions on mass loss and nutrient release in litter mixtures.

Like mass loss, we found both synergistic and antagonistic interactions in nutrient release in litter mixtures. Antagonistic effects were more common regarding N (five out of seven cases) contrary to general trends reported in literature (Hättenschwiler & Gasser 2005; Quested *et al.* 2005; Lecerf *et al.* 2007). Indeed, *V. karroo*/*P. kalachariensis* with the greatest decomposition rate also had the highest N loss compared to other litter mixtures. Considering that the absolute nutrient content during decomposition is a function of both mass loss and changes of nutrient concentration in the remaining litter (Bonanomi *et al.* 2010), and thus, the observed patterns regarding N loss can be explained by differences in decomposition rates. Further, the slow release of N from *V. karroo*/*E. ngamicum*/*V. verrucosum* and *V. karroo*/*V. verrucosum* litter mixtures also suggests that phenolic compounds released from litter species rich in these elements such as *V. karroo*, *E. ngamicum* and *V. verrucosum* (Ndagurwa *et al.* 2013) may have diffused within litter mixtures leading to either retarded decomposition or may have counteracted the positive effects of high-quality litter on decomposition, hence the antagonistic interactions. In contrast, synergistic interactions were common in P and C release similar to findings elsewhere (Lecerf *et al.* 2007; Bonanomi *et al.* 2010). The variation in additive and non-additive effects in nutrient release among litter mixtures suggests that these effects tend to be idiosyncratic, driven by positive and negative effects of individual species (Quested *et al.* 2005; Liu *et al.* 2007). As a result, the direction and magnitude of litter-mixing effects may be difficult to predict from the litter quality characteristics of the component species as also suggested in other studies (Quested *et al.* 2005; Lecerf *et al.* 2007).

In this study, synergism was greatest after 12 months, suggesting that our findings may not apply over longer time frames, and thus, we remain conservative in the extrapolation of these findings. Nonetheless, our findings show that mistletoe litter has important ramifications for decomposition, carbon storage and assimilation, and nutrient dynamics in this savanna. Soil organic carbon (SOC) accumulation depends on the quantity and quality of litter input to the soil (e.g. Vargas *et al.* 2006). Presumably, low quality litter laden with secondary compounds such as lignin or polyphenolics decomposes slowly and promote SOC accumulation (Palm & Rowland 1997; Kraus *et al.* 2003; Vargas *et al.* 2006). Thus, the accelerated decomposition rates of the litter mixtures reported here may lead to decreases in C sequestration. Given SOC improves infiltration and water holding capacity (Facelli & Pickett 1991), the reduced SOC due to faster decomposition rates suggests that mistletoes contribute to the low soil moisture content recorded beneath mistletoe-infected trees (Ndagurwa *et al.* 2014a, 2015). The low soil moisture together with high evaporative demand and the profligate use of water by mistletoes can increase the susceptibility of both mistletoe-infected trees and co-occurring species to water stress (Sala *et al.* 2001), particularly during drier periods. In addition, fires that occur in these dry conditions can also reduce C sequestration as previously reported in this study site (Bird *et al.*

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2000). In the long term, these changes are expected to negatively affect the decomposer communities and processes that they control such as nutrient cycling. Therefore, accelerated decomposition associated with mistletoe litter may create a negative feedback loop, which could affect the fitness of the host.

On the contrary, C sequestration may increase due to accumulation of slow decomposing litter mixtures and recalcitrant residues present at advanced stages of decomposition (Hättenschwiler & Vitousek 2000; Vargas *et al.* 2006), preservation of SOC by silt and clay (Mtambanengwe *et al.* 2004) or enhanced litter inputs beneath mistletoe (March 2007; Ndagurwa 2015; Ndagurwa *et al.* 2016, 2018). Also, SOC may increase due to contribution from the above- and belowground fraction of elevated plant productivity beneath mistletoe (Ndagurwa *et al.* 2016). SOC accumulation improves infiltration, water holding capacity and the diversity and function of soil decomposer communities (Facelli & Pickett 1991; Ndagurwa *et al.* 2014a) creating conditions of increased water and nutrient availability which favour the growth and performance of mistletoe and host (March 2007). However, it is not clear if the aforementioned processes are enough to compensate for the effect of the fast decomposition of mistletoe litter mixtures on SOC accumulation. Whether mistletoe litter effects on decomposition lead to an increase, decrease or no change in the SOC pool will depend on the balance between above- and belowground litter inputs and outputs from decomposition. Thus, future studies examining long-term decomposition beneath-infected hosts and stands with differing abundances of mistletoes may provide insight into how mistletoes via increased litter quality and quantity affect C sequestration in this very savanna and other environments where mistletoes occur.

Mistletoe litter, by enhancing decomposition of recalcitrant litter of the host, also ensures rapid transfer of nutrients to the system (Ndagurwa *et al.* 2015). Thus, mistletoes are unlocking nutrients that would otherwise be held in organic matter for longer periods, thereby increasing nutrient availability within their immediate vicinity (March & Watson 2010; Ndagurwa *et al.* 2013, 2014b, 2016). Furthermore, because of the patchy distribution of mistletoes (March 2007; Ndagurwa *et al.* 2012), nutrient availability is also spatially heterogeneous. These changes in nutrient availability and distribution, particularly of N and P, can affect many ecosystem characteristics including species diversity and productivity, host performance, competitive dominance, ecosystem heterogeneity and community structure (Press 1998; Press & Phoenix 2005; March 2007; Watson 2009; Ndagurwa *et al.* 2016, 2018). In this regard, because mistletoes are components of all terrestrial vegetation types (Kuijt 1969), these findings show that mistletoes have broader ramifications for ecosystem function in many environments, and understanding the ecosystem-wide influence of these plants becomes increasingly important.

CONCLUSIONS

In conclusion, not only do our findings show that parasitic plant litter may stimulate the decomposition of recalcitrant host litter, but they also show that the magnitude and direction of litter-mixing effects depend on the species composition of litter mixtures and length of litter incubation. Further, given the spatially heterogenous distribution of litter components and mixtures in natural environments, the variation in additive and non-additive effects in litter mixtures may also indicate that decomposition, nutrient release rates and nutrient availability are spatially heterogenous. Thus, we suggest that this may constitute one mechanism with which parasitic plants contribute to spatial heterogeneity in environments where they occur.

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AUTHOR CONTRIBUTIONS

Hilton G. T. Ndagurwa: Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (lead); methodology (equal); supervision (lead); validation (lead); visualization (equal); writing-original draft (lead); writing-review & editing (lead). **Tsitsi Sithandwa Maponga:** Data curation (equal); formal analysis (equal); validation (equal); writing-review & editing (equal). **Justice Muvengwi:** Data curation (equal); formal analysis (equal); validation (equal); writing-original draft (equal); writing-review & editing (equal).

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