Vegetation cover dynamics and the environmental threats to biodiversity conservation in Free State National Botanical Garden, South Africa

Master of Health Science in Environment

By

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Declaration

I declare that the ***Master Thesis*** has been composed by myself and that the work has not be submitted for any other degree or professional qualification at any university. Wherever contributions of others are involved, every effort is made to indicate this clearly, with due reference to the literature.

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Abstract

The conservation gardens under South African Biodiversity Institute (SANBI) preserve biodiversity areas with unique and threatened vegetation types including national priority Critical Biodiversity Areas (CBA's). The mandate of SANBI is to champion the exploration, conservation, appreciation, and enjoyment of South Africa's exceptionally rich biodiversity for all people. Since the SANBI mandate emphasises sustainable biodiversity conservation, however, there were no studies that have quantitatively appraised the biodiversity conservation value and natural phenomena dynamics over time for different SANBI conservation gardens. This study determined the dynamics of the vegetation cover over a 30-year period (1987-2017) and potential environmental drivers of change in Free State National Botanical Garden (FSNBG). The FSNBG is located in the broader southern African grassland biome that is under huge conservation threats in South Africa. The FSNBG preserved national priority for biodiversity conservation (CBA1) in South Africa: the "Bloemfontein Karroid Shrubland". The study applied the remote sensing technology and Geographical Information System (GIS) imagery to analyse the spatial vegetation cover changes that occurred over 30-years using 10 year's intervals.

Overall study results show that vegetation cover of the study area significantly increased (in term of woody biomass) over the 30 year period, partly attributed to the combination of factors, which include severe droughts and the rise of CO_2 concentrations in the atmosphere. In addition, it was possible that absence of the biophysical disturbance such as fire and browsers in the natural vegetation to suppressed wood species has encourage encroachment of woody cover species. Using Normalized Difference Vegetation Index (NDVI) approach, the vegetation of FNSBG was divided into four categories, namely: Dense vegetation, Moderate vegetation, Sparse vegetation and open soil. Overall, the sparse vegetation cover significantly decreased over time (R^2 = - 0.29). Similarly, the moderate vegetation cover increased by 25.1 ha while dense vegetation also increased by 8.6 ha. However, the bare soil cover was found to be relatively consistent over the 30-year period.

Since the use of the remote sensing and GIS imagery could not clearly validate the current ecological status of CBA1 vegetation type, the manual field sampling approach was used looking into documenting the typical plant species provided by Mucina and Rutherford (2006); and measuring the vegetation cover. Vegetation cover of CBA1 was relatively high (i.e., 79.6 ± 15.9%); and this was above the minimum threshold of 60% habitat intactness reported to represent the habitat functionality that is normal although overall integrity is reduced. The existence of CBA1 vegetation type was supported by a record of 27 plant species out of 77 species documented as typical in the key descriptions provided by Mucina and Rutherford (2006). Major conservation threats entailed of 27 invasive alien plant species,

human settlement encroachment have reduced the ecological buffer zone of FSNBG by 18% (119.07 ha) in the past 18 year; and 10 pests and pathogens that were interspersed in different vegetation patches of the study site. The observed changes suggested a need for regular vegetation monitoring of CBA1 vegetation type to manage the prevailing conservation threats that could have impact to ecological integrity.

Animal-plant mutualisms are important for the maintenance of both animal and plant population since both participating partners' benefit. The phenology of seed dispersal is critical to the successful recruitment of species since dispersed seeds require conducive conditions for germination. Having noted the increasing bush encroachment in FSNBG in the past 30 years, the study also investigated the seed dispersal phenology and the potential influencing factors for the encroaching tree/shrub species of FSNBG focusing on biotic interaction of flesh fruits resource foraged by birds as study model. During fieldwork sampling for the seed deposited by birds in the 17 roosting sites, I collected a total of 22 161 seeds belonging to 14 native and non-native tree/shrub species. It was found that black and red fruits were more preferred by birds since most of the dispersed tree/shrub species had those colours and attracted 22 resident and colonial migratory frugivorous birds. I found that 80% of the bird-ingested and dispersed seeds of many tree/shrub species were collected between March and July. Two species: *Ehreta rigida* and *Searsia lancea,* displayed relatively short fruiting peak times between August and December when other fruits were scarce. Whereas the observed two fruiting patterns could reduce the competition for seed dispersal services between two groups of tree/shrub species, the resident and wintering birds are also likely to benefit substance in food resources. In addition, it was found that frugivorous birds adapt to localised foraging patterns and to limited fruit diversity. It was concluded that the highly preferred tree/shrub species are likely drivers of the reported bush encroachment in FSNBG.

Frugivorous birds disperse seed away from parent plant to new microsites that have relatively lower competition for the resources, and ingested seeds get scarified during feeding on fruits and ingestion by birds. Therefore, the study also investigated whether bird-dispersed seeds benefit from improved germination after their passage through the bird's gut and the potential impact of seed density on competition at the microsites. On the germination trail results of bird-ingested seeds versus manual depulped seeds, *Ziziphus mucronata* displayed the highest germination rate of all trees/shrubs yet frugivorous birds did not improve its seed germination. However, only *S. lancea* seeds had significantly high seed germination after passage through the avian digestive tract. The test experiments for seed competition during germination at the microsite showed that only *Z. mucronata* and *Olea europaea subsp. africana* displayed significant positive performance with increasing seed density. The study concluded that the tree/shrubs species with high germination could account for the observed bush encroachment in FSNBG. It was recommended that adaptive conservation

management plan of FSNBG should include monitoring and prioritised bush encroachment control of those species.

Table of Contents

List of figures

List of tables

Chapter 1: General introduction, study rationale, study area, and aims and objectives

1.1. General introduction

The South African grassland biome has been identified as critically endangered (SANBI, 2013; Matsika, 2008; Neke and du Plessis, 2004 Reyers et al., 2001; Olsen and Dinerstein, 1998) and requires urgent attention for biodiversity conservation. Numerous studies (see Matsika, 2008; Neke and du Plessis, 2004; SANBI, 2013; Niang et al., 2014) have shown that the grassland biome is threatened by incorrect fire regimes, overgrazing, human settlement encroachment, agricultural activities, mining activities, afforestation, increased habitat fragmentation, soil erosion, bush encroachment, and alien invasive plants. This highlights a demand for protecting the grassland biome. Despite this need, SANBI (2013) and Neke and du Plessis (2004) confirmed that less than 3% of the grassland biome is located within formally protected areas.

Botanical gardens worldwide and within South Africa are meant to maintain the gene pool of unique plant species (Burgess, 2012; Hurka et al., 2004; Jackson et al., 2000; Bramwell et al., 1987). Kirstenbosch National Botanical Garden (NBG) in South Africa, which was established in 1913, was the first botanical garden established by the South African National Biodiversity Institute (SANBI) (Willis, 2015; van Wilgen, 2013). As the first garden in the country, Kirstenbosch NBG was used to establish and preserve the country's unique flora, based on the vision of Harold Pearson (van Wilgen, 2013). The vision of Pearson was to advance the role of science by establishing botanical gardens with a focus on research, indigenous plant horticulture, economic botany, and plant conservation (van Wilgen, 2013).

Even though SANBI botanical gardens are reported to be conservation gardens, they have not undergone quantitative appraisal to assess their biodiversity value and natural phenomena dynamics over time, in order to inform adaptive management (Willis, 2018, 2015). It is critical to understand the vegetation dynamics in SANBI botanical gardens (i.e., FSNBG), and to assess whether the vegetation units have changed over time because of reported increase in the biodiversity threats in the South African Biomes, such as fire, fragmentation, alien and invasive species etc. (van Wilgen et al., 2018; Mucina and Rutherford, 2006; Skowno et al., 2019). Time series analyses of vegetation can reveal the impacts of different management styles used within botanical gardens and determine which one is sustainable in the context of the desired state of the garden and in the face of threats from global environmental change (Peter et al., 2018).

The Free State National Botanical garden (FSNBG) is one of eleven national botanical gardens belonging to SANBI. SANBI is a public entity in terms of section 38 (1) of the Public Finance and Conservation Act and it was established in term of section 10 (1) of National

Biodiversity Management: Biodiversity Act (Act No 10 of 2004). SANBI's mandate comes from the National Environmental Management: Biodiversity Act (NEMBA) and consequently, SANBI botanical gardens are established in the biodiversity areas with unique plants growth and threatened vegetation types (i.e., Critical Biodiversity Areas) for scientific study, horticultural display and environmental education for public. The mandate of SANBI is to champion the exploration, conservation, appreciation, and enjoyment of South Africa's exceptionally rich biodiversity for all people (SANBI, 2019; Willis, 2015). SANBI's mandate also includes the development of conservation gardens and tourism in its eleven botanical gardens. According to Willis (2018), SANBI gardens conserve the representative biodiversity of seven out of the nine South African biomes (Grassland, Savanna, Nama Karoo, Succulent Karoo, Albany Thicket, Fynbos, and Forest). In as much as garden strategies emphasise the conservation of the South African biodiversity, there are no studies that have quantitatively appraised the biodiversity of each garden and established the potential threats from current environmental global change. Such research is essential for the sustainability and development of adaptive management strategies of SANBI's gardens, to ensure biodiversity.

1.2. Rationale for the study: Potential conservation challenge of SANBI's gardens

The FSNBG is reserved as a biodiversity conservation window in the Free State Province because the garden conserves two areas that are priority for biodiversity conservation in South Africa: the Bloemfontein Karroid Shrubland (Nama Karoo-like vegetation) that falls under Critical Biodiversity Area 1 (CBA1), and the Bloemfontein Dry Grassland that falls under the Ecological Support Area 1 (ESA1) (see the layout of the different ecological units in FSNBG in Appendix 3.1). SANBI (2017) define CBA1 as an area that is irreplaceable or near irreplaceable for meeting biodiversity targets, while ESA1 is an area that is in good or fair ecological condition with minimum degradation. In addition, ESAs play an important role with supporting the ecological function of CBAs (SANBI, 2017). With the increasing biodiversity threats in the Grassland Biome (see Matsika, 2008; Neke and du Plessis, 2004; Little, 2015; Yapi et al., 2018), the FSNBG is likely to lose critical biodiversity areas. As a conservation biodiversity area, it is critical to understand the FSNBG's vegetation dynamics and assess if the vegetation units critical within the garden are expanding or reducing due to threats surrounding the botanical garden, as well as how these threats within grasslands influence day-to-day management of the garden. Therefore, this study focused on determining the dynamics of the vegetation cover over a 30-year period (1987-2017) in the FSNBG.

1.3. Study area and location

The FSNBG was introduced under the Forest Act No. 122 of 1984 because the garden provides protection for living resources (Masilo, 1999). The FSNBG was established in 1965 and was opened officially on 22 February 1969 by the Bloemfontein City Council, which transferred control to Trustees of the National Botanic Garden of South Africa. The land was purchased after the Kirstenbosch Jubilee Year Tour in 1963. Under the International Union for Conservation of Nature (IUCN), the garden falls under Category III meaning that it has geological features and receives high numbers of visitors (Mckinnon, 1986).

The FSNBG is located approximately 10 km north of central Bloemfontein (Dan Pienaar), next to the N1 (S 29° 12′ 55.6″; E 26° 12′ 41,3″) (Figure 1.1). The garden is located within the Mangaung Metropolitan Municipality (Motheo district) in the Free State Province. The FSNBG covers an area of 66 hectares (ha), with 60 ha of natural veld and 6 ha of developed area. The study area's topography ranges from plains to lowland areas at the altitude between 1300 and 1400 m above sea level, consisting of a dolerite hill (monk head beacon) separated by valley with a dry watercourse.

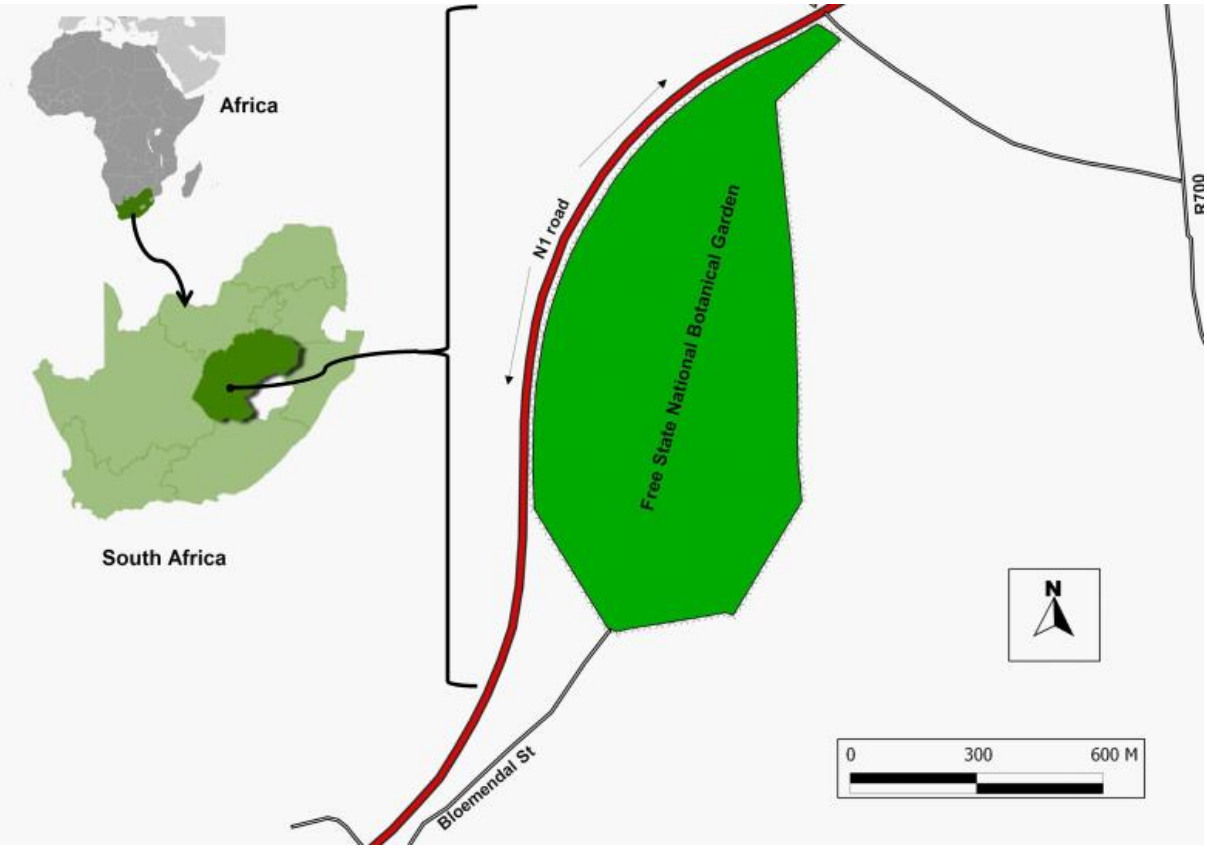

Figure 1.1: Location of the study area: Free State National Botanical Garden, Bloemfontein in South Africa.

1.4. Description of vegetation types in the Free State National Botanical Garden

The FSNBG is located in the Dry Highveld Grassland bio-region of the grassland biome (Mucina and Rutherford, 2006). According to Carbutt et al. (2011), the Dry Highveld Grassland bio-region is poorly protected because only 1.52% is under formal protection while 31.51% is already transformed. The vegetation structure is largely characterised by Gh 8 Bloemfontein Karroid Shrubland (CBA1), Gh 5 Bloemfontein Dry Grassland, Gh 7 Windburg Grassy Shrubland, and Waterbody/Riparian vegetation (Table 1.1) (Mucina and Rutherford, 2006). The ability of the FSNBG to support vegetation species may change due to different environmental factors such as alien and invasive species, which highlights the need for monitoring (Yapi et al., 2018).

Table 1.1: Vegetation types of the FSNBG described in terms of dominant taxa, geology, and altitude (adapted from Mucina and Rutherford, 2006)

Vegetation type	Dominant taxa	Geology	Altitude (m)
Bloemfontein Karroid Shrubland	*Eriocephalus ericoides, Euryops empetrifolius, Chasmatophyllum muscullium, Euphorbia mauritanica, Ruschia spinose, Stomatium mustellium, Aristida diffusa, Eragrostic nindensis, Heteropogon contortus, Oropetium capense, Cheilanthes eckloniana,* and *Senecio radicans.*	Dolerite	1400-1440
Bloemfontein Dry Grassland	*Anthephora pubescens, Aristida congesta congesta, Aristida diffusa, Cynodon dactylon, Digitaria argyrograpta, Elionurus muticus, Eragrostic chloromelas, Eragrostic lehmanniana, Eragrostic obtuse, Eragrostic plana, Eragrostic superba, Eragrostic trichophora, Heteropogon controtus, Panicum stapfianum, Seteria sphacelata, Themeda triandra, Tragus koelerioides, Selago densiflora, Oxalis depressa, Chryscoma cilliata, Felicia filifolia subsp. fillifolia, Pentzia globose,* and *Pentzia incana*	Dolerite	1320-1420
Winburg Grassy Shrubland	*Buddleja saligna, Euclea crispa subsp. ovata, Gymnosporia polyacantha, Olea europaea subsp. africana, Searsia buechellii, Searsia erosa, Pentzia globose, Aristida congesta congesta, Aristida diffusa, Cymbopogon pospischillii, Cynodon dactylon, Cynodon incompletus, Eragrositc chloromelas, Eragrostic lehmannian, Eragrostic micrantha, Eragrostic obtuse, Eragrostic trichophora, Eustachys paspaloides, Heteropogon contortus, Panicum stapfianum, Seteria lindenbergiana, Seteria sphacelata, Sporobolus fimbriatus, Themeda triandra,* and *Tragus koelerioides*	Dolerite	1360-1440
Riparian vegetation	*Vachellia karroo*, *Searsia lancea, Celtic africana, Diospyros lyciodes, Ziziphus mucronata, Asparagus* species, ferns, and *Stapelia flavirostris*.	Dolerite	1300-1360

a) Bloemfontein Karroid Shrubland (Gh 8)

According to Collins (2016) and Driver et al. (2004), the conservation status of the Bloemfontein Karroid Shrubland is ‘least threatened’ or ‘least concern’ in South Africa based on the ecosystem status of the remaining untransformed area (original size was 9452 ha and the remaining is 8561 ha). The Bloemfontein Karroid Shrubland covers almost 0.004% of area within the Free State Province, with small, scattered vegetation (Brown and du Preez, 2014, Mucina and Rutherford, 2006). Although the Bloemfontein Karroid Shrubland is not formally conserved or protected, only a small portion occurs in the FSNBG’s conservation area (Figure 1.2) (Willis, 2018; Mucina and Rutherford, 2006). The vegetation unit is threatened by climate change and human fragmentation due to activities such as mining, road construction, and residential developments (Brown and du Preez, 2014; Munica and Rutherford, 2006).

The Bloemfontein Karroid Shrubland vegetation falls under CBA1 as a priority for biodiversity conservation in South Africa (CBA map 2017; SANBI, 2013). The Bloemfontein Karroid Shrubland vegetation unit consists of unique plant species that are drought tolerant and is similar to the Nama-Karoo biome’s vegetation (Mucina and Rutherford, 2006). The Bloemfontein Karroid Shrubland vegetation is well presented with a combination of small-leaved dwarf karroid and succulent shrubs (Brown and du Preez, 2014; Mucina and Rutherford, 2006).

Figure 1.2: Bloemfontein Karroid Shrubland within the FSNBG. A patch with dolerite rock and low grassy karroid shruland dominated by prominent succulent shrub. Important species include *Cheilanthes eckloniana, Euryops empetrifolius, Ruschia spinosa, Stomatium mestellinum, Aristida diffusa,* and *Euphorbia mauritanica*.

b) Bloemfontein Dry Grassland (Gh 5)

The Bloemfontein Dry Grassland is highly fragmented due to anthropogenic activities and its vegetation conservation status is considered to be a 'vulnerable' vegetation (Little et al., 2015; SANBI, 2013; Driver et al., 2004). Mucina and Rutherford (2006) argue instead that the status of this vegetation type is 'endangered'. According to the Government Gazette 34809 (9 December 2011) and Mucina and Rutherford (2006), the original area of Bloemfontein Dry Grassland is 492 000 ha but more than 40% has been transformed due to crop production and urban development, while less than 1% is within formally protected areas.

The Bloemfontein Dry Grassland falls under ESA1 for biodiversity conservation in South Africa (CBA map 2017). The FSNBG vegetation unit is represented by tall and dense grass species with scattered patches of karroid scrub species (Figure 1.3) (Mucina and Rutherford, 2006).

Figure 1.3: Bloemfontein Dry Grassland within the FSNBG. Patch vegetation is dominated by talll dense grassland alternating with patches of karoo tree/shrub vegetation. Important species include *Themeda triantra, Aristida congesta, Cynodon dactylon, Heteropogon contortus,* and *Digitaria argyrograpta.*

c) Winburg Grassy Shrubland (Gh 7)

The Winburg Grassy Shrubland vegetation type is listed as 'least concern' under the National list of threatened ecosystems (Mucina and Rutherford, 2006; Driver et al., 2004). According to Driver et al. (2004) and Mucina and Rutherford (2006), this vegetation unit is hardly protected, with only around 1% under formal protection, while 10% (17 678 ha) has been transformed due to cultivation and urban sprawl. From the original 157 198 ha of Winburg Grassy Shrubland that existed, only 139 520 ha has remained natural (Driver et al., 2004).

The Winburg Grassy Shrubland vegetation type is located on the southwest area of the FSNBG and comprises three hills. Winburg Grassy Shrubland is typically dominated by dolerite hills, slopes, and an escarpment of mesas with a range of open grassland to shrubland of mosaic habitat (Mucina and Rutherford, 2006). The Monk's head beacon is marked on the highest peak of the hill (1436 m above sea level). The vegetation is primarily dominated by medium height evergreen shrubs with a combination of *Olea europaea* subsp. *africana*, *Ehretia rigida, Ziziphus mucronata, Searsia ciliata, Buddleja saligna,* and *Diospyros lycioides* (Figure 1.4) (Munica and Rutherford, 2006).

Figure 1.4: Winburg Grassy Shrubland within the FSNBG. The solitary hill dominated by medium height evergreen shrubs with a combination of *Olea europaea subsp. africana, Buddleja saligna, Searsia burchella, Euclea crispa, Gymnosporia buxifolia,* and *Diospyros lycioides*.

d) Riparian vegetation

The riparian vegetation occurs on the plains and foot-slopes where the habitat conditions are relatively drier. In this vegetation, the woodland species community are restricted to the streambanks and valleys. The valleys have deeper alluvial soils with a clay-loam texture (Chaplin, 1979). The vegetation is characterised by dense bushes near the watercourse. The vegetation is dominated by the deciduous trees of *Vachellia karroo* and *Searsia lancea,* followed by *Celtis africana, Diospyros lyciodes,* and *Ziziphus mucronata*, and prominent and dwarf shrubs that include *Asparagus* species, ferns, and *Stapelia flavirostris* (Figure 1.5) (Retief and Meyer, 2017; Mucina and Rutherford, 2006; Chaplin, 1979).

Figure 1.5: Riparian vegetation within the FSNBG, dominated by the deciduous trees of *Vachellia karroo* and *Searsia lancea,* followed by *Celtis africana, Diospyros lyciodes,* and *Ziziphus mucronata.*

1.5. Climate: Local temperature and precipitation

The FSNBG experiences an extremely variable continental climate with a minimum winter temperature below zero degrees Celsius (˚C), with night temperatures often below freezing and frost weather in July. Fairly high summer temperatures frequently range between 30-35˚C in January (Figure 1.6) (Haddad et al., 2019; Neethling and Haddad, 2013). The mean annual rainfall is relatively low (584 mm annually), with most of the rainfall occurring during the summer months and early autumn. August months generally tend to be the windiest (Haddad et al., 2019; Neethling and Haddad, 2013; Mucina and Rutherford, 2006).

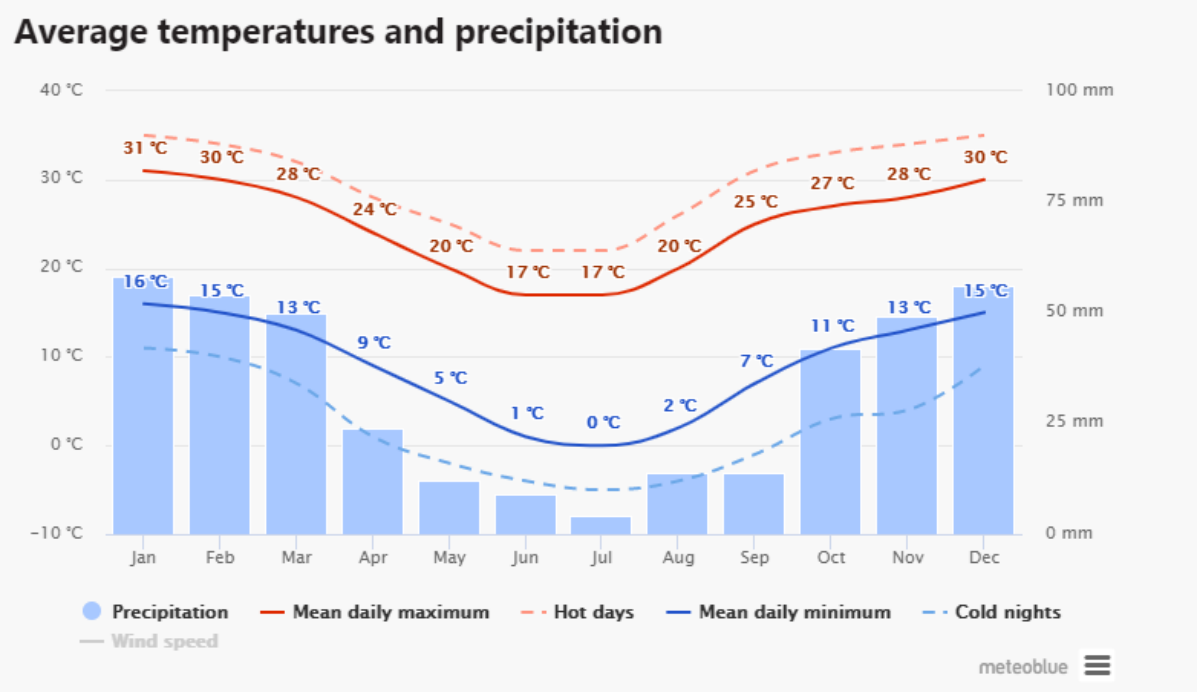

Figure 1.6: 30-year average local weather trends for the FSNBG (Precipitation is estimated from radar and satellites) (sourced from https://www.meteoblue.com/en/weather/week/princeton_united-states-of-america_5102922).

1.6. Study's aim and objectives

1.6.1. Aim

The aim of this study is to investigate and determine long-term vegetation cover change and potential environmental drivers of change and threats in the FSNBG, South Africa; and to investigate the role of animal-plant interactions in maintaining local vegetation, using birds and seeds/fruits as a model.

1.6.2 Study objectives

Objective 1: To determine the vegetation cover dynamics of the FSNBG in ten-year intervals over a 30-year period (1987-2017), and to determine conservation threats that drive vegetation cover changes in the FSNBG.

Objective 2: To evaluate ecological integrity of the CBA1's vegetation by determining the presence/absence of the flagship plant species.

Objective 3: To determine which tree/shrub species are dispersed by birds, and the timing of such dispersal for each tree/shrub species.

Objective 4: To determine the factors that potentially influence fruit consumption by birds and subsequent seed dispersal; and to identify the avian frugivores species that are potentially responsible for seed dispersal in the Grassland Biome of South Africa.

Objective 5: To investigate whether bird-dispersed seeds benefit from improved germination after their passage through the bird's gut; and the potential impact of seed density and species diversity on competition during at the microsites in the Free State Province, South Africa.

1.7. Research significance

a) **Conservation significance** – Although the FSNBG's management strategy emphasises biodiversity conservation, there are no studies that have quantitatively appraised the vegetation in SANBI's gardens. Therefore, this study will establish a new understanding of the unique and conserved vegetation types and their cover dynamics within the FSNBG. This study will also evaluate ecological integrity of the CBA1's vegetation by determining the presence/absence of the flagship plant species, as priority areas for biodiversity conservation in South Africa. The biotic interaction of plants and animals is a critical component to many ecological processes as result of co-evolution (Deveau et al., 2018). Therefore, this study will investigate the potential important/keystone species (birds and plants) to prioritise for biodiversity conservation because without those species, the environmental system could collapse.

b) **Nature-based tourism** – a core business of SANBI's national botanical gardens is to provide safe and attractive outdoor activities within botanical garden estates for visitors, which directly or indirectly depend on the natural environment (e.g., birdwatching, nature trails, etc.). However, some of the environmental threats likely to impact on these natural areas will be through tourism-mediated activities. For example, invader plants and pathogen reproduction material can be spread in the botanical gardens by human activities, such as vehicle movement and other equipment during their presence (e.g., internal, and external visitors), which can result in significant change of vegetation cover and its function. In addition, alien plant species propagule pressure at FSNBG could be obtained from home gardens by tourists' movement. The results of the study will advise adaptive management of the FSNBG and any rehabilitation of the different vegetation units, in order to achieve sustainable conservation of the FSNBG.

c) **Education and society** – SANBI leads, coordinates, and monitors biodiversity research, and provides a report on the state of biodiversity in South Africa (e.g., van Wilgen and

Wilson, 2018: National Biodiversity Assessment of 2017). Therefore, the findings of the study may be used as baseline for conducting research related to other aspects of biodiversity conservation, such as soils, microbial, hydrological, and entomological research in SANBI's botanical gardens.

1.8. Thesis outline

Chapter 1: General introduction, study rationale, study area, and aims and objectives

This chapter contains a general introduction, rationale of the study, research aim and objectives, and research significance. This chapter also provides a description of the study area and vegetation types within.

Chapter 2: Literature review

The literature review was based on more than 100 previously published scholarly literature (scientific journals and books) on the topic of vegetation threats in the grassland biome, and biotic interactions, with a focus on birds and fleshy fruits and seed dispersal, as determined by the study's main aim and objectives.

The following data chapters (Chapter 3, 4, and 5) are presented and formatted as scientific papers.

Chapter 3: Thirty years of vegetation cover dynamics, and the potential drivers of habitat change in the Free State National Botanical Garden, South Africa

This chapter mainly focuses on vegetation cover dynamics within the protected area and biodiversity conservation threats (i.e., alien invasive species and diseases) in the face of climate change and human-mediated anthropogenic pressure, using remote sensing and GIS. The montane rangelands are embedded within the grassland biome that is under huge conservation threats, including biological invasions in South Africa. Among other conservation strategies, SANBI establishes conservation gardens in areas where either a unique vegetation occurs, or certain vegetation types are threatened to extinction. Therefore, it is important to investigate the possible impact of climate change and human-mediated activities in the natural vegetation since the plants are regarded as one of the most important indicators for vegetation cover change. The results from this chapter could be used to advise the adaptive management of the FSNBG to achieve sustainable biodiversity conservation.

Chapter 4: Seed dispersal phenology of encroaching tree/shrub species in the Free State National Botanical Garden, South Africa.

This chapter focuses on biotic interactions (i.e., fruits and birds as a model) and provides a mutual overview of plants-animals seeds dispersal phenology. It is critical to understand the biotic interaction complex and adaptation of plants in response to environmental change in the protected area (FSNBG). In line with the seed dispersal cycle described by Wang and Smith (2002), the chapter's aims were: 1) to determine which tree/shrub species are dispersed by birds, and the timing of such dispersal for each tree/shrubs species; 2) to determine the factors that potentially influence fruit consumption by birds and the subsequent seeds dispersal; and 3) to identify the avian frugivores species that are potentially responsible for seed dispersal in the grassland biome of South Africa.

Chapter 5: Interspecific competition in germination of bird-dispersed seeds in a habitat with sparse tree vegetation in South Africa.

This chapter addresses the impact (i.e., positive or negative) of avian frugivores on seed germination and seed density in a new microsite. Several studies have shown the impact of avian frugivores who providing directed seed dispersal services that contribute in shaping (either positive: maintaining native species gene; or negative: introduction of alien species' propagule) vegetation units and facilitating population persistence. By transporting and scarifying the seeds during ingestion, avian frugivores reduce the competition with siblings, and may improve the germination that is critical for dispersal effectiveness. However, there is limited knowledge on how deposited seeds interact/compete in the new microsite. In this chapter, the study investigated: 1) whether bird-dispersed seeds benefit from improved germination after their passage through the bird's gut; and 2) the potential impact of seed density on competition at the microsites by determining whether seed density and species diversity influence germination in the Free State Province, South Africa.

Chapter 6: General discussion and conclusions

This chapter provides the synthesis and conclusions based on the main findings of the study, and makes recommendations for adaptive management of the study site (FSNBG).

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Chapter 2: Literature review

2.1. Global change threats to biodiversity

2.1.1. Background

Global environmental changes are likely to be a threat to biodiversity by increasing species extinction rates throughout the 21st century and late 20th century (Niang et al. 2014; Sala et al., 2000). Climate change has become a major threat to biodiversity in the current century in other parts of the world because the distribution of biological species has shifted gradually (Thuiller, 2005). For example, Niang et al. (2014) asserted that there is emerging evidence that the shifting of the home range of plant species in Africa may have resulted from microclimate change. The capacity of plants to alter their geographical distribution in response to climate change seems to be constrained by the difficulty for the species to locate a new home range with the correct survival requirements (Rutherford, 2000).

Modifying the environment to fit the needs of society has caused severe effects on the integrity of the natural ecosystems in Africa (Niang et al., 2014; Gonzalez et al., 2012). Niang et al. (2014) indicated that changes in species distribution in Africa occurs in all type of biomes, including grasslands, savanna, deserts, shrub lands, forests, and woodlands, as a result of increased anthropogenic habitat fragmentation. On the remaining natural vegetation types of Africa, Gonzalez et al. (2012) have observed that there is a decrease in the woody vegetation in western Africa, while Mitchard and Flintrop (2013) have observed an increase in wood vegetation in central, eastern, and southern Africa. This implies that there are significant environmental changes that force vegetation structure to change in different areas over time.

2.1.2. Precipitation changes

The shift in precipitation patterns due to climate change has influenced the ecosystem on all continents, particularly in recent decades (Adefisan, 2018; du Plessis and Schloms, 2017; Madhukara et al., 2016; Field et al., 2014). According to Niang et al. (2014), the reduction of precipitation is likely to occur over northern Africa and the south-western part of South Africa by the end of the 21st century. In Botswana, Zimbabwe, and western South Africa, a downward trend in rainfall, which also includes the change of certain intra-seasonal characteristics such as duration, dry spell frequencies, and rainfall intensity, has been observed (Tadross et al., 2009; Kniveton et al., 2009; Thomas et al., 2007). The shift in rainfall pattern may contribute a major threat to biodiversity because the amount and timing of rainfall influences biological processes in grasslands (Settele et al., 2014). In South Africa, Mackellar

et al. (2014) observed a downward trend in seasonal and annual total rainfall for the period 1960 to 2010; the decrease in total rainfall and the number of rainy days in the autumn season were evident over the central and north-eastern part of South Africa, while a significant increase in the number of rainy days was observed in the southern Drakensberg in the spring and summer seasons. Although studies have shown that a shift in precipitation patterns can influence an ecosystem, such studies have not been done to prove this in the central plateau of South Africa.

2.1.3. Environmental temperatures

Studies have shown that temperature changes also have a negative influence on biodiversity conservation (Sintayehu, 2018; Niang et al., 2014; Bellard et al., 2014). According to Niang et al. (2014), the global mean annual temperature is likely to increase in the late 20th century by between 3°C to 6°C, which will result in the warming of oceans. The prolonged warming in the Pacific sea's ocean surface temperature (El-Niño) when compared with average values mostly affects developing countries dependent upon agriculture and fishing (Warek, 2015). Additionally, a study focusing on the physics of the warming of Lake Tanganyika by climate change showed that the increases in surface temperatures (increased by 0.2°C at 1000 m in depth) resulted in a decrease in biomass production in the ecosystem of Lake Tanganyika (Verburg and Hecky, 2009). In addition, it has been shown that extreme warm indices have increased, with it becoming hottest during the day and the nights are also hotter than what it used to be, and a decrease in extreme cold indices (cold night and cold days) in recent decades in southern Africa (Kruger and Sekele, 2012). Consistently, Lyon (2009) indicated that summer heat waves in South Africa increased over the last two decades (20th century) compared to 1961 to 1980, a trend that was associated with the El-Niño condition. Therefore, rapid temperature changes over the current and next century is likely to lead to a major change in the vegetation cover (i.e., a shift of plant distribution). In addition, this will also affect many animal species as their ecology is linked not only to climate but also to the habitat availability.

A continuing rise in global temperatures will increase drought severity in the ecosystem (Vicente-Serrano et al., 2014). Drought is one kind of disaster that could substantially affect the vegetation in a particular habitat (Khosravi, 2017). Drought is often a result of a reduced amount of rainfall, increased temperature, and increased evaporation and transpiration (Shahabfar et al. 2012; Montandon, 2008). Drought can increase interspecific competition for water resources in the soil, which can result in constrained plant growth and seed germination (Craine and Dyzinski, 2013). The change in precipitation patterns may also pose indirect negative effects on seed banks. According to Basto et al. (2018), drought has significantly reduced the seedbank composition of the calcareous grassland by 37% after 14

years in Harpur Hill, Europe. Seed banks represent a biodiversity reservoir for ecosystems in a particular habitat (Vandvik, 2016), where they play a crucial role for maintaining genetic and species diversity (Bradbury et al., 2016; Kalamees & Zobel, 2002).

2.1.4. Environmental threats for grasslands

2.1.4.1. Conservation status of grasslands in South Africa

According to SANBI (2013), Driver et al. (2012), and Neke and du Plessis (2004), 40% of the grassland biome in South Africa has been irrevocably modified, while 60% of remaining grassland areas are threatened and losing important aspects of their composition, structure, and function. Anthropoid activities such as crop production, overgrazing, and residential and industrial development, as well as invasion by alien plant species are threatening the grassland biome in South Africa (Brand, 2011). Matsika (2008) assessed the land cover occupied by threats on the South Africa grassland biome using the National Land Cover (NLC) database for 1994 and 2000. The findings demonstrated that 55.7% of the grassland biome remained as natural grassland patches, with an increase of 2.3% over the grassland extent of 1994 NLC. The land cover loss for grasslands within a period of six years (1994 to 2000) was 25.2% due to land uses such as agriculture (19.6%), forest plantation (3.2%), mines and quarries (3.2%), and urban/built up areas (2.1%). In addition, a quantification of the transformation threats of the South African grassland biome using the NLC 1994-1995 database showed that 53.5% of the grassland biome remained as semi-pristine grassland (Neke and du Plessis, 2004). Moreover, only 1.6% is under formal protection while 44.7% is a transformed grassland area including coverage of waterbodies and wetlands, and 29.2 % has been transformed by agricultural activities (23%), afforestation (3.3%), mining (1.9%), and an urban centre (0.3%).

Bredenkamp et al. (2006) assessed the conservation value of Egoli Granite Grassland (Gm 10) in Gauteng, South Africa. Human impact (i.e., from high demand of developable land for residential, industrial. and commercial purposes in the Gauteng Province) on the Egoli Granite Grassland has resulted in the alteration of species composition, loss of endemic species, and also a change from species-rich grassland with high conservation value (dominated by *Loudetia simplex*) to a species–poor grassland with low conservation value (dominated by *Hyparrhenia hirta*). Furthermore, Egoli Granite Grassland is endemic to Gauteng Province but only 22% of its original state remained natural, while 61% has been permanently transformed by urban development (27%), smallholdings (17%), agriculture (12%), and exotic plantations, mining, and pastures (5%). The majority (97%) of the grassland landscape and the biodiversity and ecosystem it supports are at critical risk as less than 3% is within formal protected areas (SANBI, 2013). In the formal protected areas, the inclusion of road networks may have added threats on biodiversity, especially during

construction and maintenance (Matsika, 2008). Therefore, for effective habitat conservation, it is critical to understand the vegetation cover dynamics and its potential drivers, such as climatic factors (e.g., temperature and precipitation) and habitat fragmentation through human activities (e.g., agricultural activities).

2.1.4.2. Impacts of grazing and fire on grasslands

Grazing and fire are the main ecological drivers of grassland shape and land cover change; grassland vegetation alteration may occur as a result of misuse of those two elements, with significant implications for grassland biodiversity (Koener and Collins, 2014; SANBI, 2013). A case study conducted by Little (2015) in Steenkampsberg, Mpumalanga, showed that the combination of heavy grazing and annual burning led to new distinct plant community dominated by specialist plant species indicative of disturbance. Veld fire should burn in alignment with ecological objectives; for example, the frequent hot fires used to prevent bush encroachment is very different from fires used to control moribund/fuel load material (Trollope, 1974).

Overgrazing, which results in the deterioration of plant communities, is based on the equilibrium system theory. Overgrazing occurs when plant species are exposed to intensive grazing by wildlife or livestock and the grazing exceeds the carrying capacity for a long period of time without veld restoration or enough time for recovery (Zhang and Li, 2008; Hui and Chen, 2006; Mysterud, 2006). Overgrazing may be caused by a failure to match carrying capacity with a stocking rate higher than recommended (Walsh et al., 2014). Overgrazing can damage the environment by altering the vegetation cover, composition, and function of the plant community, subsequently leading to soil erosion (Rowntree et al., 2004), land degradation, and loss of various plant species. Eckhart et al. (2000) noted in the Kruger National Park, for example, that an increase in woody plants density and cover on granite were the results of decreased competition from grasses due to overgrazing by wild herbivores, due to the population which was kept high through the provision of drinking water. Grazing may increase or decrease plants' richness depending on grazing intensity (Herrero-Jauregui, 2017; Rutherford and Powrie, 2012). Hoare (2002) indicated that species richness was reduced in communal pasture areas of the Transkei due to heavy livestock grazing pressure. Therefore, heavy grazing intensity in protected areas can lead to the exclusion of palatable species intolerant to grazing and transform to an increase in unpalatable grasses (Kikoto and Mligo, 2015).

Fire is important for maintaining and conserving the health natural ecological functioning of grassland ecosystems (SANBI, 2013). There is limited information on the fire management practices that are considered for meeting the ecological objectives of

biodiversity conservation, and the information that does exist usually focuses on plant communities (Laughlin al at., 2004)**.** Cohen (2018) indicated, for example, that *Hessea cinnamomea* (Cinnamon hessea) emerged for the first time in 32 years after an accidental fire that occurred in the southern Cape Peninsula in November 2017, when previously the endangered species *H. cinnamomea* was only found in the lowland area around Cape Town. To meet management goals, accurate knowledge of all species in a particular rangeland and their response to fire regime is needed for ecologically sustainable management (Clarke, 2008; Hutto et al., 2008).

Fire intensity is an important component of fire regime that affects a variety of parameters such as grass sward and shrub and tree vegetation in the grassland biome (Trollope and Tainton, 1986). Grassland is fire prone and fire dependent. Trollope and Tainton (1986) evaluated the fire intensity on the recovery of grass sward and bush in the Eastern Cape thornveld, South Africa. After burning, the results showed that although fire intensity had no significant effect on the grass cover recovery, it had a significant positive effect on the woody plants shorter than two metres in height (Trollope and Tainton, 1986). In addition, trees must reach about four metres to avoid their stem and crown being destroyed by grass-fuelled fire; a long rest in rangelands without fire activities is necessary to allow trees to build sufficiently tall stems and grow well above flame zone (Welz, 2013). The incorrect application of fire in the grassland (vegetation, weather, and terrain) can result in the shift of species composition from grass-dominant to bush-encroachment with a decline of basal cover (SANBI, 2013; Driscoll, 2010). Fire in the grassland can also spread alien invasive species (SANBI, 2013; Wolfson, 2003), as plants are unable to adapt to subsequent fire conditions, hence causing species decline (Stephens et al., 2018; Cochrane, 2001).

2.1.4.3. Impact of habitat fragmentation on grasslands

The increase in fragmentation in grassland ecosystems has disturbed ecological processes that maintain ecosystem health (Haddad, 2015; SANBI, 2013, Schleuning, 2011, Rosenberg, 1997). The majority of lands are isolated and fragmented in small habitats, which disrupts ecological processes such as pollination, dispersal, and grazing (de Frutos, 2015; SANBI, 2013). The FSNBG is located within the Dry Highveld Grassland bioregion of the grassland biome. According to Carbutt et al. (2011), the Dry Highveld Grassland bioregion is poorly protected as 1.52% is under formal protection and 31.51% is transformed. Dry Highveld Grassland is threatened by various activities that include overgrazing, cultivation, mining, acid mine drainage, quarrying, and urban sprawl (SANBI, 2013). One of the vegetation types under the Dry Highveld Grassland bioregion preserved by the FSNBG is the Bloemfontein Dry Grassland (Gh5). According to the Government Gazette 34809 (9

December 2011) and Mucina and Rutherford (2006), the original area of the Dry Bloemfontein Grassland is 492 000 ha but more than 40% has been transformed for crop production and urban development, while less than 1% is within a formally protected area.

A habitat corridor plays a critical role in maintaining ecosystem health as animals and plants benefit from numerous ecological processes such as species migration, the recycling of nutrients, and animal-plant interactions (e.g., seed dispersal and pollination of plants) (Baum et al., 2004). Habitat corridors expand species movement and stimulate genetic diversity mostly after there has been population decline from a threat's events (e.g., disease and fires) (Christie and Knowles, 2015). Kormann et al. (2016) observed that corridors boosted forest-associated pollinator availability between neighbouring forest fragments by 14.3 times compared with unconnected equivalents, thereby increasing overall population success. However, the disruption of habitat corridors by human fragmentation (e.g., from agricultural activities) can lead to isolation of small populations and reduced genetic diversity in the habitat. Animal species can lose their natural area and ability to move between natural patches as their way of survival. Rovero and Jones (2012) assessed the two corridors (between the Udzungwa Mountains and the Selous Game Reserve) from 2007 to 2010 in southern-central Tanzania using a combination of the African elephant dung survey, habitat mapping, and questionnaires. Their study found that within five years of the study, the corridor became blocked or closed and reduced African elephant movement; these corridor closures were the result of an increase in farming activities and livestock that was associated with both population growth and local immigration. Therefore, it is important that habitat corridors are maintained and improved as a biodiversity network across all natural areas, either public or private.

2.1.4.4. Other environmental threats to grassland habitat

2.1.4.4.1. Background

Poor management practices in protected areas (e.g., grassland biome) can result in land degradation. These factors are among the major threats that are currently affecting vegetation structure within grassland vegetation (SANBI, 2013). Urbanisation and industry expansion around protected areas are transforming large areas in the grassland, increasing the impact of pollution, and limiting corridors between protected areas, which disrupts ecological processes such as plant pollination, species dispersal, and fire and grazing regimes (de Frutos, 2015; SANBI, 2013).

2.1.4.4.2. Biological invasions

Biological invasions are a result of species being intentionally or accidentally introduced outside of their native regions and successfully spreading and colonising a new

geographical region (Valery et al., 2008). Biological invasions disrupt the ecosystem structure (e.g., disappearance of native species) and ecological processes such as animal-plant interactions (Valery et al., 2008). The introduction of non-native species also impairs the native plant communities within a specific habitat (Daehler, 2003; Foxcroft, 2001) because they out-compete native species for environmental resources such as the physical space, water, light, and nutrients (Leger and Espenland, 2018). Presence of the alien and invasive species can also alter the vegetation cover of native plant communities (Bartz and Kowarik, 2019; Gallien and Carboni, 2016), which increases the risk of a decline in the native species (Singer et al., 2016). Yapi et al. (2018) studied the impact of alien invasive *Acacia* species in the South African Montane Grassland ecosystem and found that the grazing capacity of the area was reduced by 72% where the density of alien invasive plants species (*Acacia mearnsii*) had increased, and that clearing invaded areas improved grazing capacity by 66% only after five years.

Alien and invasive species (non-native species) are recognised globally as the second major threat to native biodiversity (Richardson and van Wilgen, 2004). The non-native species disrupts relationships among native species, and the colonisation, establishment, spread, and impact of biological invasions are promoted by the native species through a variety of mechanisms in the habitat (e.g., mutualism: fruits nutrients and seed dispersal) (Traveset and Richardson, 2014; Mokotjomela et al 2013; Mokotjomela et al., 2012; Stout and Morales, 2009). Gibson et al. (2012) compared the pollination rate of bees and other insects between invasive plant species *Acacia saligna* and co-flowering native plant species across four study sites of South African's Cape Floristic Region. The visitation of bees (honeybee) and other insects to native plant species *Roepera fulva* were significantly low in all study sites when *A. siligna* was present. In addition, Mokotjomela et al. (2012) compared the daily foraging activities of four avian frugivores (*Columba arquatrix, Cplius striatus, Pycnonotus capensis and Zosterops pallidus subsp capensis*) among two native plant species (*Olea europaea subsp. africana* and *Chrysanthemoides monilifera*) and two alien shrub species (*Solanum mauritianum* and *Lantana camara*). The highest daily visitation frequencies and greater consumed quantities were displayed by four avian frugivores on the fruit of the alien shrub *S. mauritianum*, as the fruits had more nutrients than other plant species. Nevertheless, frugivores (e.g., birds, insects, and animals) concentrate on their daily feeding requirement (i.e., nutrients) from a fruit resource regardless of whether the fruit is from a non-native plant species or native plant species (Mokotjomela et al., 2013).

Biological invasion poses huge threats to native vegetation (Ortega-Flores et al., 2018; Richardson and van Wilgen, 2004). Non-native species appear to have a specific trail of fast growth, rapid reproduction, and aggressive spread, which allows them to out-compete the native species in the habitat (Wong et al., 2017; Lowe et al., 2012; Marler et al., 1999). In

addition, the presence of alien invasive species can alter the vegetation cover of native plant communities (Bartz and Kowarik, 2019; Gallien and Carboni, 2016), which increases the risk of a decline in native species (Singer et al., 2016). However, the movement of alien invasive plants' fleshy fruits/seeds are linked to the role of native birds as seed dispersers (Dlamini et al., 2018; Ortega-Flores et al., 2018; Mokotjomela et al., 2013; Jordaan et al., 2011; Gosper et al., 2005). For avian frugivores' diet, alien invasive fleshy fruits are regarded as an essential fruit source of supplement (Mokotjomela, 2012; Jordaan et al., 2011; Gosper and Vivian-Smith, 2010; Mokotjomela et al., 2009). Mokotjomela et al. (2013) compared the frugivorous bird's daily visitation frequency of two plants species per category of alien shrubs, native shrubs, and emerging alien shrubs species within nine study sites of the Cape Floristic Region. The findings showed the significantly higher visitation frequency indices on the two emerging alien shrubs species by a variety of birds of different sizes (Mokotjomela et al., 2013). Nevertheless, the decline of service providers (avian population: seed dispersal) in the habitat can have ecological impacts. Egerer et al. (2018) evaluated the decline of the socially valued plant species *Capsicum frutescens* in the Mariana Islands, Micronesia. The ecosystem service loss was caused by the extinction of avian frugivores, which subsequently disrupted plant-animal interaction (mutualism) due to an alien snake *Boiga irregularis* that fed on the native frugivorous forest birds (seed disperser).

2.1.4.4.3. Diseases – Plant diseases

Plants are primary producers and an indicator of habitat change in the aspect of environmental change particularly with the impact of plant pathogens and pests in the ecosystem (Cornelissen, 2011). Plant pathogens and pests are a potential burden to either indigenous or exotic plants, as they disrupt many ecological and evolutionary processes in the natural ecosystem. Diseases can pose a threat to genetic diversity of the host plant population. Wagner and Todd (2015) indicated that the emerald ash borer (*Agrilus planipennis*) was responsible for the death of more than 200 000 million ash trees in North America since its accidental introduction in 2002 due to movement of infested firewood, infested nursey stock, and infested ash timber. A guide or knowledge on plant pathogen threats is an important component to minimise the risk of contamination and the spread of pests, diseases, and parasites.

The introduction of new pests and diseases are increasing worldwide (Freer-Smith, 2015), and one of the main spreaders is the growth of trade and human-mediated movement due to huge consignments of plants, timber, and wood products that takes place around the world daily (Potter and Urquhart, 2017). New pests and disease outbreaks may be unknown to science for years, which may pose a significant threat to native plant species (Potter and Urquhart, 2017). In the current century, climate change is likely to expand and alter patterns

of disturbance of potential plant diseases and inflict particular stresses to host plants (Masters and Norgrove, 2010; Tubby and Webber, 2010). Evans et al. (2014) indicated that the change of temperature and more frequent storms, droughts, and fires are likely to provide more opportunities for both alien and native pests to flourish.

The new diseases on plants can lead to the extinction of the host plant population if disease transformation is frequency-dependent (McCallum, 2012). It is therefore crucial to analyse every emerging disease in the protected area before it can become a major threat to native plant species. Paap et al. (2018) conducted routine surveys of tree health in South African botanical gardens. An association of an alien invasive forest pest, ambrosia beetle (Coleoptera: Curculeonidae: Scolytinaea known as Polyphagous Shot Hole Borer (PSHB), and fungus (*Fusarium euwallaceae*), was detected damaging the London plane (*Platanus x acerifolia*) as a host plant in the KwaZulu-Natal National Botanical Garden (KZN NBG). This was the first report of the PSHB in South Africa. Although PSHB is a serious threat to native plant species populations, until now there has been no eradication control successful in controlling PSHB. The relationship between biodiversity (i.e., plants as the indicator of vegetation cover change) and disease risk trends should be a priority for managing plant disease/pest in the protected areas and the establishment of conservation guidelines to protect plants (i.e., endangered plant species).

2.1.4.4.4. Soil erosion

Soil erosion as the removal/loss of topsoil (Chalise et al., 2019; le Roux et al., 2007) can either happen in the process of water or wind detaching and transporting soil materials (Holz et al., 2015; le Roux et al., 2008). Erosion is a major contributor to soil degradation and is one of the most critical environmental issues in South Africa (Gomiero, 2016; Le Roux et al., 2008; Hoffman and Todd, 2000). Le Roux et al. (2008) predicted soil erosion coverage threats on a national scale in South Africa using the regression equations between vegetation cover and MODIS-derived spectral index. The results were rated from high to low per eroded area, with approximately 61 million ha of land having moderate to severe potential risk, and more than 91 million ha of land having very low to low actual risk. However, highly eroded areas with potential huge threats to environmental soil degradation were classified to be approximately 26 million ha. The South African soil loss rate is higher due to extensive large-scale agricultural activities (e.g., cultivation and overgrazing) (Le Roux, 2011; Le Roux et al., 2007). The study conducted by Le Roux (2011) used satellite remote sensing SPOT 5 satellite imagery to detect eroded areas on a national scale in South Africa. Their study found that the Free State Province was the fourth province in South Africa affected by gully erosion, which occupied 64 674 ha (Le Roux, 2011). Furthermore, Mararakanye and Le Roux (2011)

specified that out of 664 107 ha occupied by Mangaung Metropolitan Municipality, 4 513 ha (0.68%) of land is affected by gully erosion.

Although soil erosion is a natural process, it is influenced by environmental features such as climate (e.g., drought and floods), terrain, soil, and vegetation characteristics (Mahasa, 2015). Furthermore, soil erosion is influenced by human activities during interaction with natural resources, such as clearing of permanent vegetation as a result of development (e.g., road contraction), and overgrazing in the protected areas, which all accelerate soil erosion processes (Zhao and Hou, 2019). Additionally, the Free State Province is a water scare province and often hit by prolonged droughts and high temperatures, which results in an increase of grazing intensity (Botai et al., 2016) and subsequent soil erosion events (Duniway et al., 2019). Erosion events associated with low levels of soil moisture from prolonged drought events release small organic particles through the process of wind erosion (Duniway et al., 2019). In addition, the habitat fragmentation from extensive clearing of permanent vegetation cover reduces windbreaks and can result in erosion from high winds (Leenders, 2006). Therefore, the loss of soil from land by erosion adversely impacts the production of rangeland ecosystems by decreasing rainfall infiltration and water storage capacity (Zuazo and Pleguezuelo, 2008). In this sense, protection of vegetation cover is essential to reduce exposing land and minimise soil erosion, as plants slow down runoff and allow more water to soak into the ground.

2.1.4.4.5. Bush encroachment

The grassland biome is characterised by the dominance of one contrasting plant life form, namely grasses with a sparse shrub and tree vegetation (Mucina and Rutherford, 2006). The herbaceous layer cover (grasses and forbs) of the grassland biome is reported to decline as a result of being encroached by woody species (see Matsika, 2008; Neke and de Plessis, 2004; Archer et al., 2017; Yapi et al., 2018); this is commonly known as bush encroachment. Bush encroachment refers to the thick stands of impenetrable bushes/plants either native plants or invasive species that grows vigorously close to each other that are more than three times the mean crown diameter (Archer et al., 2017; Wiegand et al., 2006). It has been attributed to co-occurrences and interactions of direct (i.e., pastoralist) and indirect factors, such as climate change (i.e., intensity of precipitation events and severity of drought conditions) (van Rooyen, 2010; Botai et al., 2016); the rise of CO_2 concentrations in the atmosphere (Ward, 2010), which favours C_3 woody plant growth (Archer et al., 2017); fire regime (van Rooyen, 2010; Trollope, 1974; Hare et al., 2020; Gordijn, 2010); and grazing/browsing regime (Wiegand et al., 2006). For example, Little et al. (2015) shows that the combination of intensive burning and grazing resulted in the detrimental impact of plant

species diversity and altered vegetation structure by replacing palatable grass species with less palatable species in the Steenkampasberg Plateau, Mpumalanga.

The abovementioned factors that can drive bush encroachment might be insufficient to warrant the dominance of woody species in the grassland. However, woody species may negatively suppress herbaceous layers because of their interception of water availability, shading, and root competition (Scholes and Archer, 1997). Wiegand et al. (2005) assumed that herbaceous and woody plants possess different adaptations to exploit soil resources (e.g., moisture and nutrients) because of rooting-niche separation depth. Woody plants typically have access to deeper moisture and nutrient resources (Ward et al., 2013) since they have larger taproot mass in the subsoil layer than grass species (Sankaran, 2005). On the other hand, herbaceous plants are assumed to be more water-use efficient because they extract waste only from the topsoil (Wiegand et al., 2005). The removal of the herbaceous layer may decouple the typical relationship between grass and tree biomass cover, and this could have a negative exponential impact on grass production because of increment of tree cover (Scholes and Archer, 1997).

2.2. Biotic interactions: Plant-animal seed dispersal

2.2.1. Background

Biotic interaction is a network pattern of activities caused by different organisms' requirements for survival among species, which may either have a positive or negative effect on the survival of other species in the community (Staniczenko et al., 2017; Morales-Castolla et al., 2015). Animal-plant interaction can either involve frugivory, nectarivory, or insectivory (Peter et al., 2016). Biotic interaction models of plants and animals have been documented as a critical component of many ecological processes, with several of those related to feeding as a result of co-evolution (Deveau et al., 2018). The parameter involved in the study of animal-plant interaction has involved a number of feeding strategies by animals, and with plants concurrently utilised this relationship to benefit in different ways (e.g., pollination and seed dispersal) (Wielkopolan and Obrepalska-Steplowska, 2016). Human dominance followed by a high rate of habitat fragmentation together with climate change has altered and threatened the natural system of animal-plant interdependence (Howe, 2016; Nyhus, 2016; Amedie, 2013). Tewksbury et al.'s (2002) study focused on the effect of fragmented corridor landscapes on animal-plant interaction (pollination and seed dispersal) in the Savannah River site of the National Environmental Research Park, South Carolina. Their study showed the impact of fragmentation on the corridor areas that reduced animal and plant movements (Tewksbury et al., 2002). From Tewksbury et al.'s (2002) findings, Common Buckeye (*Junonia coenia*) was three to four times more likely to move from central patches to connected patches than unconnected patches, and pollination was significantly higher in

connected patches; there was a higher proportion of flower-produced fruits in connected patches than in the unconnected patches. The benefit and influence of corridors in the habitat (i.e., an increase of animals and plants movement through corridors) was observed to have positive impact on the plant population and community interactions.

2.2.2. Seed dispersal

Seed dispersal is the movement of seeds away from their parent plants through a variety of dispersal vectors, either by abiotic (e.g., wind and water flow) or living vectors (e.g., birds) (Jones et al., 2017; Thomason et al., 2011; Wang and Smith, 2002; Westsott and Graham, 2000). The role of the seed disperser in plant regeneration is crucial to maintain genetic structure of many native plant species (i.e., abundance, distribution, phenotypes, and genotype composition) (Kleyheeg, 2018; Egerer et al., 2018; Castilla et al., 2017; Howe, 2016; Heleno et al., 2011). Many native tree and shrub species require animals (avian, insect, etc.) for seed dispersal as a longevity strategy in response to environmental change (Howe, 2016). However, plants rarely interact with a single mutualism strategy but regularly network with a variety ecological processes (i.e., plant pollination and plant-seed dispersal) (Thomas et al., 2007; Sharon et al., 2004). The role of avian frugivores is essential in shaping seed dispersal patterns of native vegetation structures. For example, Banos-Villalba et al. (2017) studied the role of macaws as a primary seed disperser in shaping the vegetation structure and function of the Amazonian ecosystem, Beni Savannas. The average rate of seeds/fruits dispersed by macaws was 75 to 100%, with a distance of over 1 200 m range, and they also influenced the germination success of forest plant recruitment.

After primary seed dispersers (i.e., mammals) have played their role, secondary seed dispersal contributes to extending the distance from the first seed disperser location (Padilla et al. 2012). However, it is very rare for a single disperser to determine a seed's final destination (Hamalainen et al. 2017). Urrea-Galeano et al. (2019) assessed the effect of horizontal seed dispersal by dung beetles as a secondary disperser using pig dung (pig: primary disperser) and recorded their fate and spatial location over time in the tropical rainforest of Mexico. Dung beetle activity did not increase the germination rate of the seed but resulted in extending the seed's destination by seed aggregation after seeds were deposited by mammals in faecal clumps. In addition, Vander Wall et al.'s (2005) study assessed the two phases of seed dispersal between avian frugivores as primary seed dispersal and rodents as secondary seed dispersal in the Wittell Forest of Reno (University of Navada System). Rodents removed plant seeds from bird's faeces at a rate of 8-50% per day and scatter-hoarded them into the soil and some cache sites (Vander Wall et al., 2005). Consequently, some seeds germinated and established seedlings from rodents' caches in

the spring season. Therefore, primary and secondary phases of seed dispersal were very crucial as each phase offered different benefits to plants.

Frugivores play diverse roles in dispersing seed across their communities from different seed dispersal ranges (Rehm et al., 2019). However, habitat fragmentation can change the species' (e.g., birds) movement due to altered vegetation structure and may disrupt avian frugivorous seed dispersal and pollination (Jones et al., 2017). Neuschulz et al. (2016) reported human disturbance as a global threat that affects plant-animal interactions, particularly for pollinators and seed dispersers in the process of plant regeneration. The study showed a significant negative effect of human disturbance (land-use change, over-exploitation, and defaunation) that disrupts the mutualistic interactions between plants and their animal pollinators and seed dispersers; in addition, this threat was higher for forest plant regeneration in the disturbed forest than the undisturbed forest. Lehouch et al. (2009) examined how habitat fragmentation/disturbance affected the avian frugivorous community (seed disperser) and seed removal in the Kenyan Mountain cloud forest. This study showed how deforestation disrupted avian frugivores' visitation patterns and subsequently reduced seed removal on the parent plant (Lehouch et al., 2009).

Not all species positively influence seed dispersal effectiveness. Some bird species (granivores) are seed predators as they digest seed and appear not to play any part in either seed dispersal or germination (Heleno et al., 2011). Barnea et al. (1991) compared the effects of two frugivorous birds, bulbuls (*Pycnonotus xanthopygos*) and blackbirds (*Turdus merula*), by germinating a variety of seed from different plant species after seed treatment. The seeds ingested by blackbirds had a higher germination rate than seeds ingested by bulbuls; the blackbirds have a long seed retention time in the digestive system compared with the digestive system of bulbul birds, which might cause a stronger effect of seed coat abrasion and therefore enhance better germination. Soares et al. (2015) investigated the forest cover loss using the seed of *Euterpe edulis* as a keystone palm in the Brazilian Atlatic Forest, with nine samples composed of a vertebrate exclosure versus an open treatment located in the different percentage of forest cover. The forest loss cover was due to a high number of seed predation of vertebrate species, which was the main factor limiting seed recruitment in the forest areas compared to areas with less forest cover. However, seed predation can significantly enhance germination or reproduction of the potential plant (Ferreira et al., 2016). For example, Loayza et al. (2014) assessed if rodents are an effective seed disperser of the endangered shrub *Myrcianthes coquimbensis* in the southern edge of Atacama Desert in Chile. The results showed that rodents played a dual role in the seedling recruitment dynamic of *M. coquimbensis*, as a seed predator and an effective disperser (mutualism). Rodents feed on the seed storage tissue of *M. coquimbensis* and hoard the seeds in the ground, and more than 60% of discarded fragmented seed produced seedlings. Therefore, biotic interactions

through seed predation can either have a positive or negative impact on seed recruitment or germination of potential plants' fruits/seeds.

2.2.3. Impact of avian frugivores on seed germination

Avian frugivores modify the rate of germination and enhance the chance of seedling survival (Kleyheeg, 2018; Mokotjomela et al., 2016; Mokotjomela et al., 2015; Fricke et al., 2013; Mokotjomela, 2012; Barnea et al., 1991). However, there are several factors that can cause variation in the seed germination response, such as seed source, size, retention time before seed is released in the guts, and the condition where germination is tested (Traveset et al., 2001). Traveset et al. (2001) tested germination characteristics with the seeds from different plants (*Rhubus ulmifolius, Osyris alba, Rubia peregrine, Asparagus acutifolius*, and *Phillyrea* spp) that passed through birds' guts in the western Mediterranean shrubland. The overall results showed that different germination speed/rates were promoted by birds' gut treatment. The study also revealed that different seed germination responses relied on the avian frugivores' treatment. In contrast, Alves-Costa and Eterovick (2007) investigated the seed/fruits trail of 53 species that were consumed and dispersed by coatis (*Nasua nasua*) in the Mangabeiras Park, Southern Brazil. The results showed that seed passage through coatis' guts did not alter success of germination except for the *Myrcia guajavaefolia* species, where germination success increased by approximately 50% after treatment by coatis. Nevertheless, not all the seeds treated by avian frugivores (i.e., passage through birds' gut, ingested, and excreted/regurgitated) can successfully germinate. For example, Dlamini et al. (2018) compared the effect of frugivorous birds on seed dispersal and germination of two alien invasive plants (*Schinus terebinthifolius* and *Listea glutinosa*) in the Animal House Unit, University of KZN. The overall findings showed that frugivorous birds enhance the germination rate of *Schinus terebinthifolius* after seed treatment, although no positive effect was noted on the germination of *Listea glutinosa* after seed treatment.

Seeds handle competition mediated through interspecific seed density and species diversity in the microsite (Orrock and Christopher, 2010). When seeds of different plant species interact, their ability to affect and respond to competition determines the contribution of seed germination to an individual plant species' population (Goets et al., 2018; Aschehoug et al., 2016; Johnson et al., 2008; Schupp, 1993). To overcome competition, seeds of different species can sense each other prior to their emergence (Ward, 2016; Tielborger and Prasse, 2009). Consistently, seeds may also have to overcome a variety of environmental pressures against germination to attain their subsequent plant recruitment (Orrock and Christopher, 2010; Wang and Smith, 2002). Orrock and Christopher (2010) observed an accelerated germination response of *Phytolacca americana* when seed density was increased due to physiological enhancement from the seeds of other species. Previous

studies have shown that seeds' endocarp has some hormones that play a major role in maintaining seed dormancy and in protecting the embryo against pathogens by producing phenolic compounds (i.e., tannins) (see Raviv et al., 2017) that eventually suppress the germination of neighbouring seeds.

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Chapter 3: Vegetation cover change, potential drivers, and species composition of the Free State National Botanical Garden, South Africa

3.1. Abstract

The montane rangelands in South Africa are embedded within the Grassland Biome, which faces huge conservation threats, including biological invasions. Among other conservation strategies, the South Africa National Biodiversity Institute (SANBI) establishes conservation gardens in areas where either a unique vegetation occurs, or certain vegetation types are threatened with extinction. The Free State National Botanical Garden (FSNBG) preserves Bloemfontein Karroid Shrubland (BKS) vegetation, which falls under priority Critical Biodiversity Area 1 (CBA1). Despite multiple threats, there is limited knowledge about how the long-term environmental dynamics affect the vegetation cover and how the threats can be mitigated in order to optimise the targeted vegetation protection. Therefore the study aimed: 1) to determine the vegetation cover dynamics of the FSNBG in ten-year intervals over a 30-year period (1987-2017), focusing on different vegetation classes (i.e., sparse vegetation, dense vegetation, moderate vegetation, no vegetation, and open soil); 2) to evaluate CBA1's vegetation status by determining the presence or absence of the flagship plant species in CBA1; and 3) to determine potential biodiversity conservation threats that drive vegetation cover changes in the FSNBG. Remote sensing and geographical information system analyses using the earth observation system (EOS) land viewer were applied to detect spatial vegetation cover changes. Landsat series images (path 170/row 80) of the study area were acquired for the years 1987, 1997, 2007, and 2017, with the 1987 imagery used as a baseline. Major vegetation conservation threats were also documented. The overall results showed a significant increase in the vegetation cover in the study site over the 30-year period. From 1987 to 2017, moderate vegetation cover increased by 25.1 ha, while dense vegetation increased by 8.6 ha. Vegetation cover of the CBA1 vegetation type was 79.6 ± 15.9%. Major conservation threats were posed by 27 invasive alien plant species, dominated by 13 woody plants and eight succulent species interspersed within different vegetation patches, and 10 pests and pathogens. Human settlement encroachment has also substantially increased (i.e., covering ~18% of the buffer zone) in the past 18 years, which has reduced biodiversity corridors. The study concludes that there is a need for regular vegetation monitoring and intervention to manage the prevailing threats.

Keyword: Vegetation cover, Remote sensing, Climate change, Human-mediated threats, Grassland biome, Biodiversity Conservation.

3.2. Introduction

Plants are primary producers and indicators of habitat change as an aspect of climate change (Cornelissen, 2011). Climate change has become a major threat to biodiversity in the current century in other parts of world, because the distribution of many biological species has shifted gradually (Thuiller, 2005). For example, Niang et al. (2014) assert that there is emerging evidence that the shifting of the home range of plant species in Africa may have resulted from microclimate change. The capacity of plants to alter their geographical distribution in response to climate change seems to be constrained by the difficulty the species have in locating a new home range with the requirements for their survival (Rutherford, 2000).

Modifying the environment to fit the needs of society has caused severe effects on the integrity of the natural ecosystems in Africa (Niang et al., 2014; Gonzalez et al., 2012). Niang et al. (2014) indicate that changes in species distribution in Africa occur in all type of biomes – including grasslands, savanna, deserts, shrub lands, forests, and woodlands – as a result of the increase in anthropogenic habitat fragmentation. As for the remaining natural vegetation types of Africa, Gonzalez et al. (2012) consistently observed a decrease in woody vegetation in western Africa, while Mitchard and Flintrop (2013) observed an increase in woody vegetation in central, eastern, and southern Africa. This implies that significant environmental changes force vegetation structures to change in different areas over time.

Conversely, 40% of the grassland biome in South Africa has been irrevocably modified, while 60% of the remaining grassland areas are threatened, as they are losing important aspects of their composition, structure, and function (SANBI, 2013; Driver et al., 2004; Neke and Du Plessis, 2004). Anthropoid activities such as crop production, overgrazing, residential and industrial development, and invasion by alien plant species are threats to the grassland biome in South Africa (Brand, 2011). Matsika (2008) assessed the land cover occupied by threats to the South African Grassland Biome using the national land-cover (NLC) database (NLC, 1994, 2000), with the findings demonstrating that 55.7% of the Grassland Biome remained as natural grassland patches, with an increase of 2.3% in the grassland's extent (NLC, 1994). The grassland land cover loss over a period of six years (1994 to 2000) was 25.2% owing to forms of land use: 19.6% for agriculture, 3.2% for forest plantation, 0.2% for mines and quarries, and 2.1% for urban/built up areas (Matsika, 2008).

The shift in precipitation and temperature patterns may constitute a major threat to biodiversity because the amount and timing of both phenomena influence biological processes in grasslands (Settele et al., 2014). In South Africa, Mackellar et al. (2014) observed a downward trend in seasonal and annual total rainfall for the period 1960 to 2010; the decrease in total rainfall and the number of rainy days in the autumn season were evident over the central and north-eastern part of South Africa, while a significant increase in the

number of rainy days in the southern Drakensberg in the spring and summer seasons was observed. A rapid increase in temperature over the present century and the next is likely to lead to a major change in the vegetation cover (a shift in plant distribution). According to Niang et al. (2014), Africa's mean annual temperature is likely to increase in the late 20th century by between 3°C and 6°C. This will also affect many animal species, as their ecology is ultimately linked not only to the climate but also to habitat availability.

Drought is one kind of disaster that can substantially affect the vegetation in a habitat (Khosravi, 2017). Vegetation cover undergoes severe stress during drought conditions, and vegetation function, composition, and cover can be damaged if affected areas are not identified in time. Drought is often the result of a reduced amount of rainfall, increased temperature, and increased evaporation and transpiration (Shahabfar et al., 2012; Montandon, 2008). Drought can increase interspecific competition for water resources in the soil, which can result in constrained plant growth and seed germination (Craine and Dyzinski, 2013). The change in precipitation patterns may also have indirect negative effects on seed banks. According to Basto et al. (2018), drought resulted in a significant reduction (37%) of the seed bank composition of the calcareous grassland in Harpur Hill, Europe after 14 years. Seed banks are biodiversity reservoirs for ecosystems in particular habitats (Vandvik, 2016), where they play a crucial role in maintaining genetic and species diversity (Bradbury et al., 2016; Kalamees & Zobel, 2002).

The increased fragmentation of grassland ecosystems has disturbed the ecological processes that maintain the ecosystems' health (Haddad, 2015; SANBI, 2013; Schleuning, 2011; Rosenberg, 1997). Most lands are isolated and fragmented into small habitats, disrupting ecological processes such as pollination, dispersal, and grazing (De Frutos, 2015; SANBI, 2013). The Free State National Botanical Garden is located within the Dry Highveld Grassland bioregion of the Grassland Biome. According to Carbutt et al. (2011), the Dry Highveld Grassland bioregion is poorly protected, with 1.52% under formal protection and 31.51% already transformed. Dry Highveld Grassland is threatened by various activities, including overgrazing, cultivation, mining, acid mine drainage, quarrying, and urban sprawl (SANBI, 2013). One of the vegetation types in the Dry Highveld Grassland bioregion that is preserved by the FSNBG is the Bloemfontein Dry Grassland (Gh5). According to Government Gazette 34809 (9 December 2011) and Mucina and Rutherford (2006), the original area of the Dry Bloemfontein Grassland was 492 000 ha; but more than 40% has been transformed for crop production and urban development, while less than 1% is a formally protected area.

A habitat corridor plays a critical role in maintaining ecosystem health, as animals and plants benefit from numerous ecological processes, such as species migration, recycling of nutrients, and animal-plant interactions (e.g., seed dispersal and pollination of plants) (Baum et al., 2004). Habitat corridors expand species' movement and stimulate genetic diversity,

mostly after a population decline following threat events such as disease and fires (Christie and Knowles, 2015). Kormann et al. (2016) observed that corridors boosted forest-associated pollinator availability between neighbouring forest fragments by 14.3 times compared with unconnected equivalents, and increased overall population success. However, the disruption of habitat corridors by human fragmentation (such as agricultural activity) can lead to the isolation of small populations and reduced genetic diversity in the habitat. Animal species can lose their natural area and the ability to move between natural patches as a way of survival. From 2007 to 2010, Rovero and Jones (2012) assessed two corridors between the Udzungwa Mountains and the Selous Game Reserve in the southern-central area of Tanzania, using a combination of the African elephant dung survey, habitat mapping, and questionnaires, and found that, within five years of the study, the corridor route had become blocked or closed; and reduced African elephant movement resulted from an increase in farming activities and livestock, which were associated with both population growth and local immigration. Therefore, it is important that habitat corridors are maintained and improved as a biodiversity network across natural areas, whether public or private.

Biological invasion poses huge threats to native vegetation (Ortega-Flores et al., 2018; Richardson and Van Wilgen, 2004). The non-native species appear to have a specific trail of fast growth, rapid reproduction, and aggressive spread, which allows them to out-compete the native species in the habitat (Wong et al., 2017; Lowe et al., 2012; Marler et al., 1999). Alien invasive species' presence can also alter the vegetation cover of native plant communities (Bartz and Kowarik, 2019; Gallien and Carboni, 2016), which increases the risk of a decline in the native species (Singer et al., 2016). Yapi et al. (2018) studied the impact of alien invasive Acacia species in the South African Montane Grassland ecosystem, and found that the grazing capacity of the area was reduced by 72% where the density of alien invasive plant species (*Acacia mearnsii*) had increased, and that clearing invaded areas improved grazing capacity by 66% after only five years. The movement of alien invasive plants' fleshy fruits/seeds is linked to the role of native birds as seed dispersers (Dlamini et al., 2018; Ortega-Flores et al., 2018; Mokotjomela et al., 2013; Jordaan et al., 2011; Gosper et al., 2005). For an avian frugivorous diet, alien invasive fleshy fruits are regarded as an essential fruit source (Mokotjomela, 2012; Jordaan et al., 2011; Gosper and Vivian-Smith, 2010; Mokotjomela et al., 2009). Mokotjomela et al. (2013) compared the frugivorous birds' daily visitation frequency of two plant species per category of alien shrub, native shrub, and emerging alien shrub species in nine study sites of the Cape Floristic Region. The findings showed significantly higher visitation frequency indices on the two emerging alien shrub species by a variety of birds of different sizes (Mokotjomela et al., 2013).

The FSNBG conserves critical grassland biodiversity. The garden conserves the Bloemfontein Karroid Shrubland vegetation, which falls under Critical Biodiversity Area 1

(CBA1), and the Bloemfontein Dry Grassland (threatened), which falls under ecological support areas 1 (ESA1; see the layout of the different ecological units in FSNBG in Appendix 3.1), and which are priorities for biodiversity conservation in South Africa (Mucina and Rutherford, 2006; Skowno et al., 2019). Critical biodiversity Area 1 (CBA1) contains biodiversity that is irreplaceable or near irreplaceable for meeting conservation targets, while ESA1 patches are those areas in either a good or a fair ecological condition with minimal degradation (Mucina and Rutherford, 2006; SANBI, 2017; Skowno et al., 2019). It has also been demonstrated that ESAs provide important support in the ecological functioning of CBAs (Skowno et al., 2019; SANBI, 2017). This therefore suggests that an effective conservation strategy in the FSNBG must consider various neighbouring vegetation patches. Consequently, it is critical to understand the vegetation dynamics in the FSNBG and to assess whether the vegetation units are affected by reported threats to the broader Grassland Biome, such as fire, fragmentation, and alien and invasive species (Matsika, 2008; Neke and Du Plessis, 2004). This knowledge is critical to the development and revision of the adaptive vegetation management plans of the FSNBG. In view of the above facts, the aims of the study were: 1) to use remote sensing technology and geographic information systems to determine the vegetation cover dynamics over a 30-year period (1987-2017) in the FSNBG. It was predicted that plants, as key producers and drivers of habitat change, might respond to global change threats such as extreme droughts (Del Vecchio et al., 2015, 2016; Smart, 2015); 2) to evaluate the CBA1's vegetation status by determining the flagship plant species in CBA1; and 3) to document the ecological threats that influence vegetation cover change.

3.3. Methods and material

3.3.1. Study site

The study was conducted in the Free State National Botanical Garden (FSNBG) (S 29° 12′ 55.6″; E 26° 12′ 41,3″), Free State Province, South Africa. The FSNBG occurs in the Grassland Biome under the Dry Highveld bioregion (Mucina and Rutherford, 2006). The Garden covers an area of 66 hectares, and is at an altitude between 1300 and 1400m above sea level (Masilo, 1999; Chaplin, 1979). The vegetation structure is largely characterised by Gh 8 Bloemfontein Karroid Shrubland, Gh 5 Bloemfontein Dry Grassland, Gh 7 Winburg Grassy Shrubland, and Waterbody/Riparian vegetation (Mucina and Rutherford, 2006). The botanical garden experiences an extremely variable continental climate, with minimum winter temperatures below 0˚C, the night temperature often falling below freezing, and frost in July. Fairly high summer temperatures frequently range between 30˚C and 35˚C in January (Haddad et al., 2019; Neethling and Haddad, 2013). The mean annual rainfall is relatively low (584 mm y−1), with most rainfall being recorded during the summer months and in early autumn.

3.3.2. Vegetation spatial data collection and image processing

To understand the extent of vegetation cover change over 30 years, remote sensing (RS) and geographical information system (GIS) imagery were used to analyse the spatial changes that occurred over 30 years (1987-2017) in 10-year intervals. The garden is dominated by shrub and grass species, with a small portion of dense woodland. According to Hill et al. (2005), the natural expression of the growth, mortality, and regeneration of trees and shrubs is measurable over a period of 10 years or more for significant trend detection, while that for grass species is measurable over a period of five years. High-resolution imagery was generated for the study area (the FSNBG) as an area of interest (AOI), and imported from a Google Earth shapefile in KML format (Figure 3.1A) (Yukhnovskyi and Zibtseva, 2020). The EOS land viewer satellite images had already undergone pre-processing to overcome image error and remove distortions (EOS, 2020). The scene search tool was used to select various satellite images of the AOI (Figure 3.1A). Satellite imagery – from Thematic Mapper^{TM} and the Operational Land Imager and Thermal Infrared Sensor (OLI/TIRS) – of the study area (path 171/row 80) was acquired for the years of interest: 1987, 1997, 2007 and 2017, with the 1987 image being a baseline (Table 3.1; Figure 3.1A) (Yukhnovskyi and Zibtseva, 2020; Peter et al., 2018). Extra care was taken to select images in the same season/month in order to increase the accuracy of the research results and the vegetation reflection data (Table 3.1). The cloudiness was set to 10%, with a sun elevation of more than 30° (Figure 3.1A).

Table 3.1: Characteristics of satellite images

Satellite	Acquisition date and path/row	Cloudiness (%)	Sun elevation (◦)	Spectral resolution (µm): band combination NDVI
Landsat 5^{TM}	14 Feb 1987 (171/080)	10	46	Band 3 & Band 4
Landsat 5^{TM}	30 April 1997 (171/080)	0	32	Band 3 & Band 4
Landsat 5^{TM}	10 April 2007(171/080)	0	41	Band 3 & Band 4
Landsat 8 (OLI + TIRS)	21 April 2017 (171/080)	0	39	Band 5 & Band 4

Because the study was focused on the vegetation cover dynamics, I selected the band combination of channels that is used to obtain coverage of the vegetation indices' characteristics (Figure 3.1B). The normalised difference vegetation index (NDVI) imagery set was used to classify the Landsat satellite pattern for the 30-year period (1987–2017), and the change in the vegetation cover distribution of the study area was determined (Figure 3.1C). The NDVI remote sensing method displays the healthiness and greenness (relative biomass) of the vegetation (Prabhakara et al., 2015). The NDVI measures the state of the plant health based on the plants' reflection of light at certain frequencies (EOS, 2020). This index takes

advantage of the contrast in the characteristics of two bands from a multispectral raster dataset, the chlorophyll pigment absorptions in the red band, and the high reflectivity of plant materials in the near-infrared (NIR) band (EOS, 2020). For example, the Landsat^{TM} sensor reflects both the near-infrared spectrum in band 4 and visible red (RED) in band 3 (Table 3.1). The vegetation cover was compared with the reflection in the NIR spectrum and the visible red range of the spectrum (RED) with the formula:

$$NDVI = \frac{NIR - RED}{NIR + RED}$$

The processing of NDVI indices was carried out by an automated software tool type of coverage, with the transfer of the indicators and the subsequent analysis and interpretation of the received data. The types of vegetation (i.e., dense woodland, grass, and shrubs), the presence of open ground, and the absence of vegetation were generated in percentages or absolute units of area (m^2 or ha) (Figure 3.1C). The NDVI EOS land viewer software provides the automatic distribution of the vegetation cover (Figure 3.1C). Both supervised and unsupervised classification methods were used to classify the images in this study. The NDVI value ranges between -1.0 and +1.0, and reflects the healthiness of plants (greens). A low NDVI value of 0.1 and below corresponds with rocky and sandy areas or water (an NDVI below zero means no vegetation). A value from 0.1 to 0.2 represents open soil; sparse vegetation (shrubs and grassland) values are from 0.2 to 0.4; moderate vegetation values are from 0.4 to 0.6; and a large value from 0.6 to 1.0 indicates dense vegetation (forest) (EOS, 2020).

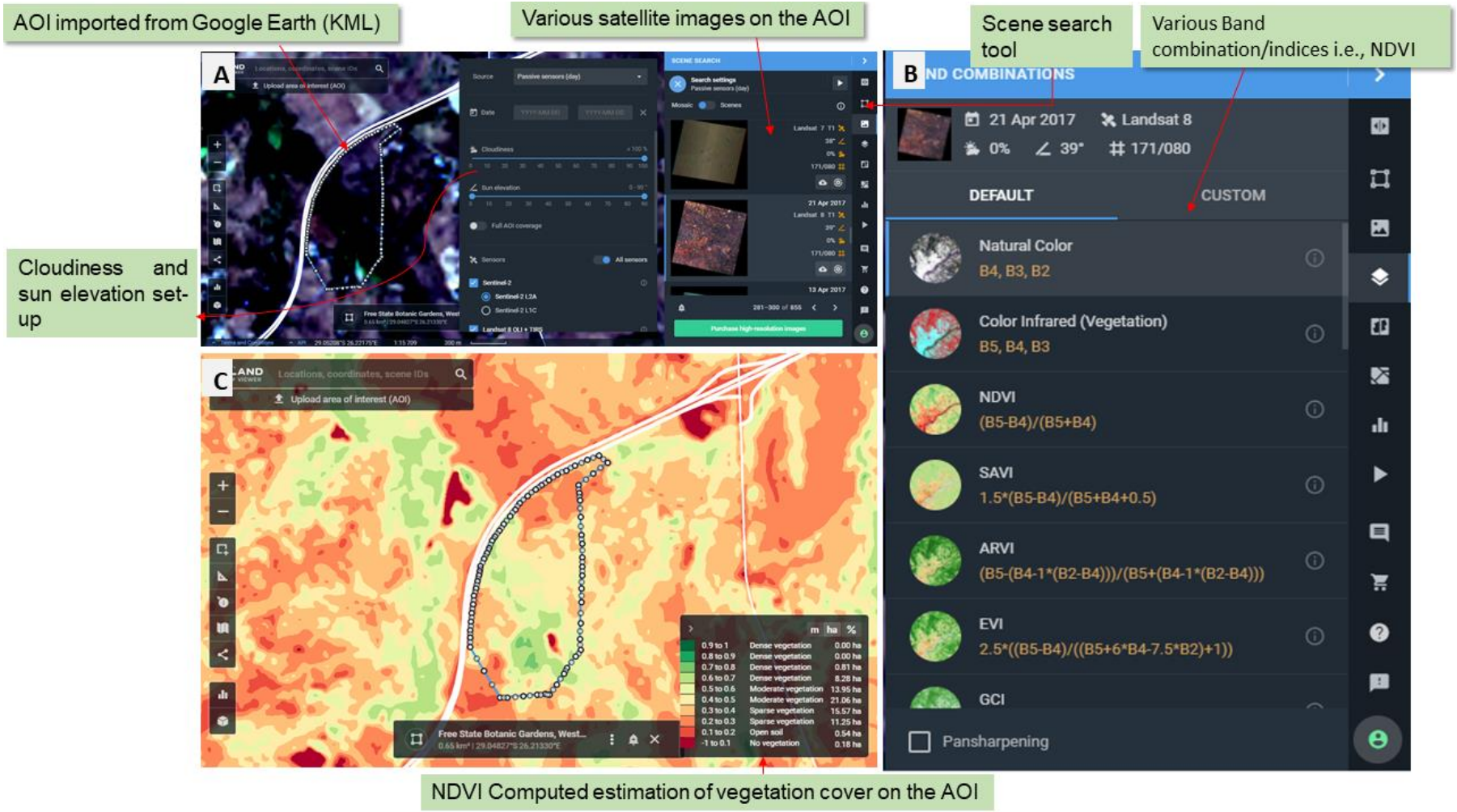

Figure 3.1: Overall step-by-step layout of the process of vegetation cover data analysis: A) a process for sourcing satellite images; B) the selection of a combination of channels; and C) the distribution of the vegetation cover in the study area according to the NDVI index analysis.

3.3.3. Ground-truthing the vegetation types

In the study, the vegetation units within the FSNBG were validated using descriptive methods. To evaluate the status of the vegetation type described in Mucina and Rutherford (2006), I used field surveys that entailed the systematic quadrat method to sample the vegetation species and number species (Hill et al., 2005; Mokotjomela et al., 2009). The survey was conducted during the active growing season in December, 2020.

The FSNBG conserves the Bloemfontein Karroid Shrubland, which falls under CBA1 as a national priority for biodiversity conservation (SANBI, 2017). A quadrat method was used to assess whether the typical plant species still exist within the CBA1 vegetation patch (Mokotjomela et al., 2009; Hill et al., 2005). A conventional frame quadrat (i.e., a mini-quadrat) of 1m^2 was employed to quantify the presence or absence of CBA1 vegetation in the set of systematic located samples. The quadrat method is recommended for a visual estimation of plant cover (the presence or absence of species) to describe vegetation (Hill et al., 2005). The over-shoulder-toss method was used to position the mini-quadrat within the patch boundary (Mokotjomela et al., 2009; Bullock, 1997; Hill et al., 2005). The high ratio edge of plant species intercepted by a grid line (i.e., in or out decision) was applied (Elzinga et al., 1998). Fifty-two mini-quadrats were sampled within the CBA1 vegetation type, in which I recorded the frequency and vegetation cover of all rooted plant species (i.e., herbaceous or woody layer).

Within the quadrat, the overall vegetation cover per quadrat was estimated (using a modified Braun-Blanquet scale; Mokotjomela et al., 2009; Hill et al., 2005). Plant species recorded in each quadrat sample were identified using existing knowledge, different plant field guides (e.g., Ulian et al., 2019; Van Wyk and Van Wyk, 2013; Fish et al., 2015; Joffe and Oberholzer, 2012) and a South African database (www.plantzafrica.com). When positive field identification was not possible, plant species were assigned a nickname (e.g., unknown1), and both physical samples (e.g., leaves, flowers) and digital records were collected for further identification. Some species were also collected for the South African National Biodiversity Institute (SANBI) herbarium located in Pretoria for further identification. The environmental parameters were recorded at each quadrat/plot (i.e., slope, geomorphological units, surface rock percentage, and geographic co-ordinates) using a digital handheld GPS (Garmin Montana® 600).

3.3.4. Habitat ecological threats

I quantified the local threats (i.e., biological invasions, human settlement encroachment, bush encroachment, and plant diseases) using a direct observation method and historical information based on the most frequent threats (as reported in the study of Schulze et al., 2017) that could alter the vegetation cover of the FSNBG. The threats were

compared with the most frequently reported threat data in southern Africa's protected areas (PAs), as reported by Schulze et al. (2017).

3.3.4.1. Alien and invasive plant species

A survey (i.e., a walk-through) was conducted that covered most of the gazetted areas in the Botanical Gardens. The field surveys were undertaken during the period of April 2018 to March 2019. The method was based on observable features – i.e., plant growth form, leaf shape (length and width), flower shape, and fruits (i.e., colour, coat, length, and width) – to identify the species (Ulian et al., 2019; Bromilow, 2018; Van Wyk and Van Wyk, 2013). For small plant features (i.e., fruits and inflorescence) I used the portable pocket 100x metal mini science microscope (Song et al., 2018). Different plant field guides (i.e., Van Wyk and Van Wyk, 2013; Bromilow, 2018) and a South African database (www.invasives.org.za) were used for plant species identification.

The digital handheld GPS (Garmin Montana® 600) was used to collect the individual species' locations. The GPS gives position coordinates with an accuracy of within 1 to 10 metres (Heselton, 1998). The data collected each day were stored on the GPS device in keyhole markup language (KML/KMZ) tracks before being imported to the Google Earth Pro app. KML/KMZ is a file format used to display geographical data in an earth browser (Marek et al., 2015). The collected GPS coordinates and attributes were imported to Google Earth Pro. The Google Earth plot was overlaid on the Google Earth interface. Different alien invasive plant species' density/abundance, habitat type, and area of infestation were also recorded and mapped using Google Earth Pro.

Invaded areas (i.e., both lightly and densely invaded areas) were mapped to estimate the aerial vegetation cover of alien and invasive species to guide managements' efforts at prioritisation.

3.3.4.2. Human settlement encroachment

To evaluate the extent of human settlement encroachment on the FSNBG buffer zone over 18 years (2002-2020), I generated a time series analysis of habitat cover for the years 2002, 2007, 2012, 2017, and 2020 (see Ridding et al., 2020). Google Earth Pro was used to estimate the human settlement aerial cover that occurred over 18 years at five-year intervals until 2020 (Figure 3.2). A standard buffer area of 1 km for biodiversity protected areas was applied around the perimeter (Margules and Pressey, 2000; Madsen et al., 2014). This buffer area is an area that should not be disturbed as a way of protecting a specific conservation area within a protected area (Ebregt and de Grave, 2000). I computed the total human settlement area as a proxy for habitat transformation by human impact (Chowdhury et al., 2018). Polygons were drawn around the anthropogenically altered landscape (i.e., built

infrastructure), and the area was measured in hectares and converted into proportions (percentages). Extra care was taken in measuring between points on the built-up perimeter areas in order to increase the accuracy of the results. Thereafter Google Earth automatically estimated the area size in hectares according to settings.

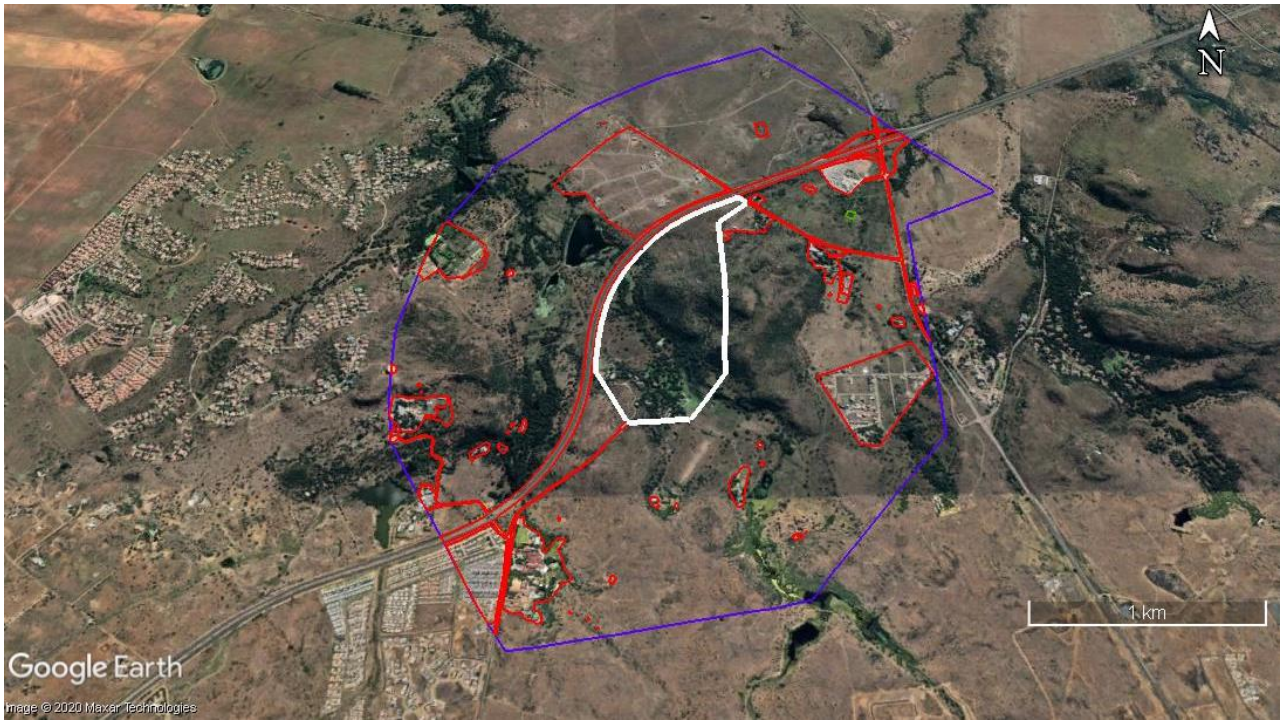

Figure 3.2: The human settlement encroachment around the FSNBG. The white line shows the FSNBG perimeter, the blue line indicates the Garden buffer area of 1 km width outside the FSNBG boundary, and the red lines show the human settlement cover in 2020 within the buffer zone *(Satellite imagery of 2020)*.

3.3.4.3. Monitoring plant diseases

In order to provide an early warning of the risk of emerging pests and pathogens, and to increase the chances of their eradication and control, the garden plant species were surveyed for the presence or absence of pests and diseases. To sample the pests and pathogens, a walk-through survey was conducted covering the gazetted forested areas in the FSNBG (Paap et al., 2018). The trees were inspected for signs of pest/disease attack, such as the polyphagous shot hole borer beetle (PSHB) (*Fusarium euwallacea*), which is emerging in South Africa (Figure 3.3) (Paap et al., 2018). The survey was conducted during the active growing season, which allows detectability (early summer to late summer: September 2019 to February 2020). De Villiers and Schoeman (1988) have shown that most pests and diseases are drastically reduced during winter compared with summer. For plant species suspected of

having positive signs of the occurrence of PSHB (i.e., suspected insect entry holes), a sterilised chisel was used to remove a sample of the bark for further identification and observation. A stainless-steel double-sided scale straight ruler was used to measure the suspected shallow entry hole associated with the emerging alien invasive species, polyphagous shot hole borer (*Fusarium euwallacea)*. Specimens of the plants (e.g., infected stems, leaf samples) were collected in plastic bags with labels stating the plant name and location. Later the specimens were split open to check for the presence of adult beetles, eggs, larvae, and pupae (Paap et al., 2018). Digital evidence in the form of photographs was taken for each sampling site. For all inspected trees, the digital handheld GPS (Garmin Montana® 600) was used to determine the location of the pest and host species that had been noted. The pest and pathogen, including the host plant species, were identified using different guidebooks (e.g., De Villiers and Schoeman, 1988; Van Wyk and Van Wyk, 2013; Bromilow, 2018).

Figure 3.3: Sampling observations for the signs encountered during pest/pathogen survey. A: Stainless steel double-sided scale straight ruler was used to measure the shallow entry holes on the tree suspected of showing signs of shot hole borer attack. B: Gerstner's aloe *Aloe gerstneri* attacked by aloe white scale *Duplachionaspis exalbida*. C & D: Aloe weaver damage to bitter aloe *Aloe ferox*.

3.4. Data analysis

3.4.1. Vegetation spatial data collection and image processing

To analyse the NDVI trends during the study period (1987-2017), I used different proportions of vegetation cover class in hectares across the 10-year intervals, using the 1987 data as the baseline to quantify the potential vegetation cover change on the study site. The

change was plotted over time, and the trendline was used to determine the relationship between the vegetation cover/surface change and time (coefficient of determination: R^2).

3.4.2. Ground-truthing the vegetation types

To validate the vegetation type in the FSNBG through ground-truthing, the plant species (i.e., all the rooted plant species) identified within the sampling quadrats were used to generate frequency as a response variable in order to compare species dominance. The plant species' names were specified as ordinal response variables, and a descriptive statistic was applied to compare the number (i.e., the frequency) of plant species at a significance of $P \leq 0.05$, using Statistical Package for Social Sciences (SPSS, version 20) software.

The extent of the threats posed to the CBA1 vegetation type Bloemfontein Karroid Shrubland by habitat transformation and degradation (SANBI, 2013), was applied to assess their overall ecological integrity. This was defined as the ability of the habitat to be balanced and integrated in terms of community of organisms having a species composition (Karl and Dudley, 1981; Kleynhans, 1996). A modified generalised habitat integrity evaluation method adopted from Mokotjomela and Nombewu (2019) was used, in which a habitat with a minimum threshold of 60% habitat intactness was applied and used to infer the relative ecological integrity of the CBA1 vegetation type. A 'category C' habitat is a moderately modified habitat through some loss or alteration of the natural habitat and biota, but with its basic ecosystem function mainly unchanged (Kleynhans et al., 2008).

After sampling the species diversity of the CBA1 vegetation type, the results were analysed and compared with the reference plant species described in Mucina and Rutherford (2006).

3.4.3. Habitat ecological threats

3.4.3.1. Alien and invasive species

The native range description at the continental level of each recorded alien and invasive species was classified and specified from different sources: books (see Bromilow, 2018), and a global invasive database to guide adaptive biosecurity management (Mokotjomela et al., under review). Each species was classified into its life forms following field guides by Bromilow (2018) and Henderson (2001), and its native range, since this information provides bases for important management decisions in South Africa, and because life forms can help to predict the potential impacts of species (Dorrepaal, 2007; Pysek et al., 2012; Mokotjomela et al., under review). Alien and invasive plant species lists were classified into four categories according to the South African National Environmental Management: Biodiversity Act (NEMBA, Act 10 of 2004) Alien and Invasive Species Regulations (hereafter called NEMBA-

AIS Regulations; Department of Environmental Affairs, 2014), which were gazetted in 2014 to guide management prioritisation (Mokotjomela et al., under review). Below is a brief explanation of each alien and invasive species category in the A&IS regulations of 2014.

I. **Category 1a**: Invasive species that must be combated and eradicated. Any form of trade or planting is strictly prohibited.
II. **Category 1b**: Invasive species that must be controlled and, wherever possible, removed and destroyed. Any form of trade or planting is strictly prohibited.
III. **Category 2**: Invasive species deemed to be potentially invasive, in that a permit is required to carry out a restricted activity. Category 2 species include commercially important species such as pine, wattle, and gum trees. Plants in Riparian areas are Category 1b.
IV. **Category 3**: Invasive species that may remain in prescribed areas or provinces. Further planting, propagation, or trade is prohibited. Plants in Riparian areas are Category 1b.

The distribution of different alien and invasive plant species was mapped using Google Map Pro software to determine the invasion spatial patterns and to guide and prioritise their management and containment in the FSNBG.

The data on the total frequencies of species in each of the three classes (predictor variables) were analysed using the chi-square (χ^2) contingency test to compare significant differences between the classes ($P \leq 0.05$) in SPSS version 20 software.

3.4.3.2. Human settlement encroachment

To analyse the rate of habitat transformation attributed to human settlement in the FSNBG buffer since 2002, portions of transformed – i.e., anthropogenically altered – landscape, such as the physical development of built infrastructure and plantations, were estimated in hectares for the five-year intervals, using the 2002 data as a baseline, up to 2020. The habitat change was plotted over time, and the regression analysis was used to determine the strength of the relationship between change and time (coefficient of determination – R^2).

3.4.3.3. Monitoring plant diseases

The list of different species and their host plants was documented. The history of the impacts of each was also assessed from the published literature.

3.5. Results

3.5.1. Determinations of vegetation cover change: Vegetation spatial data collection

The Landsat images were taken at 10-year intervals, and reflect the field where there are still signs of vegetation cover. The vegetation cover was seen to increase the biomass in hectares over time from 1987 to 2017, as R^2 ranges from 0.72 to 0.76 (Figure 3.4. and 3.5). The sparse vegetation cover decreased over time (R^2 = 0.29; Figure 3C); moderate vegetation cover increased by 25.1 ha (Figure 3.5A); while dense vegetation increased by 8.6 ha (Figure 3.5B) from 1987 to 2017 (Figure 3.4). The bare soil cover was found to be relatively consistent (Figure 3.5D; R^2 = 0.0198). Alternatively, the sparse vegetation cover (i.e., the reference cover change) decreased progressively by 11.07 ha from 1987 to 2017 (Figure 3.4 & 3.5C).

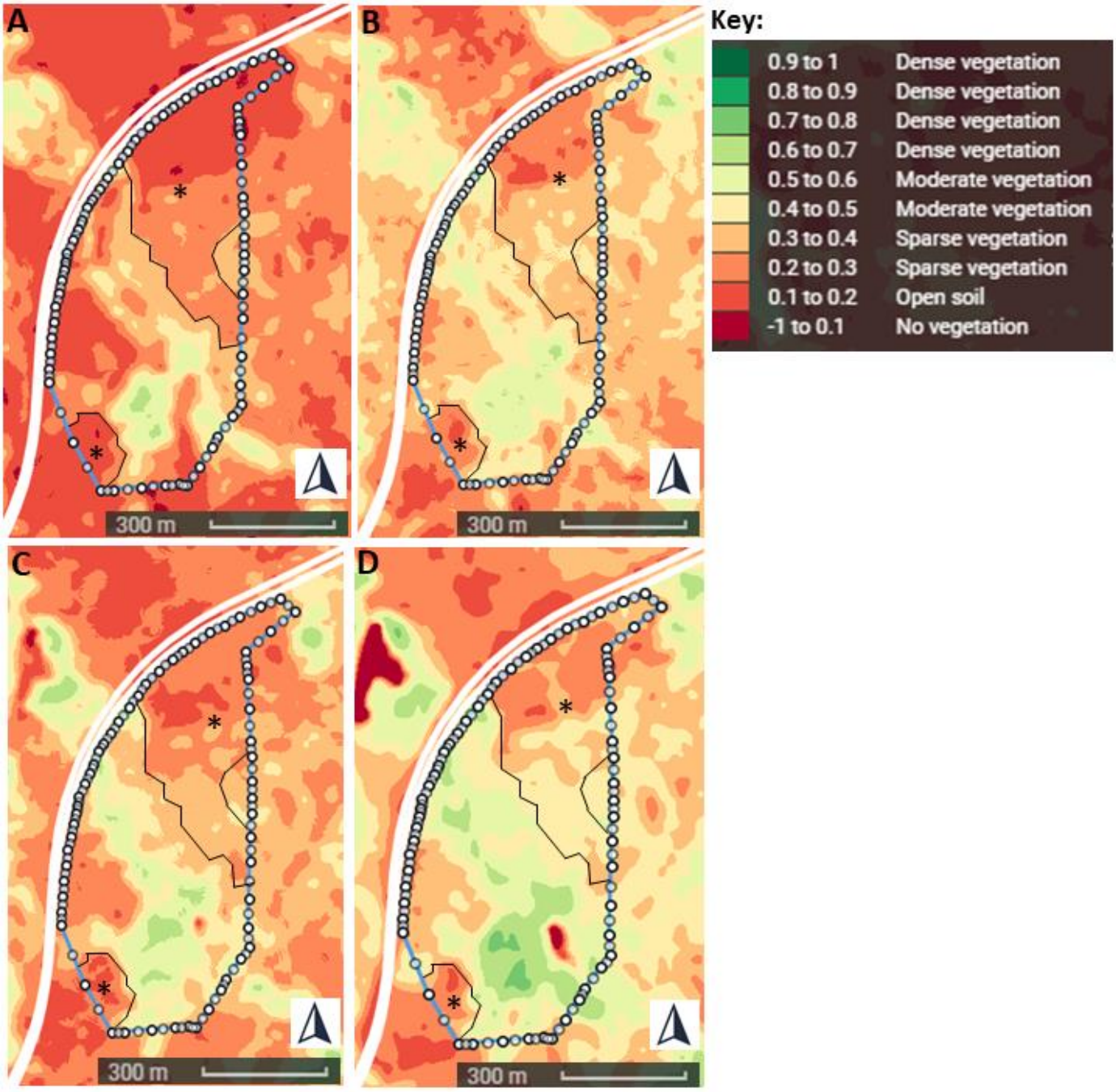

Figure 3.4: NDVI (vegetation cover) images demonstrate the 30 years of vegetation cover dynamics of the Free State National Botanical Garden, from 1987 to 2017, in 10-year intervals (NDVI vegetation cover: A – 1987, B – 1997, C – 2007, and D – 2017). The blue line with white dots indicates the perimeter of the FSNBG. The black lines enclosing the black stars (*) show the coverage of the CBA1 vegetation (Bloemfontein Karroid Shrubland).

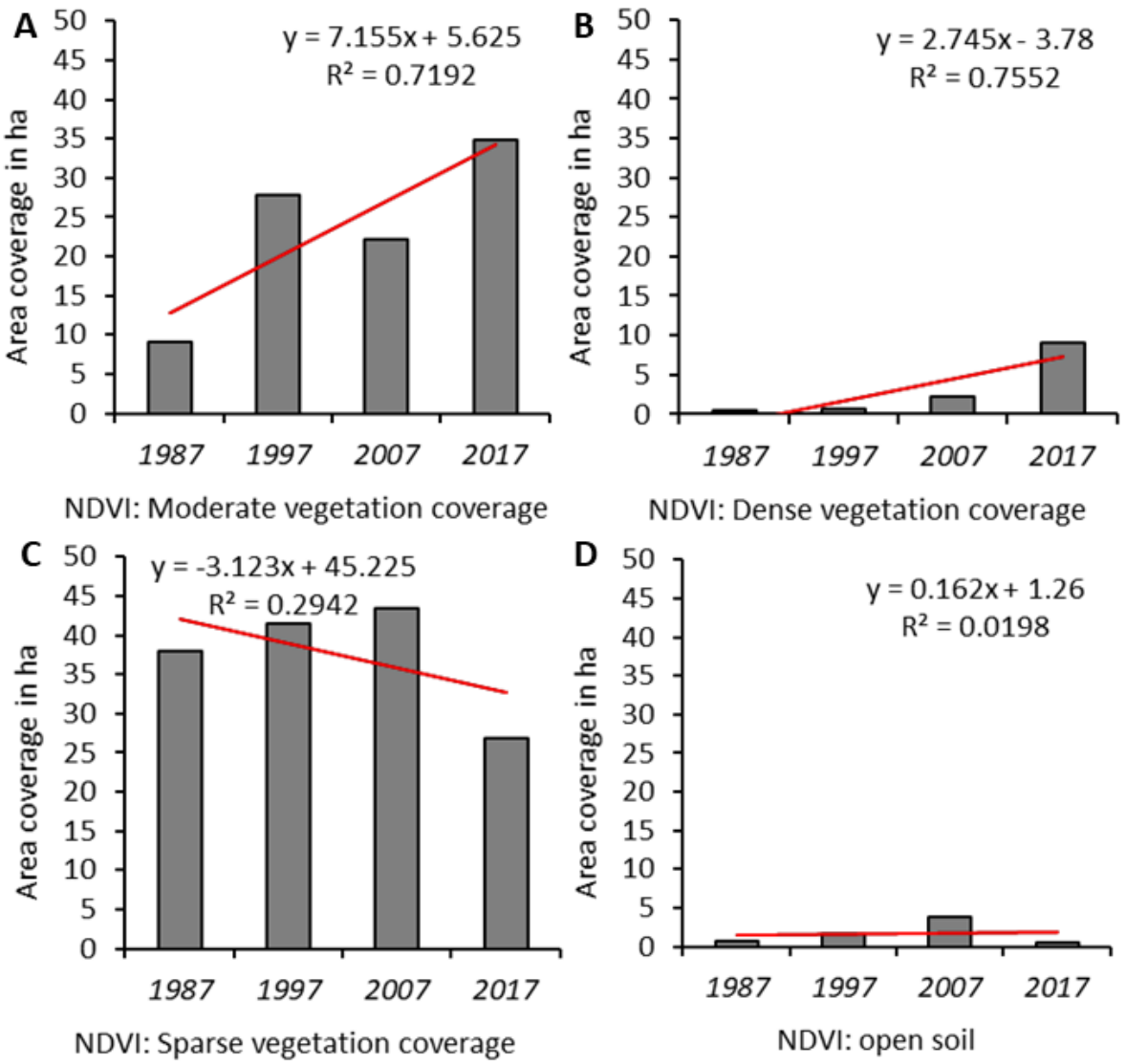

Figure 3.5: NDVI (vegetation cover dynamics' satellite classification results and its cover areas over the 30-year period. The red line represents the trend of change and the relationship with time in years. A – D: vegetation coverage trends with the coefficient of determination (R^2) that determined the strength of the relationship between change and time.

3.5.2. Ground-truthing plant species in the Critical Biodiversity Area 1 vegetation type

The overall cover of the CBA1 vegetation type (Bloemfontein Karroid Shrubland) was 79.6 ±15.9% per square metre (Figure 3.6). Also, the ecological integrity determined using habitat intactness was relatively high (above the 60% threshold for habitat intactness; *sensu* Mokotjomela and Nombewu, 2019; Kleynhans et al., 2008).

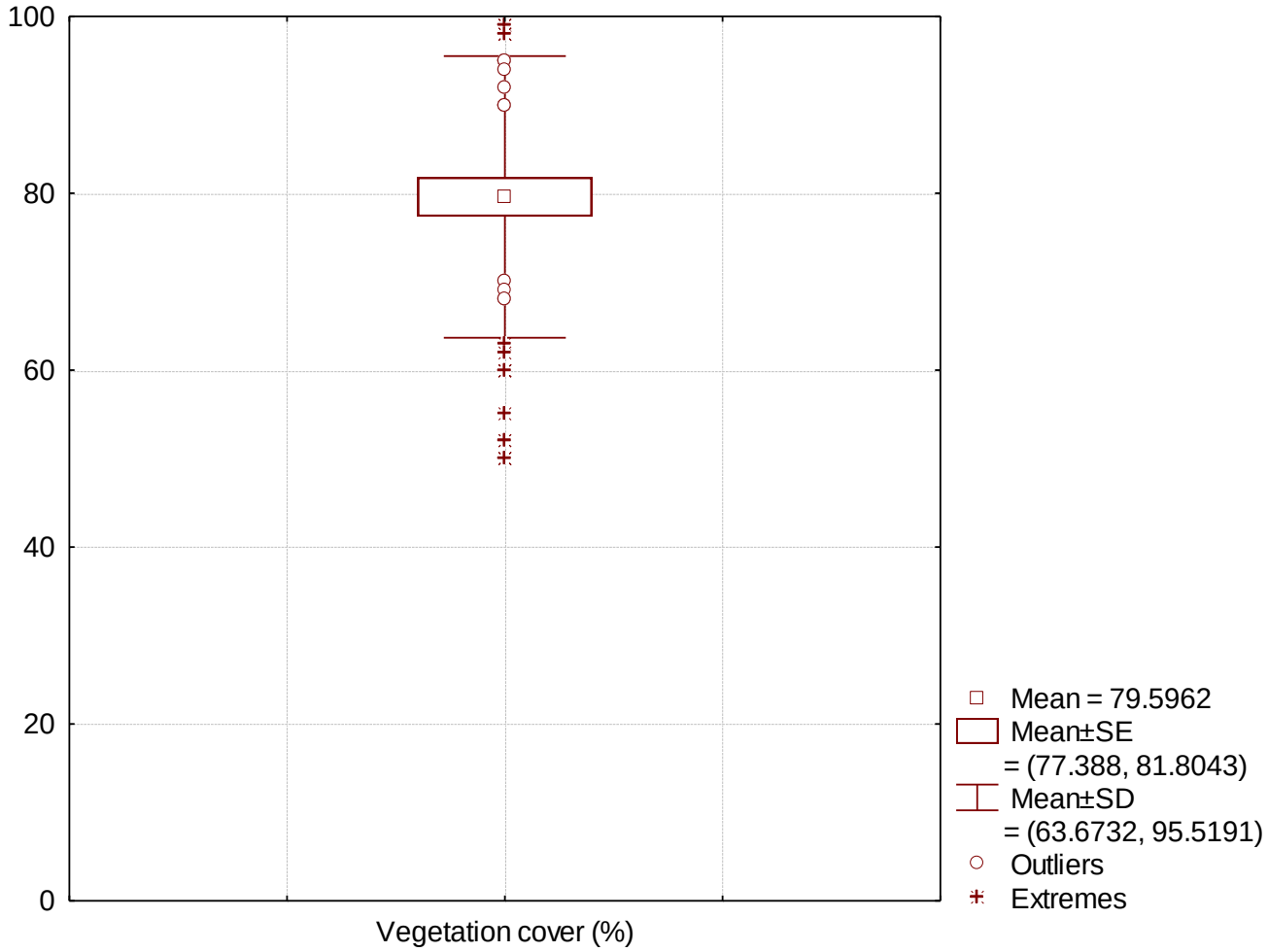

Figure 3.6: Overall vegetation cover per square meter in the CBA1 vegetation type: Bloemfontein Karroid Shrubland.

A total of 55 plant species belonging to 17 families was recorded from 52 quadrats in the 22.37 ha of CBA1 vegetation type (Bloemfontein Karroid Shrubland). The dominant plant family was Poaceae (25.6%), followed by Asteraceae (19.6%).

Out of the 77 plant species documented by Mucina and Rutherford (2006), 27 (35%, N = 77) plant species still exist, and were identified in the field. Of the 12 reference plant species described as dominant by Mucina and Rutherford (2006), 11 species were still found in the CBA1 vegetation type: *Eragrostic nindensis* (10.2%; N = 352); *Cheilanthes eckloniana* (7.1%; N = 352), *Euphorbia mauritanica* (4.8%; N = 352), *Ruschia spinosa* (4.8%; N = 352), *Albuca setosa* (3.7%; N = 352), *Aristida diffusa* (2.8%; N = 352), *Somatium mustellium* (2.3%; N = 352), *Euryops emetrifolius* (2%; N = 352), *Eriocephalus ericoides* (2%; N = 352), *Curio radicans* (2%; N = 352), and *Heteropogon contortus* (0.6%; N = 352) (Figure 3.7).

In addition, 16 subsidiary plant species were documented in the CBA1 vegetation patch, with *Aloe grandidentata* (4.3%; N = 352) being the most frequent (Figure 3.7).

Conversely, 25 plant species and three alien invasive plant species were recorded as invader native species, with *Anacampseros subnuda* (2.5%; N = 352) and *Ledebouria sp*. (2.5%; N = 352) being the most common (Figure 3.7).

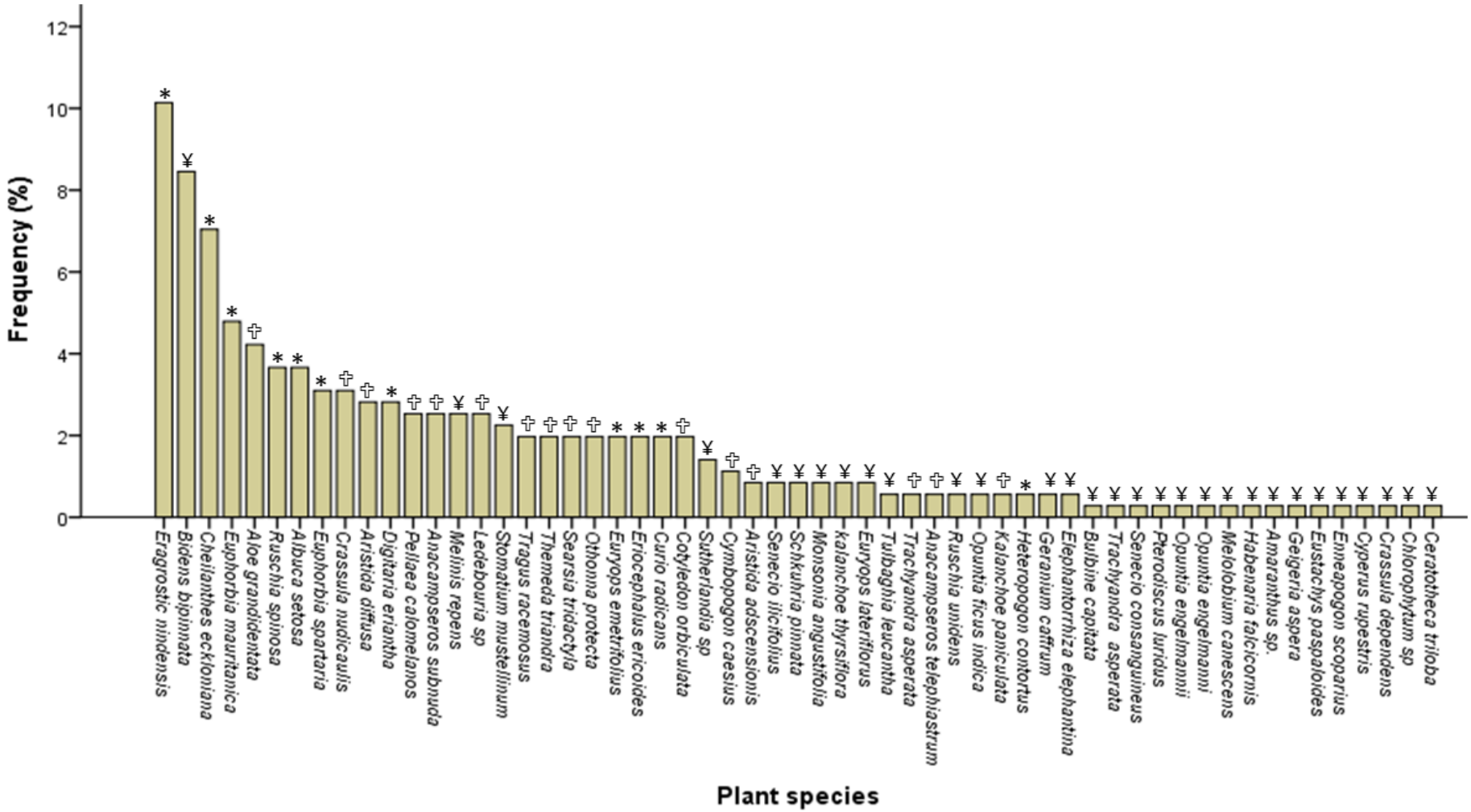

Figure 3.7: Frequency (%) of sampled plant species within the CBA1 vegetation type (Bloemfontein Karroid Shrubland). The ✶ sign shows the reference plant species described in Mucina and Rutherford (2006); the ✞ sign indicates the subsidiary plant species; ¥ is the invader plant species, and ☠ indicates non-native plant species.

3.5.3. Habitat ecological threats

3.5.3.1. Alien and invasive plant species

Within the 66ha perimeter of the FSNBG, I documented a total of 27 alien and invasive plant species belonging to 16 families. The dominant plant family was Cactaceae (22.2%; N = 27), followed by Rosaceae (14.8%; N = 27; see Figure 3.8A).

There were significant differences among the various alien and invasive plant growth forms (Wald χ^2 = 12.21; df = 4; p = 0.016; Figure 3.8B; Table 3.3). Of the five plant growth forms, woody species occurred most often (58%; N = 27; Figure 3.8B).

Among the four vegetation types (Bloemfontein Dry Grassland, Bloemfontein Karroid Shrubland, Winburg Grassy Shrubland and Riparian vegetation) conserved in the FSNBG, the Riparian vegetation was the most invaded vegetation type (n = 16; Figure 3.7), and *Cestrum laevigatum* (inkberry) was found to be the most invasive species (Figure 3.7).

The number of alien plant species recorded in the different NEMBA A&IS regulations (i.e., NEMBA A&IS category 1a, 1b, 2, & 3) was not statistically different (Wald χ^2 = 4.35; df = 3; p = 0.23; Figure 3.8C; Table 3.3). Out of 27 recorded plant species, 74% (N = 27) of the species were classified under Category 1b in the FSNBG, followed by 19% (N = 27; Figure 3.8C) of the Category 3 species. Also, only four percent (N = 27; Figure 3.8C) of the alien invasive species were not listed in NEMBA A&IS. Conversely, no emerging plant species (Category 1a) was recorded.

There were also no significant differences in the numbers of alien and invasive plant species introduced from different continental levels (Wald χ^2 = 4.23; df = 4; p = 0.38; Figure 3.8D; Table 3.3). However, most of the recorded alien invasive plant species were introduced from the Americas (64.5%; N = 27; Figure 3.8D).

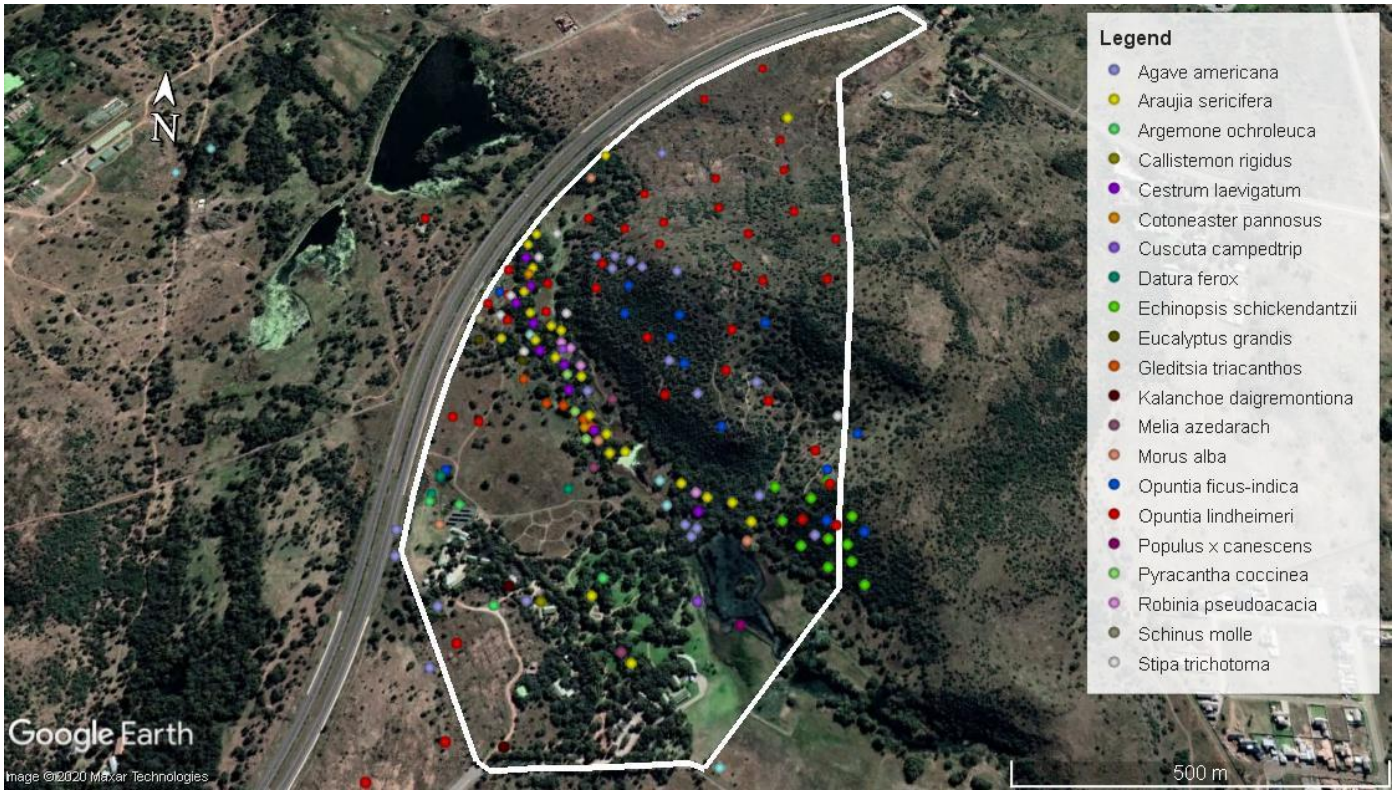

Figure 3.8: AIS infestation (i.e., baseline data) in the Free State National Botanical Garden (white line shows the perimeter). The coloured dots represent the distribution of different species – see the key in the figure.

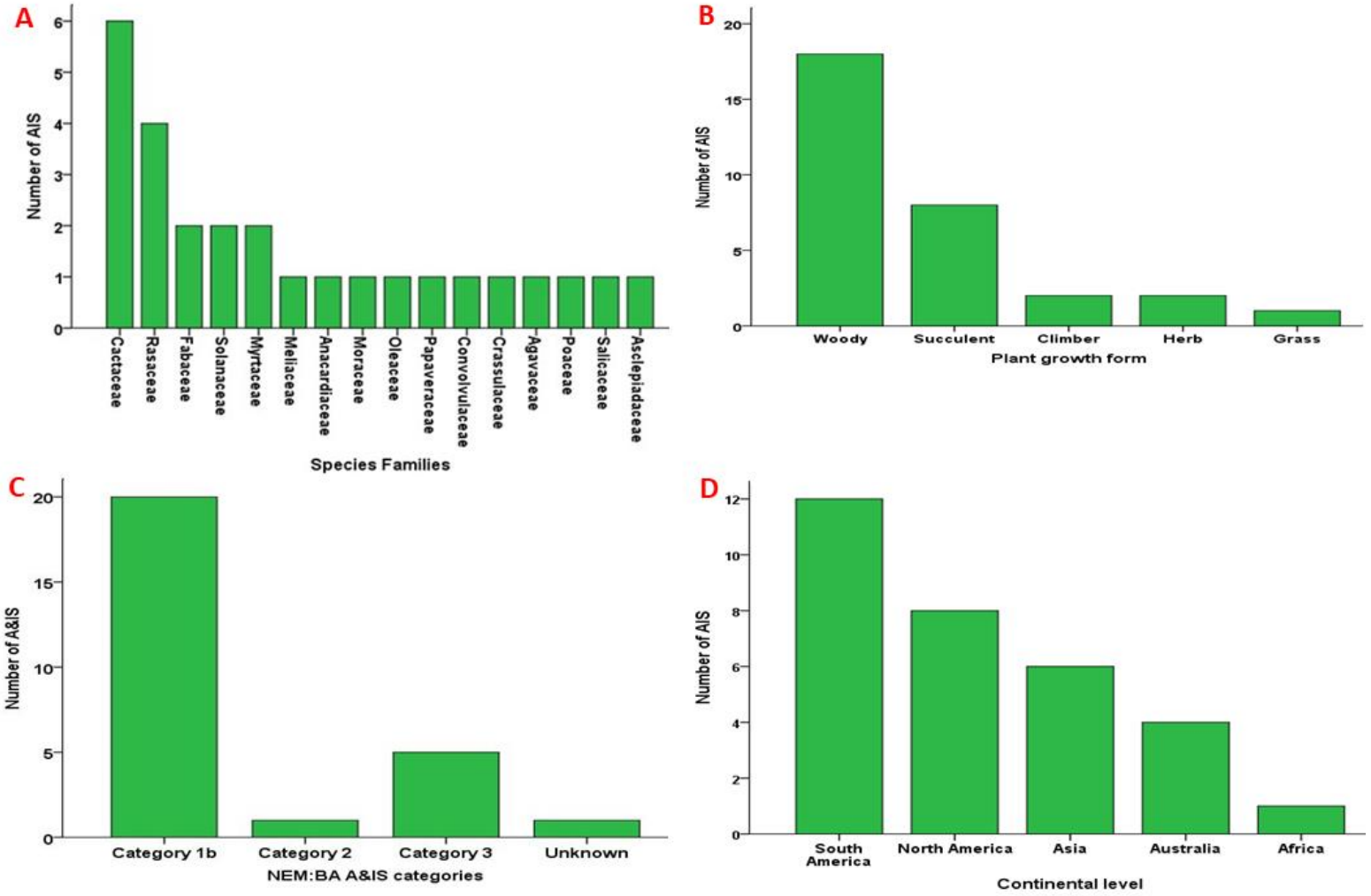

Figure 3.9: Classification of alien and invasive plant species recorded in the FSNBG. A: species family; B: plant growth form; C: NEM:BA A&IS categories; and D: continental level.

Table 3.2: Wald statistics for comparison of counts of alien and invasive plant species in different classification criteria (country of origin, NEMBA categories, and life growth forms) recorded in the FSNBG (* denotes significant difference).

				95% Wald confidence interval		*Hypothesis test*		
Predictor variables	***Parameter***	***B***	***Std. error***	***Lower***	***Upper***	***Wald chi-square***	***df***	***P-value.***
Continental level	Africa	.521	1.1000	-1.635	2.677	.224	1	0.636
	Asia	-.368	.7288	-1.796	1.060	.255	1	0.613
	Australia	-1.435	.8057	-3.015	.144	3.174	1	0.075
	North America	-.123	.7153	-1.525	1.279	.030	1	0.863
	South America	0[a]						
NEMBA categories	Category 1b	-1.371	1.1125	-3.552	.809	1.519	1	0.218
	Category 2	-1.328	1.5678	-4.401	1.745	.718	1	0.397
	Category 3	-2.258	1.2198	-4.649	.133	3.427	1	0.064
	Unknown	0[a]						
Plant growth form	Climber	-2.566	.8901	-4.310	-.821	8.308	1	0.004
	Grass	12.405	10343.8664	-20261.200	20286.011	.000	1	0.999
	Herb	-2.206	.9203	-4.010	-.403	5.748	1	0.017
	Succulent	-1.614	.7448	-3.074	-.154	4.697	1	0.030
	Woody	0[a]						
	(Scale)	1[b]						

3.5.3.2. Human settlement encroachment

For spatial analysis, human settlement has been encroaching on the FSNBG buffer zone (*ca* 1 km) during the past 18 years. In 2002, 6.6% (45.59 ha) of the total area of the FSNBG buffer zone was found to be covered by human settlements. Since 2002, almost 12% (73.48 ha) of the FSNBG buffer area has become human-modified habitat. while by 2020 this area had increased to 17.9% (119.07 ha) (Figure 3.9). Thus, there is a strong increase of human settlement encroachment coverage over time ($R^2 = 0.79$; Figure 3.9).

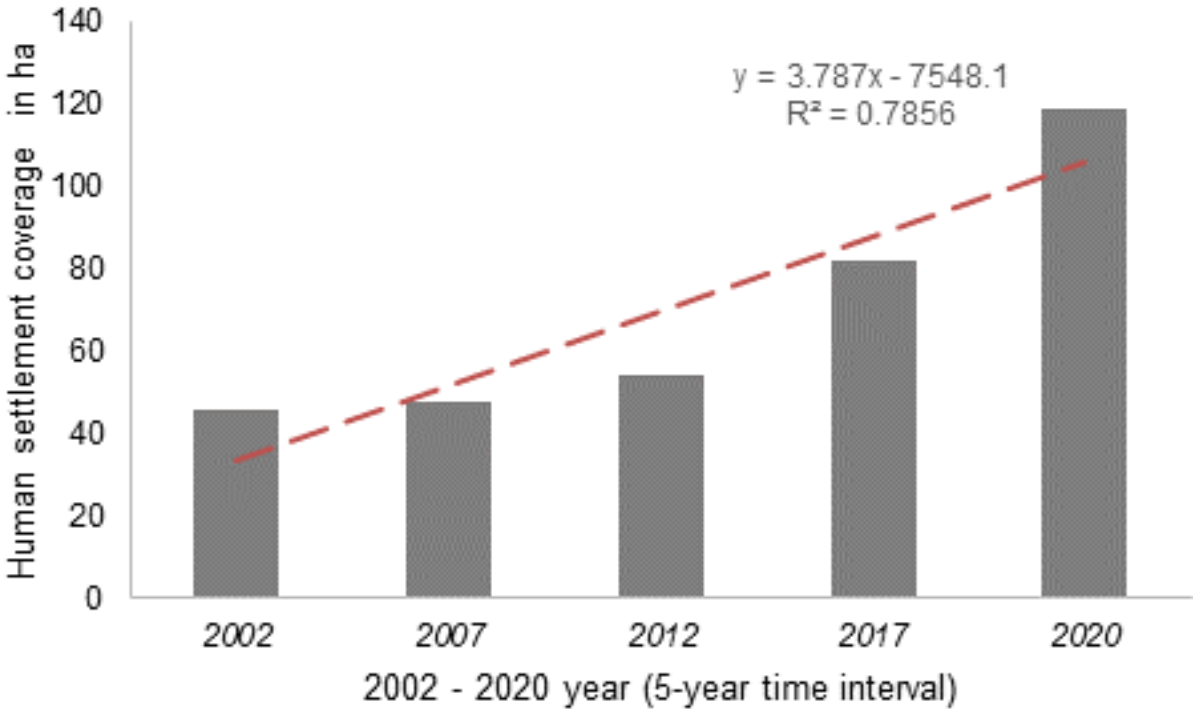

Figure 3.10: A bar graph of human settlement encroachment measured in hectares from 2002 to 2020, with habitat change estimated at five-year intervals. The red line represents the trend of change and the strength of the relationship with time in years.

3.5.3.3. Detecting plant pests/diseases

A total of 10 native/non-native pest/pathogen species was recorded (Table 3.4). Species were classified into ten genera and nine families from 18 different native (and one non-native *Opuntia ficus-indica*) plant host, with no detection of the emerging polyphagous shot hole borer species (*Fusarium euwallacea*). Among the 10 non-native/ native species recognised as causing reportable damage in the study area, seven were sap-feeding insects, two were foliage-feeding insects, and one was a pathogen.

The non-native pest species that were identified were introduced from different continental levels; three from North America, two from Australia, two from Asia, and one from South America, with three native pest species (see Table 3.4).

Most of the pests/pathogens (n = 9; Table 3.4) were recorded in the horticulture landscape; they could have reached the FSNBG through horticulture practices (i.e., humid microclimate conditions from the irrigation systems), and they had limited natural enemies.

Table 3.3: Documented pests and pathogens in the Free State National Botanical Garden: continental origin and history, type of impact, host plant species, and vegetation type. The bolded species are non-natives.

Pest/pathogen species	Scientific name	Family	History (source)	Type of impact	Host plant species	Vegetation type
Cochineal	*Dactylopius austrinus*	Dactylopiidae	South American and North American, introduced in 1935 as a biological control for invasive cacti (Bromilow, 2018).	Sap-sucking insect	***Opuntia ficus indica***	Bloemfontein Karroid Shrubland
Aloe white scale	*Duplachionaspis exalbida*	Diaspididae	Southern Africa (Africa)	Sap-sucking insect	*Aloe striata, Aloe marlothii, Aloe microstigma* and *Aloe van balonii*	Horticulture landscape
White scale	*Duplachionaspis exalbida*	Diaspididae	Southern Africa (Africa)	Sap-sucking insect	*Gymnosporia buxifolia*	Horticulture landscape
Aloe weevil	*Gonipterus scuttelatus*	Curculionidae	Australia, introduced in 1916 (Mally, 1924)	Foliage-feeding insects	*Aloe gerstneri* and *Aloe broomi*	Horticulture landscape
Amaryllis borer	*Brithys pancratia*	Amaryllidaceae	Dominican Republic (North America)	Foliage-feeding insects	*Crinium moorie, Crinum bulbispermum* and *Nerine laticoma*	Horticulture landscape
Red scale	*Aonidiella aurantii*	Diaspididae	Southeast Asia, introduced in 1857 (Compere, 1961).	Sap-sucking insect	*Ziziphus mucronata*	Bloemfontein Dry Grassland
Arimalia root	*Armillaria heimii*	Physalacriaceae	Africa, introduced into South Africa with potted plants from Europe in the 1600s (Coetzee et al., 2001)	Pathogen	*Vachellia tortilis*	Horticulture landscape
Aphids	*Aphis fabae*	Aphididae	Central America (North America), introduced in 1978 (Walters, 1984).	Sap-sucking insect	*Artemisia afra*	Horticulture landscape
Wax scale	*Ceroplastes destructor*	Coccidae	China (Asia), introduced in 1654 due to citrus trade (Wakgari, 2000).	Sap-sucking insect	*Artemisia absinthium*	Horticulture landscape
Australian mealybug	*Pseudococcus longispinu*	Pseudococcidae	Australia, introduced in 1983 as a biological control for invasive cacti (Moran and Zimmermann, 1991).	Sap-sucking insect	*Carpobrotus edulis* and *Felicia amelloides*	Horticulture landscape
Psylla	*Trioza erytreae*	Psyllidae	Africa	Sap-sucking insect	*Cussonia spicata* and *Hypoestes aristata*	Horticulture landscape and Winburg Grassy Shrubland

3.6. Discussion

3.6.1. Determination of vegetation cover change

Overall, the results of the study confirmed the change in vegetation cover over the past 30 years, with the woody plants' canopy cover having increased. The woody state could be attributed to a combination of factors, including severe drought conditions (Van Rooyen, 2010; Botai et al., 2016) and the rise of CO_2 concentrations in the atmosphere induced by global climate change (Ward, 2010), which enhance the growth of woody species (Buitenwerf et al., 2012; Welz, 2013; Sintayehu, 2018) and suppress the herbaceous plant cover (Kgosikoma and Mogotsi, 2013). Previous studies have shown that drought conditions favour the growth of woody species, since the roots of grasses can extract water only from the topsoil layer, while woody species have access to deeper water sources (Van Rooyen, 2010; Walker and Noy-Meir, 1982; Ward et al., 2013). These differences are likely to increase the negative impacts of the tree-grass interactions in the grassland biome and result in increased bush encroachment (Ward, 2005). Several studies have consistently noted that the Grassland Biome in South Africa has undergone extensive modification, and has seen a fundamental shift from its original state to resemble savanna vegetation (see Matsika, 2008; Neke and Du Plessis, 2004; Little et al., 2015).

It is possible that the observed bush encroachment also is a result of the absence of the biophysical disturbances that are essential for grassland health (Eldridge et al., 2011). For example, the exclusion of fire as an essential component of successional changes in the grass communities of the FSNBG vegetation could result in the recruitment of woody tree/ shrub species (Neary and Leonard, 2020; Van Rooyen, 2010; Trollope, 1974; Hare et al., 2020; Gordijn, 2010), since periodic fires suppress woody plants' recruitment in the grasslands (Le Maitre and Midgley, 1992; Trollope and Tainton, 1986; Van Rooyen, 2010). In addition, the encroachment of woody plants in the study could be attributed to the absence of browsers that suppress the population growth in palatable woody species (Van Rooyen, 2010). It has been shown that trampling by animals could also strongly suppress the woody vegetation cover in savanna (Oliveras and Maihi, 2016). This finding is consistent with the report that woody vegetation in grasslands and savannas has increased worldwide over the past 100–200 years (Archer et al., 2017).

In addition, several studies have demonstrated that the woody vegetation cover in South Africa can be associated with the propagule pressure of native and non-native fleshy-fruited woody plant species that are consumed and dispersed by frugivorous birds, such as reported for the savanna habitat by Milton et al. (2007). Therefore, I argue that the observed changes in vegetation cover, which indicate an increase in woody species' cover between 1987 and 2017, can be partly attributed to the substantial seed rain associated with resident frugivorous bird species and colonial wintering birds (Vukeya et al., 2020; also see Appendix

3.2 for satellite imagery analysis of change in vegetation cover between 1941 and 2019), since it has been shown that their gut treatment of the seeds promotes the germination of the local woody plant species (Mokotjomela et al., 2021). Frugivorous birds observed in the FSNBG were characterised by localised movements, possibly to avoid the disturbance in the urban areas (Gomes et al., 2008). Woody cover encroachment reportedly jeopardises grassland biodiversity and transforms grasslands and savannas into shrublands and woodlands (Acher et al., 2001; 2017). Indeed, Mogashoa et al. (2020) found that an increase in tree encroachment decreased grass species' richness by 53% in South African savannas. Since bush encroachment represents a conservation threat (Acher et al., 2001; 2017), there is an urgent need to mitigate the potential impacts of encroaching woody species on the vegetation types preserved in the FSNBG.

Of critical importance in the conservation of vegetation in the FSNBG is the Bloemfontein Karroid Shrubland, which is listed in national conservation priorities (SANBI, 2017). The validation of ecological integrity showed that the Bloemfontein Karroid Shrubland still has excellent vegetation cover (79.6 ±15.9%), which suggests effective management by the FSNBG. Termansen et al. (2019) indicated that vegetation cover reflects both the natural habitat health and the adaptive management responses. For example, Hesp (1991) reported that excellent vegetation cover reduces splash erosion by intercepting rainfall, decreasing the overland flow, and improving the infiltration of precipitation into the soil.

In addition, the vegetation intactness was above the threshold of 60%, suggesting partial vegetation loss, but with the habitat functionality maintained (i.e., the Category C integrity measure in Kleynhans et al., 2008; Mokotjomela and Nombewu, 2019). Indeed, most of the flagship plant species (11 out of 12) (Mucina and Rutherford, 2006) were still occurring and could be supporting the patch functionality. However, the study found that 50 of the 77 plant species (Mucina and Rutherford, 2006) in the Bloemfontein Karroid Shrubland have disappeared, thus confirming the recorded loss of ecological integrity. Two major climatic drivers that might account for the reported change are extreme frost and drought in the Free State Province (Tilman and Haddi, 1992; Basto et al., 2018; Abubakar, et al., 2020). Tilman and Haddi (1992) found that the above-ground living plant mass of grassland vegetation decreased by an average of 47% during an extreme drought condition in 1988, which led to an increased rate of species extinctions.

It is possible that the loss of plant species could have created a niche for the 25 recorded invader native plant species to colonise the Bloemfontein Karroid Shrubland (Begon et al., 1990). Among the species, *Anacampseros subnuda* and *Ledebouria sp.* were found to be dominant. *A. subnuda* in South Africa has thus far been recorded in the Rand Highveld Grassland of Mpumalanga and Gauteng (Von Staden and Castro, 2016), with their conservation threats being currently unknown. The observed species niche change could be

a response to climate change, by shifting their distribution to suit their niche requirements following the change in environmental conditions in the Free State Province and in South Africa as a whole.

3.6.3. Habitat ecological threats

3.6.3.1. Alien and invasive plant species

The fact that I recorded 27 alien and invasive species (of which 13 were woody species) in the study site highlights the vulnerability of the Grassland Biome to the negative impact of alien species (O'Connor and Van Wilgen, 2020). Many alien trees and shrubs have a substantial impact on South African biodiversity (Roura-Pascual et al., 2009). For example, it was reported that the estimated extent and impact of the Black wattle (*Acacia mearnsii*) invasion was higher on the Grassland Biome surface water runoff in South Africa than that of other plant species (Henderson and Wilson, 2017; Roura-Pascual et al., 2009; Van Wilgen et al., 2018). Of the 27 species, inkberry (*Cestrum laevigatum*) was found to be the most invasive species in the Riparian vegetation, and it is likely that the extensive seed bank could worsen the invasion risk to wetland biodiversity. Inkberry also prevents the regeneration of native species after the formation of dense stands, and it is highly toxic to some wildlife (Weber, 2003). This could well lead native species to their extinction (Winter et al., 2009). Invasive alien plant species are also known to disrupt native mutualism by attracting native pollinators and seed dispersal agents to their abundant pollen and fruit resources (Traveset and Richardson, 2006; Mokotjomela et al., 2013; Le Roux et al., 2020), subsequently decoupling native mutualist partner organisms (Le Roux et al., 2020). This could compromise the critical ecological services in the local habitat (Traveset and Richardson, 2006; Boucher et al., 1982; Bond, 1994; Aslan et al., 2013) and consequently transform the preserved vegetation types in the FSNBG.

The results also highlighted that there were many Category 1b plant species in NEMBA A&IS (74%, N = 27), and that these invaders require that effective management be put in place to mitigate their negative impact. Van Wilgen et al. (2020) indicated that, since 1995, the South African government has spent R15 billion on alien plant control operations (e.g., the Working for Water programme) across South Africa, since the benefit of clearing biological invasion has been shown to be a good investment (*sensu* Van Wilgen et al., 2001). In addition, Ziller et al. (2020) indicated that protected area managers should prioritise and maximise their efforts to control biological invasions to achieve large-scale positive results at as low a cost as possible. I found that four per cent (N = 27) of the alien species were not listed in NEMBA A&IS categories; and this highlights the need for further research and risk analysis into alien taxa to outline the likelihood of invasion, the realised and potential impacts in the ecosystem, and options for management needs (Kumschick et al., 2020; IUCN, 2020; Keller and

Kumschick, 2017; Blackburn et al., 2014). Some species are not listed because of limited capacity in documenting alien species (Bouchard et al., 2015).

The results also highlight the dominance of Cactaceae plant families (22.2%; N = 27), which already have a negative impact and have been targeted for management in South Africa since 1995 (Henderson and Wilson, 2017; Novoal et al. 2015, 2019; Walter et al. 2011; Van Wilgen at al., 2020). Masocha and Dube (2018) reported that cacti invasions may threaten native biodiversity in many countries. In South Africa, for example, Du Toit (1942) and Dean and Milton (2000) both reported that about 900 000 ha were abandoned by many landowners in the 1940s owing to the thicket invasion of *Opuntia ficus-indica* (sweet prickly pear). The results also highlight the dominance of South American native plant species; this supports the report by Turbelin et al. (2017) indicating that South America is the main source of the global spread of alien and invasive species through either deliberate or unintentional introduction by human-mediated activities for economic development (the agricultural industry) and horticultural purposes (such as ornamental plants) (Turbelin et al., 2017; Mokotjomela et al., under review). The results could guide the risk assessment of biosecurity and alien species management in the FSNBG (Turbelin et al., 2017). Preventing the introduction of alien species from their native countries is recommended as the most effective method for managing alien invasion (*sensu* Myers, 2000).

3.6.3.2. Human settlement encroachment

The spatial assessment of human settlement encroachment has shown that the FSNBG buffer area (*ca* 1km) has been decreasing in the past 18 years, which suggests an increasing conservation threat to local biodiversity. Increases in natural habitat loss may disrupt ecological processes such as the pollination and seed dispersal that is mediated by long-distance agents visiting the FSNBG (Haddad et al., 2015; SANBI, 2013; Carey et al., 2000). It has been shown that many protected areas in South Africa also struggle to manage pressure from the loss of natural habitat through human settlement encroachment in areas adjacent to those protected areas (Taylor and Atkinson, 2012; Kiringe and Okello, 2007). Since the FSNBG is also a protected area, the destruction of biodiversity corridors leads to the isolation of small populations, thus reducing their genetic diversity and persistence (Rosenberg et al., 1997; Haddad et al., 2015; De Frutos, 2015; SANBI, 2013). Rosenberg et al. (1997) indicated that habitat corridors in the protected areas help to increase population persistence by allowing the continued exchange of genetic material between the connected populations. This study's findings therefore indicate an urgent need for conservation and restoration measures to protect habitat corridors and to help maintain ecosystem health in the FSNBG, since they would preserve the CBA1 vegetation type.

3.6.3.3. Detecting plant pest/diseases

Botanical gardens in South Africa are known to provide an opportunity for sentinel plant research and biosecurity (Gossa and Paap, 2020; Paap et al., 2017), but botanical gardens have exacerbated spread of pest/pathogen threats that caused catastrophic impacts (Gossa and Paap, 2020). This study documented the pests and pathogens of 10 plants, 90% of which are associated with horticulture through the creation of artificial growth media with conditions conducive for their recruitment (Shipp et al., 2003). Many alien pests and pathogens have a substantial impact on the native trees of South African (Graziosi et al., 2020). The Armillaria root rot pathogens, for example, have been reported to cause significant and costly damage to the native plant species of South Africa such as *Vachellia sp*. (see Graziosi et al., 2020, 2019; Roux et al., 2005; Van der Walt, 2008). Also, since the FSNBG has no restriction on the movement of untreated firewood, the trees' susceptibility to certain pests and pathogens such as the emerging pest PSHP may increase (Jacobi et al., 2012; Meurisse et al., 2018; Klapwijk et al., 2016). Although the study did not record the presence of PSHP, the movement of untreated firewood could increase the vulnerability of trees in the FSNBG to incursions by pests and pathogens.

3.7. Conclusions

It is critical to understand the vegetation cover dynamics and to assess whether the vegetation units are affected by conservation threats reported in the Grassland Biome. With the use of remote sensing and GIS, I investigated the vegetation cover dynamics of the FSNBG over a period of 30 years. The results showed that the FSNBG vegetation cover has been encroached on by woody species. Such long-term vegetation cover change was attributed to a combination of factors, including severe drought conditions (Van Rooyen, 2010; Botai et al., 2016) and the rise of CO_2 concentrations in the atmosphere induced by global climate change (Ward, 2010). The sparse vegetation cover has decreased over time, while moderate and dense vegetation cover shows an increase (25.11 ha and 8.64 ha respectively) from 1987 to 2017. The major conservation threats were biological invasions – 27 invasive alien plant species dominated by 13 woody plants and eight succulent species interspersed within the different vegetation patches – and human settlement encroachment covering 18% of the buffer area (*ca* 1km) over the past 18 years, thus decreasing the biodiversity corridors. It is also possible that the absence of biophysical disturbances such as fire and browsers in the natural vegetation to suppress wood species partly contributed to the increase of woody cover, since both factors are critical to plant species' niches in the Grassland Biome (Neary and Leonard, 2020). These absences therefore suggest the need

for partial burning by fire of, and the introduction of game species to, the natural vegetation, which might help to restore the grassland species' structure and composition. In addition, the propagule pressure from native and non-native fleshy-fruited woody plant species, which are consumed and dispersed by resident and colonial wintering bird species observed on the study site, is a likely driver of the reported bush encroachment in the FSNBG (Vukeya et al., 2020). Birds' gut treatment is known to improve seed germination for certain species, and thus expedite their population growth (Mokotjomela et al., 2021). Therefore, there is a need regularly to monitor vegetation in the study site in order to manage the biodiversity conservation threats. Since remote sensing cannot estimate individual plant species' cover, further research to estimate the vegetation cover of individual species per vegetation type will be required.

The FSNBG preserves the national biodiversity conservation priority vegetation type of the Bloemfontein Karroid Shrubland. This category of biodiversity (i.e., CBA1) is prioritised because it is irreplaceable (SANBI, 2017). In this study, the conservation status of the CBA1 vegetation type within the FSNBG was validated by comparing the currently occurring species with those described by Mucina and Rutherford (2006). The ground-truthing results highlighted that the vegetation intactness of CBA1 is still above the threshold of 60%, which suggests that this patch still has habitat functionality (Mokotjomela and Nombewu, 2019; Kleynhans et al., 2008). However, the ecological integrity of the CBA1 vegetation type could have been reduced by several biodiversity conservation threats, including biological invasion. The study therefore suggests the need for regular vegetation monitoring and intervention to manage the prevailing conservation threats. Since ecological support areas (ESAs) play an important role in supporting the ecological function of CBAs' vegetation (SANBI, 2017), further research will be required to ground-truth other ESAs (e.g., Winburg Grassy Shrubland, Riparian vegetation, and Dry Bloemfontein Grassland).

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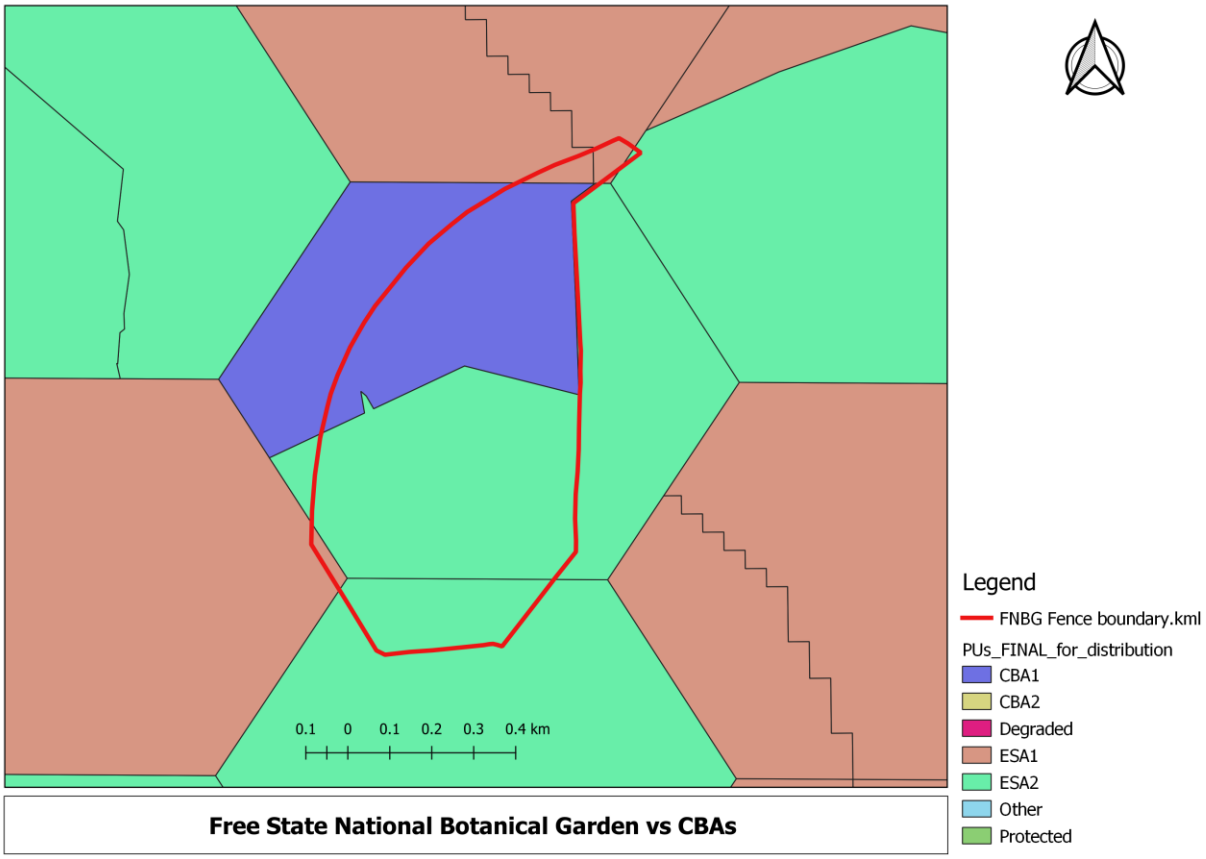

Appendix 3.1: Relative location of preserved vegetation type that falls under critical biodiversity areas (CBAs) in the Free State National Botanical Garden.

Appendix 3.2: Aerial photographs of the study site (FSNBG) showing the encroachment of woody cover over 78 years.

Chapter 4: Biotic interactions 1: Seed dispersal phenology of encroaching tree/shrub species in Free State National Botanical Garden, South Africa

4.1. Abstract

Free State National Botanical Garden vegetation has been experiencing bush encroachment that transforms the preserved national priority 'critical biodiversity vegetation'. Critical biodiversity areas are irreplaceable, and so must protected from any ecological threats. To understand the tree/shrub species' population dynamic, I investigated the seed dispersal phenology of the encroaching tree/shrub species and the influential factors. The aims of this study were: 1) to determine which tree/shrub species are dispersed by birds, and the timing of such dispersal for each tree/shrubs species; 2) to determine the factors that potentially influence fruit consumption by birds and the subsequent seeds dispersal; and 3) to identify the avian frugivores species that are potentially responsible for seed dispersal in the Grassland Biome of South Africa. The results from seed collection in 17 roosting sites show that a total of 22 161 seeds belonging to 14 tree/shrub species under 11 families were eaten and defecated by foraging birds. The fruits of six tree/shrub species were significantly foraged and their seeds dispersed during the study's month of monitoring. Out of five fruit colours, the avian frugivores preferred the red (*Z. mucronata, E. rigida,* and *V. rotundifolium*) and black fruits (*O. subsp. africana* and *D. lyciodes*). *Ziziphus mucronata* and *Olea europaea subsp. africana* presented the significantly highest number of fruit/seeds between March and July, while *Searsia lancea* and *Ehretia rigida* presented theirs between August and December – a dichotomy that could minimise the competition for dispersal agents while sustaining the resident bird species. Over 480 hours of trapping 22 bird species were captured in camera traps, of which seven displayed a high forging frequency and were resident in behaviour, while six species were migratory and colonial avian frugivores, including two alien bird species. I conclude that the reported bush encroachment is a result of high propagule pressure from resident frugivorous birds in combination with low fruit diversity in the grassland habitat.

Keywords: Avian frugivory, Bush encroachment, Keystone fruits resource, Grassland biome, Conservation.

4.2. Introduction

Seed dispersal is important for reducing competition among siblings, species' colonisation of new sites, and population persistence (Howe and Smallwood, 1982; Clark et al., 1998; Nathan et al., 2003). Therefore, the phenology of seed dispersal is critical to the successful recruitment of species (Stiles, 1980; Jordano, 2000), since dispersed seeds require conducive conditions for germination (Footitt et al., 2018); and where dispersal vectors are migratory, fruit ripening and the arrival of the vectors should be synchronised (Stiles, 1980; Johnson et al., 2019). Phenology is the study of seasonal and interannual variations in the life and growth cycle of plants (e.g., leafing, flowering, fruiting, seeding etc.) (Rathcke and Lacey, 1985; Willson and Whelan, 1993; Scranton and Amarasekare, 2017; Wolf et al., 2017; Gonzalez-Varo et al., 2019; Johnson et al., 2019). Temporal availability fruit resources are an example of fruiting phenology in plants (Willson and Whelan, 1993; Peres, 2000; Daiz-Martin et al., 2014; Maruyama et al., 2019). For four seasons of the year, plants are exposed to periodic environmental conditions that change with respect to the physiological state that influences fruits' development and seed maturation (Willson and Whelan, 1993; Traveset et al., 2014; Johnson et al., 2019). Such changes in the environment can also affect seed dispersal in plants (Johnson et al., 2019).

Fruit traits (i.e., fruit size, colour, and phenology) play an important role in the fruits' selection and the success of seed dispersal (Gautier-Hion et al., 1985; Jordano, 2000; Gosper and Vivian-Smith, 2009; Galetti et al., 2011). Previous studies have reported a positive relationship between fruit size and the number of seeds dispersed by birds (Gosper et al., 2005; Cazetta et al., 2008; Galetti et al., 2011). Large fruits are preferred by birds, and this enhances the probability of their being consumed and have their seeds dispersed away from the maternal plant by frugivores (Schupp et al., 2019; Galetti et al., 2013). Large flesh fruits may have a higher nutrient content and many seeds, thus increasing the probability of their being dispersed (Moles and Westoby, 2006; Schurr et al., 2009; Velázquez-Rosa et al., 2017). In this regard, Mokotjomela et al. (2013) showed that avian frugivores concentrate their foraging activities on the more abundant alien and invasive fruits with higher sugar content than on native plant species in the South African Mediterranean-climate. Consistent with this finding, Schupp et al. (2019) stipulated that, for avian frugivores that swallow the entire fruit, their selection is driven by the fruit's size, which is positively correlated with the width of the bird's bill. However, Johnson et al. (1985) and Ally (2010) both indicated that birds' bill size appears not to affect fruit consumption over a range of fruit sizes.

It has been shown that fruit colour could influence the foraging preferences of birds for fleshy fruits (Wilson and Thompson, 1982). Schaefer et al. (2008) confirmed that birds use fruit colour as a foraging signal. Bright-colour ripe fruits also attract avian seed dispersers (e.g., avian frugivores). Birds also commonly prefer black fruits (Knight and Siegfried, 1983;

Wilson and Whelan, 1990; Jordano, 2000; Mokotjomela et al., 2013; Ally, 2010; Schaefer et al., 2014) because they are rich in lipid content (Schaefer et al., 2014) with higher water content (Wilson and Downs, 2010). Consistent with this, Gagetti et al. (2015) reported that red and black fruits were those most consumed in the state of São Paulo, Southeastern Brazil.

The effectiveness of seed dispersal mediated by avian frugivores is calculated as the quantity of seeds dispersed (Schupp, 1993; Mokotjomela eta al., 2016, 2013). The quantity component of seed dispersal is dependent on a number of interactive events between the maternal plant and the avian vectors (Schupp et al., 2010; Traveset et al., 2014; Ganzalez-Varo et al., 2018). It has been shown that the quantity of seeds dispersed can be influenced by fruit crop size, bird body mass, and the foraging visitation frequency of the vector (Jordano, 2000; Schurr et al., 2009; Mokotjomela, 2012; Mokotjomela et al., 2013, 2016). Previous studies have reported that vectors (i.e., birds) with a large body mass are likely to disperse large numbers of seeds more effectively than small vectors (Schupp, 1993; Jordano and Schupp, 2000), which then increases the probability of species' recruitment success (Levine and Murrel, 2003). Nevertheless, Mokotjomela et al. (2013) demonstrated that a high foraging visitation frequency by small frugivorous birds improves the quantity of seeds being dispersed, thereby offsetting the body size limitation. This was confirmed by Godinez-Alvarez et al. (2020) in a report showing that small and medium birds have a relatively higher seed dispersal effectiveness than large birds.

The Free State National Botanical Garden (FSNBG) is a protected area with vegetation that is classified as critical biodiversity areas (CBAs). There is a growing conservation concern that rapid environmental degradation owing to global climate change and anthropogenic habitat fragmentation threatens animal/plant interactions in the Grassland Biome in South Africa (see Neke and du Plessis, 2004; Matsika, 2008; Brown and du Preez, 2014). Disturbance by unplanned frequent fires and changing land use leads to the decoupling of mutualist partner organisms; and this may comprise the ecological services in the local habitat (Boucher et al., 1982; Bond, 1994; Aslan et al., 2013). In addition, a 40-year vegetation cover study suggests that bush encroachment is threatening vegetation integrity in FSNBG (Vukeya et al., 2020). That tree/shrub cover has increased could suggest that FSNBG is receiving seeds of the species that are effectively colonising the local habitat. However, the main drivers of vegetation cover change are not currently known to the FSNBG – but this is critical information for the effective adaptive management that would sustainably conserve the biodiversity of the FSNBG and its tourism functions. In view of these factors, the aims of this study were: 1) to determine which tree/shrub species are dispersed by birds, and the timing of such dispersal for each tree/shrub species; 2) to determine the factors that potentially influence fruit consumption by birds, and the subsequent seed dispersal; and 3)

to identify the avian frugivores species that are potentially responsible for seed dispersal in the Grassland Biome of South Africa.

4.2. Methods and material

4.2.1. Study site

The study was conducted in the Free State National Botanical Garden (S 29° 12´ 55.6˝; E 26° 12´ 41,3˝), Free State Province, South Africa. The conservation garden covers an area of 66 hectares, and is at an altitude between 1300 and 1400m above sea level (Masilo, 1999; Chaplin, 1979). The study site is located within the Dry Highveld Grassland bioregion of the Grassland Biome in the central plateau of South Africa (Mucina and Rutherford, 2006). The vegetation's structure is a predominance of grasses, with sparse tree/shrub vegetation restricted by rocky slopes (Mucina and Rutherford, 2006). The topography ranges from plains and pans to lowland, with a dolerite hill (Monk Head), separated by a valley and a dry watercourse. The conservation garden experiences a severe continental climate, with minimum temperatures below 0˚C with frost conditions in winter, and maximum temperatures of 35˚C in summer (Haddad et al., 2019; Neethling and Haddad, 2013). The annual rainfall ranges between 443.6 mm and 718.4 mm, with most rain being recorded in early autumn and in summer.

4.2.2. Collection of bird-dispersed seeds

Seeds were collected from various identified bird roosting areas (17 locations) within FSNBG from March 2019 to February 2020. Only seeds that had been ingested and treated (i.e., that had passed through frugivorous birds' gut and been trapped in the faecal samples) were collected (Figure 4.1). The seeds that were free from insects and diseases were stored in paper bags in a seed storeroom before the individual seeds were counted. I used the seeds' morphological structure (size, shape, coat, length, and width) to identify them (Ulian et al., 2019). For small seeds, I used a portable pocket 100x metal mini science microscope to identify each seed (Song et al., 2018). For further identification, existing knowledge and different plant field guides (e.g., Ulian et al., 2019) and a South African database (www.plantzafrica.com) were used.

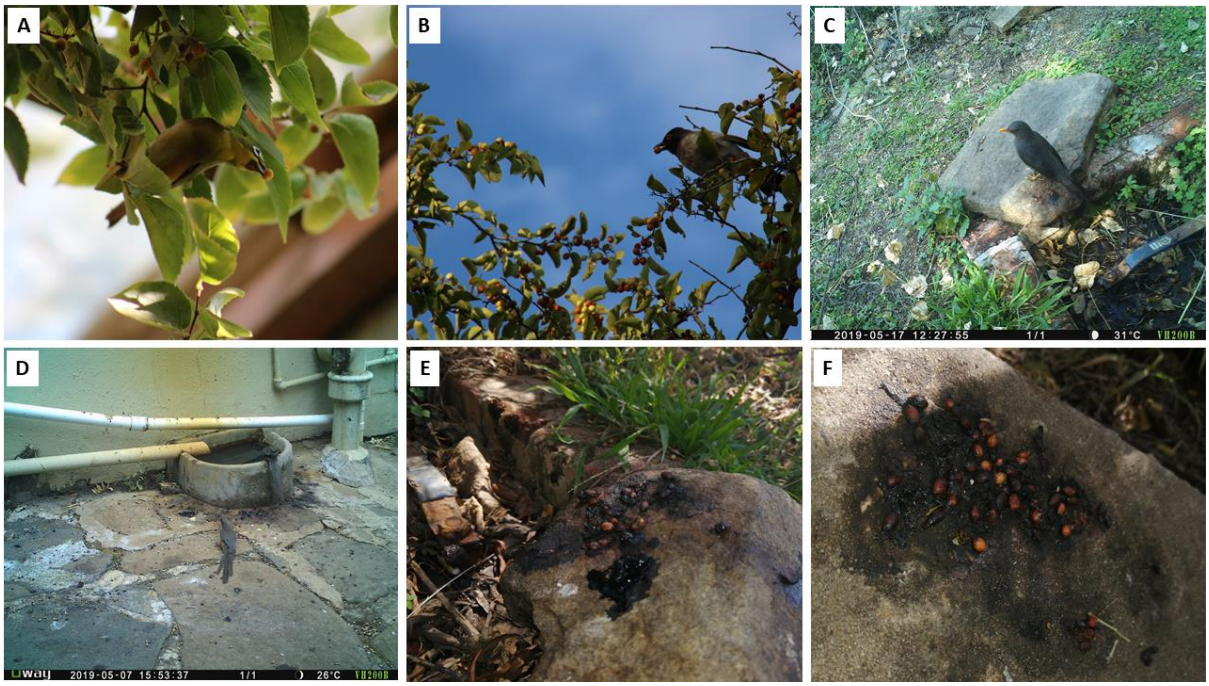

Figure 4.1: Interactions between birds and fruits in Free State National Botanical Garden: A: White stinkwood “Celtis africana” fruits being consumed by Cape white-eye “Zosterops capensis”; B: Buffalo thorn ‘’Ziziphus mucronata” with flesh ripe fruits being consumed by Red-eyed bulbul “Pycnonotus nigricans”. (C) Olive thrush “Turdus olivaceus”, and (D) Speckled mousebird “Colius striatus” were captured in the roosting sites with faecal samples. E and F show the defecated seeds.

4.2.3. The role of fruits’ characteristics

To determine the variations in the seed sizes of species fruits foraged by avian frugivores, the seed size of all identified tree/shrub species was measured. A digital calliper was also used to measure the length in millimetres (mm) of each fruit and seed per species (i.e., 30 replicates) as a surrogate measure size.

The fruits’ nutrient composition characteristics were obtained from the published literature (Table 4.1).

Table 4.1: Fruit characteristics of the tree/shrub species occurring in the Free State National Botanical Garden (*** non-native species): fruit type and colour, nutrients composition, fruits and seed size. The bolded species showed significant variation in quantity of seed dispersal (number seeds) across the months.

Plant species	Family	Life-form	Fruit type	Fruit colour	Fruit size: Length (mm): mean ± SE (N = 30)	Seed size ± SE (N = 30) Length (mm)
Ziziphus mucronata	**Rhamnaceae**	**Shrub**	**Drupe**	**Reddish-brown or deep red**	**10.28 ± 0.15**	**8.72 ± 0.13**
Olea europaea subsp. africana	**Oleaceae**	**Shrub**	**Drupe**	**Purple- black**	**7.73 ± 0.17**	**7.61 ± 0.14**
Grewia occidentalis	**Malvaceae**	**Shrub**	**Drupe**	**Reddish brown**	**13.08 ± 0.13**	**4.72 ± 0.13**
Ehretia rigida	**Boraginaceae**	**Shrub/tree**	**Berry**	**Orange red- black**	**6.94 ± 0.11**	**3.52 ± 0.10**
Viscum rotundifolium	**Santalaceae**	**Parasite**	**Berry**	**Orange-red**	**4.19 ± 0.056**	**3.61 ± 0.09**
Diospyros lyciodes	**Ebenaceae**	**Shrub**	**Berry**	**Orange red or dark red**	**14.83 ± 0.11**	**8.57 ± 0.14**
Celtis africana	**Ulmaceae**	**Tree**	**Drupe**	**Yellow or brownish**	**5.07 ± 0.09**	**4.26 ± 0.09**
Clutia pulchella	Paraceae	Shrub	Capsule	Orange	11.26 ± 0.23	7.45 ± 0.26
Searsia lancea	Anacardiaceae	Tree	Drupe	Dull yellow to brown	4.88 ± 0.029	4.23 ± 0.05
Rhamnus prinoides	Rhamnaceae	Shrub	Drupe	Reddish to purple-black	5.84 ± 0.075	3.68 ± 0.06
****Pyracantha angustifolia*	Rosaceae	Shrub	Berry	Yellow-orange	9.66 ± 0.92	3.25 ± 0.03
****Melia azedarach*	Meliaceae	Tree	Berry	Yellow-brown	9.85 ± 0.86	8.25 ± 0.24
****Cestrum laevigatum*	Solanaceae	Tree	Berry	Purple-black	9.91 ± 1.11	3.45 ±0.09
****Ligustrum japonicum*	Oleaceae	Shrub	berry	Purple-black	6.67 ± 0.05	5.79 ± 0.06

4.2.4. Documenting bird species

The digital surveillance camera traps (Uway VH200B) were set up to capture birds' frequency of visitation in different fruiting tree/shrub species during the fruit peak period. The camera traps were set up for eight hours a day: from 08h00 (at sunrise) until 17h00 afternoon (Malan et al., 2007). At sunset (18h00), camera traps were occasionally left operational in an attempt to identify the nocturnal mammals that might provide secondary seed dispersal services to the deposited seeds, and even predation. Five camera traps were set up, and each represented a replicate in each trapping site. Trapping was done on days without rain and wind conditions. At each trapping site, the data birds that were captured consecutively were treated as one sample. The identification of the bird species was done using both sound and visuals, supported by different bird field guides (e.g., Sinclair et al., 2011) and online information (https://www.birdlife.org.za). Each bird species was classified by family, adult body, foraging movement behaviour, and whether alien or native (Hockey et al., 2005).

4.3. Statistical analyses

4.3.1. Collection of bird-dispersed seeds

The bird-dispersed seeds from each plant species were counted (i.e., the response variable) and identified at species level. The seed count data was not normally distributed, and then it was log-transformed. Because of the unbalanced experimental design, the nonparametric statistical model – Kruskal-Wallis ANOVA – was used to compare the numbers of seeds dispersed (i.e., the count data) between the native and non-native tree/shrub species.

The general linear model – factorial analysis of variance – was applied to determine the significant differences in the seed counts for different tree/shrub species in different months, and to identify the most highly preferred species, using SPSS Version 20. The response variable consisted of the counts of the seeds. The tree/shrub species and months were specified as the predictor variables in order to allow an assessment of the interactions between the different tree/shrub species and months.

4.3.2. The role of fruits' characteristics in the seeds' dispersal phenology

I used the fruits' visual attributes (i.e., size and colour) to infer the potential drivers of fruit preference and the subsequent seed dispersal.

The fruit size data sets were tested for correlations with the number of seeds dispersed for different tree/shrub species, using SPSS Version 20.

4.3.3. Documenting bird species

The birds' foraging visitation frequency was determined from images from the camera traps. Thereafter the frequency was converted into proportions (%) to determine the important potential avian dispersers.

4.4. Results

4.4.1. Collection of bird-dispersed seeds

A total of 22 161 seeds, belonging to 14 tree/shrub species in eleven (11) families and 14 genera, along with two unknown seeds, were collected from 17 birds' roosting points. The 14 tree/shrub species included 10 native species and four alien species.

Overall, the number of seeds dispersed between the native species versus the alien and invasive species differed significantly ($H(1, 2688) = 115.9$; $p < 0.001$); the native species had a larger number of collected seeds than the alien and invasive species (mean ± SE (medians; %): 11.43 ± 1.46 (1411.5; 99%) vs 0.27 ± 0.05 (1177.1; 1%).

Also, there were significant differences in the number of dispersed seeds collected for the different tree/shrub species ($F(15, 2880) = 40.9$; $p < 0.001$; Figure 4.2), with *O. subsp. africana* being more dispersed than all the other tree/shrub species (mean ± SE: 47.1 ± 11.6) (Figure 4.2).

Over a one-year monitoring period, I noted a significantly different number of seeds dispersed each month ($F(11, 2880) = 35.971$; $p < 0.001$). For the seven tree/shrub species, most of the seeds were dispersed from March to July, with the common peak dispersals occurring in May and December, thus suggesting the presence of fruits during these times. There were significant interactions between the number of dispersed seeds from different tree/shrub species and the months of the year during the monitoring period ($F(165, 2880) = 10.5$; $p < 0.001$; Figure 4.2). The fruits of seven tree/shrub species (*Olea europaea subsp. africana, Ziziphus mucronata, Diospyros lycioides, Grewia occidentalis, Celtis africana, Ehretia rigida,* and *Viscum rotundifolium*) had their fruits significantly foraged and their seeds dispersed during the study's months of monitoring (Figure 4.2; Table 4.2*). Olea. subsp. africana* (mean ± SE: 47.1 ± 11.6) and *Z. mucronata* (51.4 ± 9.0) were mostly dispersed in April to June, along *with Diospyros lycioides, Grewia occidentalis, Celtis africana, Rhamnus prinoides*, and *Viscum rotundifolium*; Figure 4.2), while only two species (*Ehretia rigida*: 373.6 ± 91.5 and *Sersia lancea*: 221.6 ± 74.1; Figure 4.2) had seeds dispersed during the local fruit scarcity period between August and December.

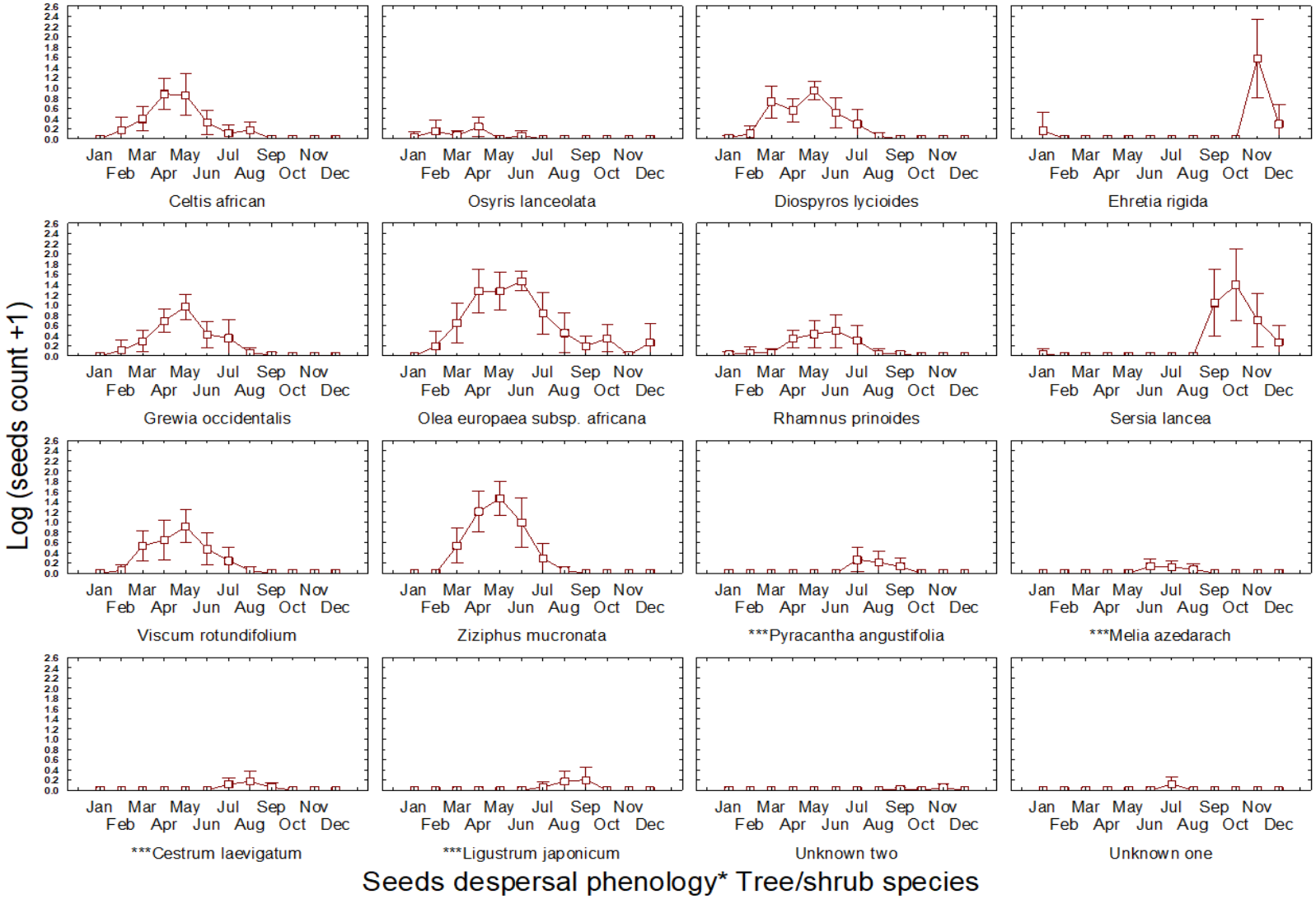

Figure 4.2: Variation in mean numbers of bird-dispersed seeds for different trees/shrub species (*** alien and invasive species) across different months of the year collected in FSNBG. Error bars represent ± Standard error of mean.

4.4.2. The role of fruits' characteristics

There was no correlation between the fruit size and the number of seeds of different tree/shrub species dispersed by birds.

Overall, I noted five fruit colours that were foraged by avian frugivores, with the red *(Z. mucronata, E. rigida*, and *V. rotundifolium*) and black fruits (*O. subsp. africana, D. lyciodes* and *R. prinoides*) being more preferred than all the other fruit colours (e.g., brown: *G. occidentalis* and *C. africana*) of the tree/shrub species.

4.4.3. Bird species

Among the 168 bird species documented in FSNBG's data, a total of 22 bird species belonging to 12 families were captured by the camera trap at the 17 bird trapping points of the study area (Table 4.2). Out of the 22 bird species, seven displayed a high frequency of capture and were resident in behaviour, while six species were migratory and colonial avian frugivores, including two alien species (Table 4.2).

Table 4.2: Documented avian frugivores captured from different birds' roosting areas in the Free State National Botanical Garden (*** non-native species and ¥ showing colonial bird species): body size, behaviour and % frequency of capture. The bolded bird species represent the important dispersal vectors.

Bird species (scientific name)	Common name	Family	Body size (g)	Behaviour	Frequency of capture (%) N = 2850
Pycnonotus nigricans	**African red-eyed bulbul**	**Pycnonotidae**	**30.8**	**Resident & Nomad**	**12.6**
Turdus olivaceus	**Olive thrush**	**Turdidae**	**78.0**	**Resident**	**11.6**
Cossypha caffra	**Cape robin-chat**	**Muscicapidae**	**28.4**	**Resident**	**9.5**
Columba guinea	**Speckled pigeon**	**Columbidae**	**344**	**Resident**	**8.4**
Zosterops capensis	**Cape white-eye**	**Zosteropidae**	**10.9**	**Resident**	**7.4**
Streptopelia senegalensis	**Laughing dove**	**Columbidae**	**101.5**	**Resident**	**5.3**
Passer domesticus	**House sparrow**	**Passeridae**	**24.0**	**Resident**	**5.3**
¥**Sturnus vulgaris	**Common starling**	**Sturnidae**	**77.6**	**Migratory**	**2.1**
¥Onychognathus morio	**Red-winged starling**	**Sturnidae**	**78.0**	**Migratory**	**2.1**
¥Creatophora cinerea	**Wattled starling**	**Sturnidae**	**67.0**	**Migratory**	**2.1**
¥Spreo bicolor	**Pied starling**	**Sturnidae**	**23.0**	**Migratory & Resident**	**2.1**
¥Colius colius	**White-backed mousebird**	**Coliidae**	**39.9**	**Migratory & Resident**	**2.1**
¥Urocolius indicus	**Red-faced mousebirds**	**Coliidae**	**56.2**	**Migratory & Resident**	**4.2**
Colius striatus	Specked mousebird	Coliidae	55.0	Resident & Nomad	2.1
Streptopelia semitorquata	Red-eyed dove	Columbidae	252.0	Resident	4.2
Streptopelia capicola	Cape turtle-dove	Columbidae	152.9	Resident	4.2

Ploceus capensis	Cape weaver	Ploceidae	57.0	Resident	3.2
Upupa africana	African hoopoe	Upupidae	30.8	Resident, Nomad & Migratory	3.2
Trachyphonus vaillantii	Crested barbet	Lybiidae	73.6	Resident	2.1
***Acridotheres tristis*	Common myna	Sturnidae	35.0	Migratory	2.1
Lanius collaris	Common fiscal	Laniidae	29.0	Resident	2.1
Ploceus velatus	Southern masked weaver	Ploceidae	17.0	Resident	2.1

4.5. Discussion

The fact that I found only 10 native and four non-native tree/shrub species in the study site could be explained by the predominance of the Grassland Biome that inherently possesses few fruiting woody plant species (Mucina and Rutherford, 2006). Although the Grassland Biome is reportedly the second most highly invaded biome after the Fynbos Biome in South Africa (Van Wilgen and Wilson, 2018); and the finding of a few seeds of alien species can be partly attributed to the limited movement of birds between home gardens as the sources of the alien fruits (McLean et al., 2017, 2018) and the natural vegetation in FSNBG. Previous studies have shown that anthropogenic habitat fragmentation is associated with colonisation by alien plant species (Hobbs and Huenneke, 1992; Alston and Richardson, 2006; Catford et al., 2012; Van Rensburg, 2017). It has also been shown that frugivorous birds avoid foraging in highly disturbed habitats (Gomes et al., 2008), which could affect their movement behaviour. Consequently, it is suggested that limited human-mediated disturbance in FSNBG, in combination with resident seed dispersers (i.e., resident birds) could be the important driver of the increasing wood plant species cover in FSNBG.

The interaction between birds and fruits depends on the fruiting phenology of the plants and the nutritional demands of the birds (Jordano, 2000; Holland and Bronstein, 2008). I found that 80% of the bird-ingested and dispersed seeds of many tree/shrub species were collected between March and July, which could suggest the availability of ripe fruit resources on the site during this period. A similar fruiting pattern among tree/shrub species in Free State Province, South Africa was reported by Zietsman et al. (1989); and this observation indicates that the large populations of wintering bird species observed in FSNBG during this period may be relying on a fruit diet for sustenance (De Swardt, 2014). Out of 14 tree/shrub species, I observed a higher consumption of the seven red and black fleshy fruits than of the other fruit colours; and birds' preference for the red and black fruit colours has been reported elsewhere (Knight and Siegfried, 1983; Ally, 2010; Schaefer et al., 2014; Gagetti et al., 2015). It is believed that frugivorous birds use fruits' colour as a signal to select specific fruits during foraging (Schaefer et al., 2008). The results also highlight the mutual importance of wintering sites for conservation (Johnson et al., 2019), since these tree/shrub species may rely on wintering birds to disperse their seeds (Viana et al., 2015). I argue that the colonial wintering bird species may be partly responsible for the high propagule pressure owing to localised movement, and thus, may partly account for the increasing bush encroachment (Vukeya et al., 2020). Although previous studies have reported a significant correlation of seed dispersal quantities with fruit size (see Jordano, 1995; Gosper et al., 2005; Cazetta et al., 2008; Galetti et al., 2011; Palacia and Ordano, 2018), the results show no correlation of fruit size with the number of dispersed seeds. A plausible explanation is that low levels of fruit diversity prevented a comparison of the fruit size preferences and the number of seeds.

I note that *O. subsp. africana's* fruits were most preferred by foraging frugivorous birds in the study site. Having the longest fruiting period (i.e., 10 months), together with the large fruit crop of *O. subsp. africana* (Mokotjomela, 2012), which birds prefer, is a typical attribute of keystone fruit resources (Lambert and Marshall, 1991; Mills et al., 1993; Mokotjomela et al., 2009). Similarly, this criterion can be applied to the *Z. mucronata* species, as it displayed attributes like those of *O. subsp. africana* at the study site between April and June. The keystone fruit resource species may help to define an ecosystem/habitat's identity (Paine, 1969), and may act as a key indicator of ecosystem/habitat change.

By contrast, two shrub species, *E. rigida* and *S. lancea,* displayed relatively short fruiting peak times between August and December, when other fruits were scarce, thereby sustaining the resident bird species. By fruiting in periods of scarcity, these tree/shrub species may also avoid possible competition for the birds' seed dispersal services and thus for access to safe microsites (Stiles, 1980; Hulme, 1998; Bronstein, 2001). Indeed, different plant species evolve different mechanisms for survival during their seed phases (Stiles, 1980; Hulme, 1998; Wang and Smith, 2002; Schup et al., 2010). However, it is not clear whether the observed fruit presentation patterns in these two species are an evolutionary response to avoid competition with other tree/shrub species for migrant dispersal agents, or the result of microclimatic influences on the tree/shrub species.

4.6. Concluding remarks

Biotic interactions provide the opportunity for important ecological services such as the dispersal of the seed that is ingested by birds during foraging (Whelan et al., 2015; Le Roux et al., 2020). I investigated biotic interactions, focusing on the fruit resource plant species used by birds in the study area. The results confirmed that biotic interactions between birds and fruiting plants assist in the seed dispersal of 10 native and four non-native fruiting tree/shrub species whose seeds were extracted from faecal samples collected from different roosting areas of birds. Such dispersal services were provided by the 22 foraging species, including two alien birds, that were sampled with camera traps. From the bird sampling, it was noted that since frugivorous birds avoid high disturbance areas (Gomes et al., 2008), they adapt to localised foraging patterns and to limited fruit diversity. I argue that this adaptation could be reflected in a few alien fruit/seed species that are usually foraged when birds visit home gardens in urban areas. When put together with the high seed rain by colonial wintering bird species observed on the site, I have shown that this is a contributing driver of the reported bush encroachment in FSNBG. Among the fruiting tree/shrub species, it was also found that *Z. mucronata* and *O. subsp. africana* co-occurred and presented the largest number of fruits/seeds for foraging birds in the study area; and this is indicative of their keystone function on the site. Finally, I suggest that the dichotomy in the fruit presentation of

the above species from that of *E. rigida* and *S. lancea* may represent an evolutionary response to avoid competition for seed dispersal services; while, from a conservation perspective, I suggest that this pattern provides sustenance for local frugivorous birds during periods of fruit scarcity.

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Chapter 5: Biotic interactions 2: Interspecific competition in germination of bird-dispersed seeds in a habitat with sparse tree vegetation in South Africa

5.1. Abstract

Avian frugivores provide directed seed dispersal services that contribute in shaping the native plant communities and facilitate population persistence. By transporting and scarifying the seeds during ingestion, avian frugivores reduce the competition with siblings, and may improve seed germination which is critical for dispersal effectiveness. However, generally, there is limited knowledge on how deposited seeds interact/compete in the new microsite. Therefore, I investigated 1) whether bird-dispersed seeds benefit from improved germination after their passage through the bird's gut; and 2) the potential impact of seed density and species diversity on competition during at the microsites in the Free State Province, South Africa. Overall, the results partly supported the hypothesis. Germination trials with defecated seeds of five plant species compared with the manually depulped seeds showed that despite prolific seed germination observed in *Ziziphus mucronata*, only *Searsia lancea* seeds had significantly high seed germination after passage through the bird gut. Overall, there was a significant correlation between seed size and the germination of bird-ingested seeds except in *Olea subsp. africana* possibly due to possession of extremely hard protective seed cover. Seed competition experiments pointed to *Z. mucronata* and *O. subsp. africana* having significant germination performance that was positively correlated to seed density and seed size while *E. rigida* did not germinate at all. Seed species diversity in the germination trays did not have significant impact since only two former plant species consistently displayed significantly higher germination across the competition levels. It is concluded that not all bird-defecated seeds have their germination improved, and that further long-term tests for germination physiological responses of the seeds' samples used in this study are required since poor germination observed in other tree/shrub species cannot be attributed to competition solely.

Keywords: Avian frugivory, Grassland biome, Seeds, Bush encroachment

5.2. Introduction

Seed dispersal is the movement of seeds away from their parent plants through a variety of dispersal vectors, either abiotic or living (Jones et al., 2017; Thomason et al., 2011; Wang and Smith, 2002; Westcott and Graham, 2000). Seeds in fleshy fruits (i.e., drupe) are dispersed or discarded, often after animals (e.g., birds) have consumed the soft edible portion of the fruit, called the mesocarp, and dispersed the ingested endocarp containing the seeds (Dardick and Callahan, 2014). Seed dispersal is crucial in plant regeneration, since it promotes the maintenance of the genetic structure of many native plant species (Kleyheeg, 2018; Egerer et al., 2018; Castilla et al., 2017; Howe, 2016; Heleno et al., 2011). In addition, many native tree and shrub species require animal-mediated seed dispersal as a longevity strategy in response to environmental change (Howe, 2016). Plants (i.e., angiosperms) interact with multiple mutualists in a network pattern to acquire a variety of ecological services (e.g., pollination and seed dispersal) (Jordano, 2016; Mokotjomela, 2012; Thomas et al., 2007; Strauss and Irwin, 2004). Avian frugivores have been consistently reported to be essential in shaping the seed dispersal pattern of native vegetation structures (Le Roux et al., 2020; Rehm et al., 2019; Trakhtenbrot et al., 2005; Jordano, 2000). For example, Banos-villalba et al. (2017) reported that macaws dispersed 75% to 100% of seeds or fruits over ranges of up to 1 200 m, and influenced the recruitment success of some forest plant species, thus shaping the vegetation structure and function of the Amazonian ecosystem, Beni Savannas.

The effectiveness of seed dispersal is a product of the quality and quantity components of the multiple vertebrate vector species that consume fruits and seeds of different plant species in each habitat (Mokotjomela et al., 2016; Schupp et al., 2010; Schupp, 1993). The germination of ingested seeds represents the quality component, which is regarded as more influential in plant recruitment than the quantity component (Mokotjomela et al., 2016; Schupp et al., 2010; Schupp, 1993). Avian frugivores occasionally modify the rate of germination and enhance the chances of seeds' survival (Kleyheeg, 2018; Mokotjomela et al., 2016 & 2015; Thabethe et al., 2015; Fricke et al., 2013; Mokotjomela, 2012; Barnea et al., 1991). For example, Traveset et al. (2001) tested the germination characteristics of the seeds from different plants (*Rhubus ulmifolius, Osyris alba, Rubia peregrine, Asparagus acutifolius,* and *Phillyrea sp*.) that passed through birds' guts in the western Mediterranean shrubland, and found that different germination speeds/rates were promoted by the treatment in the birds' gut. However, Alves-Costa and Eterovick (2007) investigated the seed/fruit trail of 53 species that were consumed and dispersed by coatis (*Nasua nasua*) in Mangabeiras Park, Southern Brazil, and found that the seeds' passage through the coatis' guts did not affect germination success, except for *Myrcia guajavaefolia*, which enjoyed a 50% increase in germination success. Also, Dlamini et al. (2018) compared

the effect of frugivorous birds on the seed dispersal and germination of two alien invasive plants (*Schinus terebinthifolius* and *Listea glutinosa*) in South Africa, and found that frugivorous birds enhance the germination rate of *Schinus terebinthifolius* after seed treatment, although no positive effect was noted on the germination of *Listea glutinosa* after seed treatment.

The dispersal of large numbers of seeds over long distances from their maternal plants reduces intra- and inter-species competition (Mokotjomela et al., 2013; Schurr et al., 2009; Higgins et al., 2003), and provides essential genetic links between disconnected plant populations (Schupp et al., 2010; Nathan et al., 2008). Long-distance dispersal also increases the chances of recruitment in the absence of seed predators by maximising the seeds' access to safe microsites (Howe, 1986). In competitive conditions, the early dispersal of seed species leads to their occupation of safe microsites both above and below the ground, and consequently increases the species' recruitment success (Korner et al., 2007). Plant species that germinate early may have an advantage in survival and growth, due to having more space and greater access to resources (Guido et al., 2017). The interspecific competition between seeds may differ from year to year, depending on the availability of resources and weather conditions, as well seed density in the microsite.

There are other factors that can cause variation in the seed germination rate, such as seed size (Mandal et al., 2008), the seed's endocarp (i.e., its hormones) (Negash, 2010; Dalling et al., 2011; Bekele, 2000), and the conditions where the seeds are deposited (Carlo and Tewksbury, 2013; Hulme, 1998). The size of the seed will interact with local abiotic conditions and other co-deposited seeds to influence germination. Studies have shown that plant species with large seeds have greater success in their germination rate, and a positive effect on the seedling's survival than do small seeds (Kolodziejek, 2017; Souza et al., 2014; Mandal et al., 2008; Kahmen and Poschlod, 2008; Cordazzo, 2002). Mandal et al. (2008) consistently found that variation in seed size influences the germination rate, after recording a 78% germination rate for the large seeds of *Hyptis suaveolens*, while smaller seeds had a 41% rate of germination success. However, Moles and Westoby (2004) found no correlation between seed mass and the species' germination of viable seeds. The seed endocarp provides physical protection against disease (Dardick and Callahan, 2014), but conversely it is a barrier to the germination rate/speed (Negash, 2010; Bekele, 2000). For example, Negash (2010) and Cuneo et al. (2010) found that the hardened endocarp of *Olea europaea subsp. africana* reduced rapid germination by blocking moisture and oxygen from reaching the seed.

It has been shown that post-dispersal seed competition is important in determining plant recruitment (Ferreira et al., 2016; Wang and Smith, 2002; Hulme, 1998). After primary seed dispersers (i.e., mammals and avian) have played their role, secondary seed dispersal

may contribute to extending the distance from the first seed deposition location and finding safe new microsites (Padilla et al., 2012). For example, Vander Wall et al. (2005) reported a two-phase seed dispersal between avian frugivorous as the primary seed dispersal, and rodents as the secondary seed dispersal, in the Wittell Forest of Reno (University of Nevada System), and found that rodents removed plant seeds from birds' faeces at a rate of 8-50% per day, and scatter-hoarded them into the soil and some caches sites (Vander Wall et al., 2005). Loayza et al. (2014) also assessed whether rodents are an effective seed disperser of the endangered shrub *Myrcianthes coquimbensis* at the southern edge of the Atacama Desert in Chile. They found that rodents played a dual role in the seedling recruitment dynamic of *M. coquimbensis* as both seed predator and effective dispersal agent. Rodents fed on the seed storage tissue of *M. coquimbensis*, thus releasing seed dormancy, and hoarded in the ground more than 60% of the discarded fragmented seeds, which eventually germinated (Loayza et al., 2014). However, some secondary dispersal vectors (e.g., granivores) are seed predators that damage seeds (Heleno et al., 2011). Mokotjomela and Hoffmann (2013) reported a substantial seed predation of the invasive alien tree Acacia cyclops in South Africa, and found that this, in combination with seed production-suppressing biological control agents, may reduce the invasion potential of the species. Also, Soares et al. (2015) investigated forest cover loss using the seed of Euterpe edulis as a keystone palm species in the Brazilian Atlantic Forest and reported that high seed predation by vertebrates was the main factor limiting seed recruitment.

While it is relatively well-known how seeds are affected by passage through the gut and the associated variable retention times (Le Roux et al., 2020; Mokotjomela et al., 2015; Mokotjomela, 2012; Traveset, 2001), how post-dispersal seed predation affects recruitment (Hulme et al., 1998), and how scatter-hoarding also benefits species recruitment (Vander Wall and Beck, 2012), limited attention has been given to how the seeds of different plant species that either remain at the deposition location or survive post-dispersal predation interact to complete the recruitment processes. Hulme (1998) argued that both variation in post-dispersal seed predation and differences in predation between plant species are important elements that facilitate the coexistence of different plant species, and that the same seed predation directly influences competition for these microsites. I therefore postulated that, in areas where perching material for birds is rare – such as the grasslands-dominated habitats of the Free State Province, South Africa – seeds dispersed by birds accumulate in the areas they frequent most, thereby increasing the possibility of density-dependent competition – which, however, must be minimised by the long-distance dispersal of seeds. Studies have shown that the distribution of perching material, as well as selective fruit foraging by birds, influences the patterns of seed deposition (Aukema and Rio, 2002; Howe, 1989; Stiles and White, 1986), with the perching points, and the most preferred fruiting plants

receive large numbers of seeds (Carlo and Tewksbury, 2013). In view of these, the aims of this study were: 1) to determine whether bird-dispersed seeds benefit from improved germination after their passage through the bird's gut; and 2) to investigate potential impact of seed density on competition at microsites, determining whether species diversity influence competition with a focus on the fruit-bearing and avian-dispersed species of tree/shrubs in the Free State Province, South Africa. I tested the hypothesis that the passage of seeds through birds' gut improves germination, and found – against the hypothesis – that not all bird-ingested seed germination was improved in four of the five tree/shrub species.

5.3. Methods and material

5.3.1. Study site

The study was conducted in the Free State National Botanical Garden (S 29° 12´ 55.6˝; E 26° 12´ 41,3˝), Free State Province, South Africa. The garden covers an area of 66 hectares and is at an altitude between 1300 and 1400m above sea level (Masilo, 1999; Chaplin, 1979). The vegetation's structure is largely characterised by Bloemfontein Karroid Shrubland, Bloemfontein Dry Grassland, Winburg Grassy Shrubland, and Waterbody/Riparian vegetation (Mucina and Rutherford, 2006). The botanical garden experiences a severe continental climate, with minimum winter temperatures below 0˚C, the night temperature often below freezing, and frost in July. Fairly high summer temperatures frequently range between 30˚C and 35˚C in January (Haddad et al., 2019; Neethling and Haddad, 2013). The mean annual rainfall is relatively low (584 mm annually), with most of rainfall being recorded during the summer months and in early autumn.

5.3.2. Seed collection and seed viability test

Seeds were collected on weekly bases during dry weather from various identified bird roosting areas (i.e., 17 locations) within FSNBG from March 2019 to February 2020. Only seeds that had been ingested and treated (i.e., that had passed through frugivorous birds' gut and been trapped in the faecal samples) were collected. The seeds, which were free from insects and diseases, were stored in paper bags in a seed storeroom before the individual seed-counting and germination trials. I used the seeds' structure (size, shape, coat, length, and width) to identify the different seeds (Ulian et al., 2019). For small seeds, I used the portable pocket 100x metal mini science microscope to identify each seed (Song et al., 2018). However, since moisture can disturb seed weight measurements, a digital calliper was also used to measure the length of each seed per species (i.e., 30 replicates) as a surrogate measure size.

Seed viability can be tested using the tetrazolium method, which was recommended by Van der Walt and Witkowski (2017) after applying it for a quick estimation of seed viability

and vigour for *Adenium swazicum* in the Skukuza indigenous nursery, Kruger National Park. The tetrazolium test indirectly determines the respiratory activities in the cell found in the seed tissues (Franca-Neto and Krzyzanowski, 2019). It is recommended that at least 100 seeds should be tested in replicates of 50 or less to increase the accuracy (Patil and Dadlani, 2009; Deloache, 1961). Due to the limited number of seeds, I did not experimentally test seed viability, but used the published results for similar species in South Africa (Table 5.1).

Table 5.1: Seed viability (%) of different tree/shrub species that were included in the germination trials in this study. Seed treatment and source of information are provided (** viability of another Grewia species was used).

Species	Family	Seed viability %	Study area/country	Natural/ treated seeds	Source
Ziziphus mucronata	Rhamnaceae	71-100%	South Africa	Natural	Weiersbye and Witkowski, 2002
Olea europaea subsp. africana	Oleaceae	32-39%	South Africa	Natural	Mokotjomela, 2012
***Grewia flava (c. G. occidentalis)*	Malvaceae	29%	South Africa	Natural	Weiersbye and Witkowski, 2002
Ehretia rigida	Boraginaceae	8-45%	South Africa	Natural but sterilised	Wilman et al., 2014
Searsia lancea	Anacardiaceae	40-76%	South Africa	Natural	Weiersbye and Witkowski, 2002

The seeds of all the four species and the extra species were viable in various ways (Table 5.1). Two tree/shrub species, *Z. mucronata* and *S. lancea*, displayed a viability of above 50%, while the other species had a seed viability that ranged from 8% to 45%.

5.3.3. Seed germination success: Ingested seed vs depulped seeds

One of the study's hypotheses was that frugivorous birds modify the rate of seed germination for seeds ingested during foraging (Mokotjomela et al., 2015, 2016). To study the impact of birds' ingestion of seeds on the germination success for plant species, I conducted seed germination trials, using the seed of four dominant native plant tree/shrub species*: Ziziphus mucronata, Olea europaea subsp. africana, Sersia lancea*, and *Ehretia rigida*, whose seeds had passed through the gut of frugivorous birds. I also used manually

depulped seeds as experimental control. All the ingested seeds were collected from the 17 birds' roosting areas in the FSNBG.

However, I did not have appropriate infrastructure for keeping trapped birds for feeding trials in captivity, and thus research ethics could not endorse bird trapping. The surveillance digital camera traps (Uway VH200B) are considered a nonintrusive method to sample wildlife activities (Kay et al., 2011; Mokotjomela and Hoffmann, 2013). The camera traps were used to document bird species that defecated ingested seeds in different roosting sites during the pick fruiting time. Camera traps were set up following the bird foraging activity pattern: at sunrise (6h00), and monitoring at noon (12h00), and reset at 03h00 for afternoon. At sunset (18h00), image data was downloaded from cameras and occasionally, they were left in attempt to determine the nocturnal mammals that might provide secondary seed dispersal services to the deposited seeds.

Common bird species that ingested and defecated intact seeds included (iv) Olive thrush *Turdus olivaceus*, (v) Speckled mousebird *Colius striatus* and (vi) Cape robin-chat *Cossypha caffra* recorded in one roosting site in the study area (Figure. 5.1).

Figure 5.1: Birds roosting areas: faecal samples were collected during the study (i) – (iii). Red circle shows regurgitated seeds and blue circle shows defecated seeds. Common bird species that ingested and defecated intact seeds included (iv) Olive thrush *Turdus olivaceus*, (v) Speckled mousebird *Colius striatus* and (vi) Cape robin-chat *Cossypha caffra* recorded in one roosting site in the study area.

For depulped seeds, ripe and flesh fruits were harvested directly from four native tree /shrub species. All the fruits from the four plant species were soft and moist; the seeds' chaff was rubbed off in water by hand, except for the fruits of *Z. mucronata*, for which a wire mesh screen was used: the fruit was rubbed off the seeds by moving them back and forth against the screen. A total of 100 seeds per plant species (50 ingested seeds and 50 depulped seeds) were used for the germination trials.

Before sowing the seeds, the trays were washed with household bleach to sterilise them against fungal attack and insect pests (Ulian et al., 2019; Mokotjomela et al., 2015). Forty trays were washed and placed in the sun to dry for a week before sowing (Nichols, 2005). Each tray's dimension was 16cm x 14cm x 4.5cm; and each had drainage holes. The seeds were sown in the trays using 30 dm3 of potting soil, comprising milled pine bark, black loam, coarse river sand, and coconut palm fibre without any other components. Potting soil is a free-draining mixture that can hold moisture for the seed germination process to start (Nichols, 2005). The potting soil was mixed with water to make it moist before it was placed in the trays. The seeds were sown at least 1-1.5 cm deep from the top of the tray to allow enough room during watering, and 2-4 cm apart, depending on the species (Ulian et al., 2019). For each plant species, 10 ingested seeds (for the treatment) and 10 manually depulped seeds (for experimental control) were sown in different trays, and each tray was replicated five times. The seedlings' emergence was monitored on a daily basis, and the number of seedlings that emerged in each germination tray was recorded. Irrigation was done as necessary, either in the mornings before 10h00, or later, after 17h00. The seed germination trials were monitored over a period of at least three months, from November 2019 to the end of March 2020, to allow for possible delays in the seed germination of other species.

The study also hypothesised that variations in seed size (i.e., their weight and length) may influence their successful germination, with large seeds having a greater germination success than smaller seeds (see Souza et al., 2014; Mandal et al., 2008; Kahmen and Poschlod, 2008; Cordazzo, 2002). Therefore, the seed size of different tree/shrub species was measured. Because some seeds were too small for the scale, twenty seeds of each plant species were randomly selected and had their weight measured using a SWAN series digital scale in grams, which was replicated 30 times. Each measure of weight for 20 seeds was divided by the total number of seeds (i.e., 20) to get the weight of a single seed. Also, since moisture can confound seed weight, 30 seeds had their length as a surrogate measure of size measured using a digital calliper (i.e., the longitudinal dimension of each seed) for each plant species (Kleyheeg et al., 2018). Environmental conditions where seeds were collected, and the seed storage time had minimal impact on the results since almost all faecal

samples was collected from dry surfaces and all seeds were sown within minimal dormancy time threshold (Table 5.2).

Table 5.2: Seeds attributes for different tree/shrubs that were included in the germination trails.

Species	Family	Mean seed size ± Standard Error		Seed viability storage period (source)
		Seed length (mm)	Seed weight (g)	
Ziziphus mucronata	Rhamnaceae	8.72 ±0.13mm	0.19 ± 0.004g	Seed viability improved with an age up to1 year in the dry storage (Weiersbye and Witkowski, 2002)
Olea europaea subsp. Africana	Oleaceae	7.61 ± 0.14mm	0.09 ±0.003g	Improves seed germination after being stored for three year (Fabbri et al., 2004)
Grewia occidentalis	Malvaceae	4.71 ± 0.13mm	0.04 ±0.002g	***Grewia bicolor (c. G. occidentalis),* seed can be stored up to 1 year before sowing (Heuzé et al., 2015)
Ehretia rigida	Boraginaceae	3.52 ± 0.10mm	0.03 ±0.007g	** *Ehretia cymosa (c. E. rigida*) seed can be stored up to 12 months (Angaine et al., 2020)
Searsia lancea	Anacardiaceae	4.21 ± 0.05mm	0.02 ±0.001g	Seed viability persist for up to 1 year (Weiersbye and Witkowski, 2002)

5.3.4. Interspecific seed competition across different densities between plant species

In this experiment, I investigated the competitive interactions during germination among seeds deposited by avian frugivores for five tree/shrub species that are likely to compete for dispersers' vectors (i.e., birds): *Z. mucronata*, *O. subsp. africana, G. occidentalis* (seeds dispersed between March to July), and *S. lancea* and *E. rigida* (seeds dispersed between August and December). The hypothesis tested in this experiment was that the germination of bird-dispersed seed is free from competition near the maternal plant, and thus germinates much better than undispersed seeds. The bird-ingested seeds were sown in the trays (size: 16 cm x16 cm x 4.5 cm), which were filled with potting soil and had drainage holes in the bottom. The seeds of each tree/shrub species were spread randomly in the trays and covered with a 1 cm layer of potting soil to mimic natural conditions (Nichols, 2005). Species that were dispersed first (i.e., from March to July), were tested against each other first on the assumption that they would be the first to reach the microsite; and they were followed by two species that were dispersed between September and December. Since *Z. mucronata* displayed the highest level of germination in the first trials when testing the impact of passage through the gut, it was used as the reference species, and all the other species were added in decreasing order of seed size. Six replicates of each density set-up were sown: these entailed the low-density treatment of five seeds per species through to the high-density treatment of 20 seeds per species in each tray. Competing species (in groups of two, three, four, and five tree species) were sown according to four density classes at the same time: (1) six replicates of one container with five seeds per tree/shrub species; (2) six replicates of one container with ten seeds per tree/shrub species; (3) six replicates of one container with 15 seeds per tree/shrub species; and lastly, (4) six replicates of one container with 20 seeds per individual species. The seedlings' emergence was recorded on a daily basis, and the number of seedlings in each tray was counted. Seed germination trials were monitored over a period of four months, from 22 November 2019 to 30 March 2020.

5.4. Statistical analyses

5.4.1. Germination trials: Bird-ingested vs depulped seeds

To compare the germination rates between the bird-ingested seeds and the manually depulped seeds, the experimental design was balanced, with equal numbers of seeds in each tray. To test the hypothesis on the impact of the treatment of seeds during their passage through birds' gut, the germination rate of the bird-ingested seeds was compared with that of the manually depulped seeds (i.e., the experimental control). The generalised linear model analysis of variance (GLM-ANOVA) with negative binomial was applied to determine the

significant differences in the seed germinated for each tree/shrub species and treatments. The dependent variable consisted of the count of the number of seeds germinated, while the treatments (i.e., the bird-ingested and the depulped seeds) and the tree/shrub species were defined as predictor variables. The Statistical Package for Social Sciences (SPSS, version 20) software was used to perform the analyses.

Spearman's correlation was used to test for significant correlation between germination rate and seed size – i.e., length and weight.

5.4.2. Interspecific competition for germination of bird-ingested seeds among tree/shrub species

The seedling counts data were obtained from the balanced experimental design and I ran the comparisons of the seed germination for the different tree/shrub species across the different seed density levels and in the different combinations of tree/shrubs species per tray. Seedling counts data for competition level 1, was tested for normality using Shapiro-Wilk test (SW = 0.844, N = 49, P = 0.0001) since sample size was less than 50 (Zar, 2010). To reduce the inequality of variance, the germination counts were log transformed prior statistical analyses. Due to the complexity of the experimental design, a General Linear Model - Factorial Analysis of Variance was applied to determine the significant differences in seed germination at different seed density levels and number species (species diversity) using SPSS Version 20. The response variable consisted of the counts of the germinated seeds. The tree/shrub species and the seed density levels were defined as the predictor variables in order to allow an assessment of the interactions between the different combinations of plant species and the seed density per unit area. The Tukey HSD test was used to separate significantly different means at $P \leq 0.05$. A similar procedure was used to analyse data for other competition levels 2, 3 & 4.

Furthermore, for any tree/shrub species that showed significant different during any competition level, an overall analysis was run to compare how the germination rate varied between different seed density using the nonparametric model Kruskal-Wallis test. Also, I determined if the germination rate has relationship with total numbers of seeds per tray using the Spearman rank order correlation.

5.4.3. Germination of Z. mucronata across the seed density levels

A nonparametric Kruskal-Wallis ANOVA was applied to compare the bird-ingested seed germination rate of *Z. mucronata* across the different seed densities levels. The multi-comparison test distinguished different seed germination rates among the different seed density levels. Also, the seed germination of *Z. mucronata* was correlated with the seed density (the total numbers of seeds per tray) to determine whether there was any relationship.

5.5. Results

5.5.1. Germination trials: Bird-ingested vs manually depulped seeds

The comparisons of the germination rate between the bird-ingested and the manually depulped seeds were highly significant for the tree/shrub species (Wald $\chi2 = 142.9$, df = 3, $p < 0.001$; Figure 5.2). *Z. mucronata* had the highest germination rate for its seedlings (mean ± SE: 60.0 ± 8.0%; N = 20) (Wald $\chi2 = 55.0$, df = 5, $p < 0.001$), but bird-ingestion did not improve seed germination (Wald $\chi2 = 1.4$, df = 1, $p = 0.226$; Figure 5.2.). However, there were significant interactions between the seed germination of the different tree/shrub species and the seed treatments (Wald $\chi2 = 14.1$, df = 3, $p = 0.003$; Figure 5.2), with *S. lancea* having a much more significant germination rate for the bird-ingested seeds than for the manually depulped seeds (Wald $\chi2 = 12.7$, df = 1, $p = 0.001$; Figure 5.2; Mean ± SE: 56.0 ± 5.0; N = 20 vs 14.0 ± 2.0; N = 20). The Tukey HSD test showed that there were no significant differences between the germination of the bird-ingested seeds and the depulped seeds of *O. subsp. africana* (Wald $\chi2 = 1.1$, df = 1, $p = 0.288$; Figure 5.2), and *E. rigida* (Wald $\chi2 =$ 0.3, df = 1, $p = 0.619$; Figure 5.2).

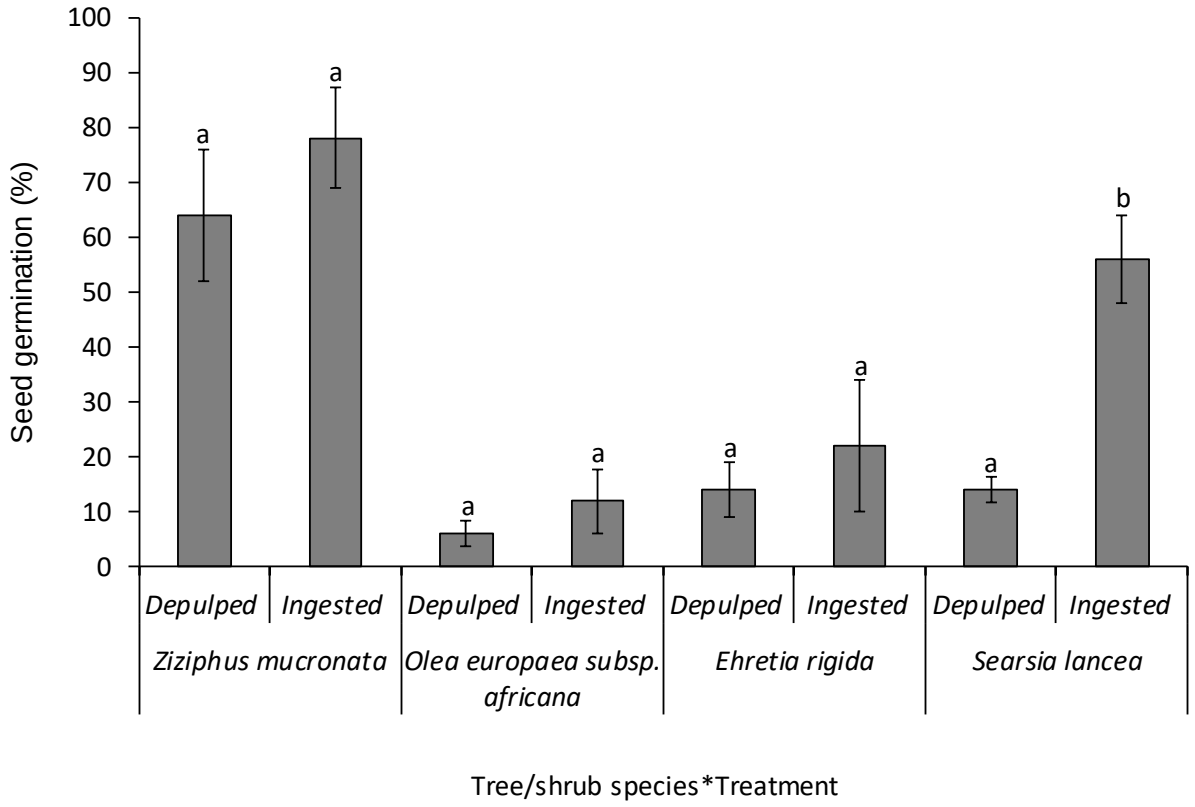

Figure 5.2: Variation between average numbers of seeds germinated in four tree/shrub species with seeds treated by passage through bird gut and the manually dupulped. The error bars represent the standard error of sample mean with the letters above indicating significance differences per species.

There was a significant correlation between seed size and the germination of bird-ingested seeds (Spearman Rank Order Correlation: r = 0.30; N = 640; P < 0.001), with the tree/shrub species that have large seeds having a greater germination than species with smaller seeds – except for *O. subsp. africana*.

5.5.2. Interspecific competition for germination of bird-ingested seeds among tree/shrub species

i) Level 1 competition test: Two species (*Z. mucronata* and *O. subsp. africana*)

The comparison of the bird-ingested seed germination between two competing tree/shrub species, *Z. mucronata* (mean ± SE: 48.9 ± 7.1%; N = 24), and *O. subsp. africana* (11.8 ± 4.8%), was highly significant (F (1, 40) = 42.4; p < 0.001; Figure 5.3). Similarly, different levels of seed density were significantly different in the seed germination of those two competing shrubs species (F (3, 40) = 3.3, p = 0.030; Figure 5.3). Significant interactions were observed between the seed density and the seed germination for the different tree/shrub species (F (3, 40) = 8.0, p = 0.0003; Figure 5.3), with *Z. mucronata* showing a

significantly higher seed germination *than O. subsp. africana* across three seed density levels – at seed densities of 10 (86.7 ± 4.2%), 20 (43.3 ± 14.8%), and 30 (55.6 ± 7.4%) – but not at a seed density of 40 (10.0 ± 1.8%).

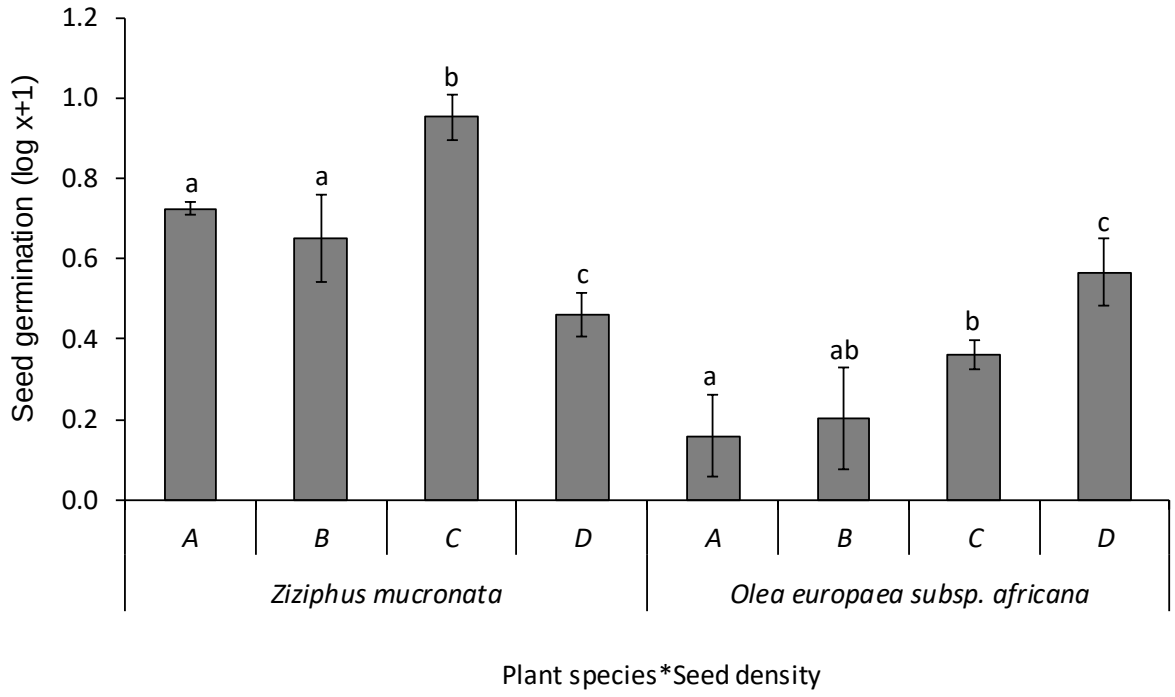

Figure 5.3: Mean seed germination for different plant species in competition level 1 between two tree/shrub species: *Z. mucronata* and *O. subsp. africana*. A, B, C, & D represented the seed densities of 10, 20, 30, and 40 seeds per tray. The error bars represent the standard error of sample mean with the letters above indicating significance differences per species.

ii) Level 2 competition test: Three tree/shrub species (*Z. mucronata, G. occidentalis,* and *O. subsp. africana*)

The comparisons of the bird-ingested seed germination rate among three competing tree/shrub species – *Z. mucronata* (mean ± SE: 49.2 ± 9.3%; N = 24*), O. subsp. africana* (17.8 ± 3.9%), and *G. occidentalis* (12.9 ± 5.2%) was highly significant (F (2, 60) = 36.2; p < 0.001; Figure 5.4). The Tukey HSD test showed that, while the germination rate for *Z. mucronata* was significantly high, the latter two tree/shrub species had equivalent germination rates. Also, there were significant differences in the seed germination of the tree/shrub species across the different seed density levels (F (3, 60) = 4.7; p = 0.054; Figure 5.4). There were no significant interactions between the seed germination of the different tree/shrub species with the sown seed density levels (F (6, 60) = 1.6; p = 0.153; Figure 5.4), with *Z. mucronata* showing a higher germination than the other tree/shrub species at seed densities of 30 (66.7 ± 12.8%), and 60 (23.3 ± 2.9%).

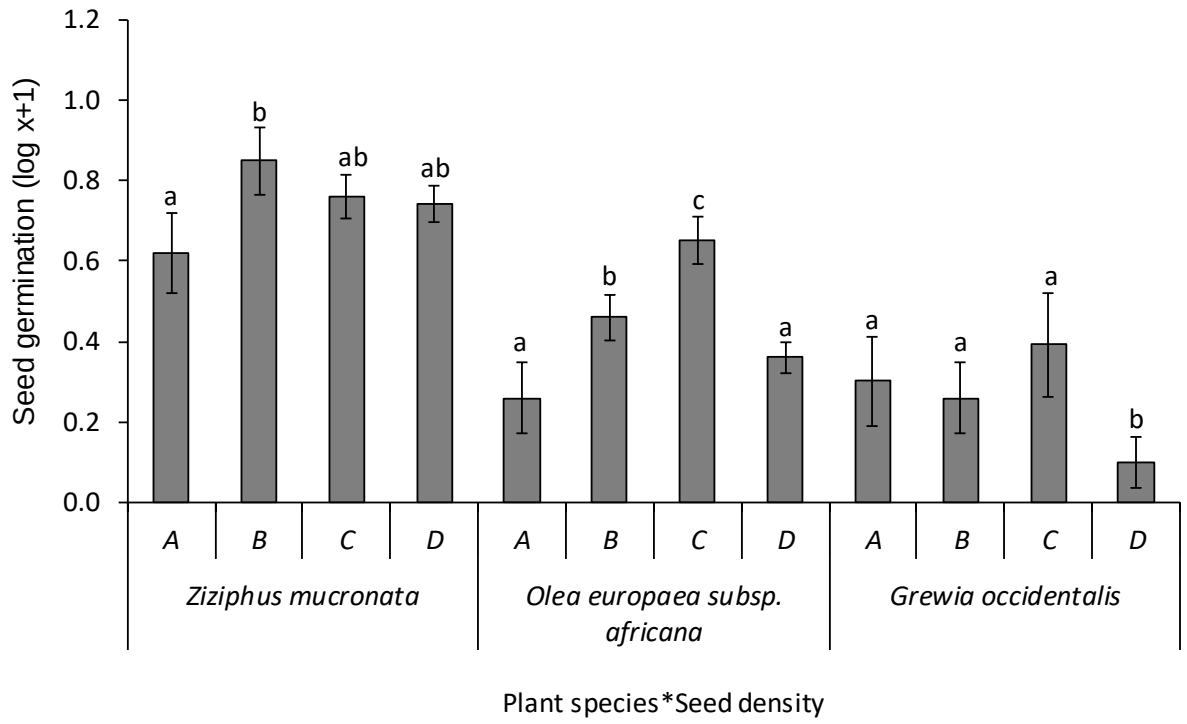

Figure 5.4: Mean seed germination for different plant species in competition level 2 between three tree/shrub species: *Z. mucronata, O. subsp. africana* and *G. occidentalis*. A, B, C, & D represented the seed densities of 15, 30, 45, and 60 seeds per tray. The error bars represent the standard error of sample mean with the letters above indicating significance differences per species.

iii) Level 3 competition: Four tree/shrub species (*Z. mucronata, G. occidentalis, S. lancea,* and *O. subsp. africana*)

The comparisons of the bird-ingested seed germination rates among four competing tree/shrub species – *Z. mucronata* (mean ± SE: 59.9 ± 10.3%; N = 24), *S. lancea* (23.3 ± 6.9%), *G. occidentalis* (12.5 ± 2.3%), and *O. subsp. africana* (5.8 ± 2.1%); were highly significant ($F(3, 80) = 39.2$; $p < 0.001$; Figure 5.5), and there were significant differences in the seed germination across the different seed density levels ($F(3, 80) = 7.3$; $p = 0.0002$). The Tukey HSD test showed that, while the germination rate for *Z. mucronata* was significantly high, *S. lancea* and *G. occidentalis* had equivalent germination rates, but they were significantly greater than those for *O. subsp. africana*. There were significant interactions between the tree/shrub species and the density levels ($F(9, 80) = 1.9$; $p = 0.051$; Figure 5.5), with *Z. mucronata* having a significantly higher seed germination rate in seed

densities of 40 (76.7 ± 11.2%), 60 (64.4 ± 13.4%), and 80 (45.0 ± 9.1%) than the other shrubs, although all of the shrubs were similar at a seed density of 20 (Figure 5.5).

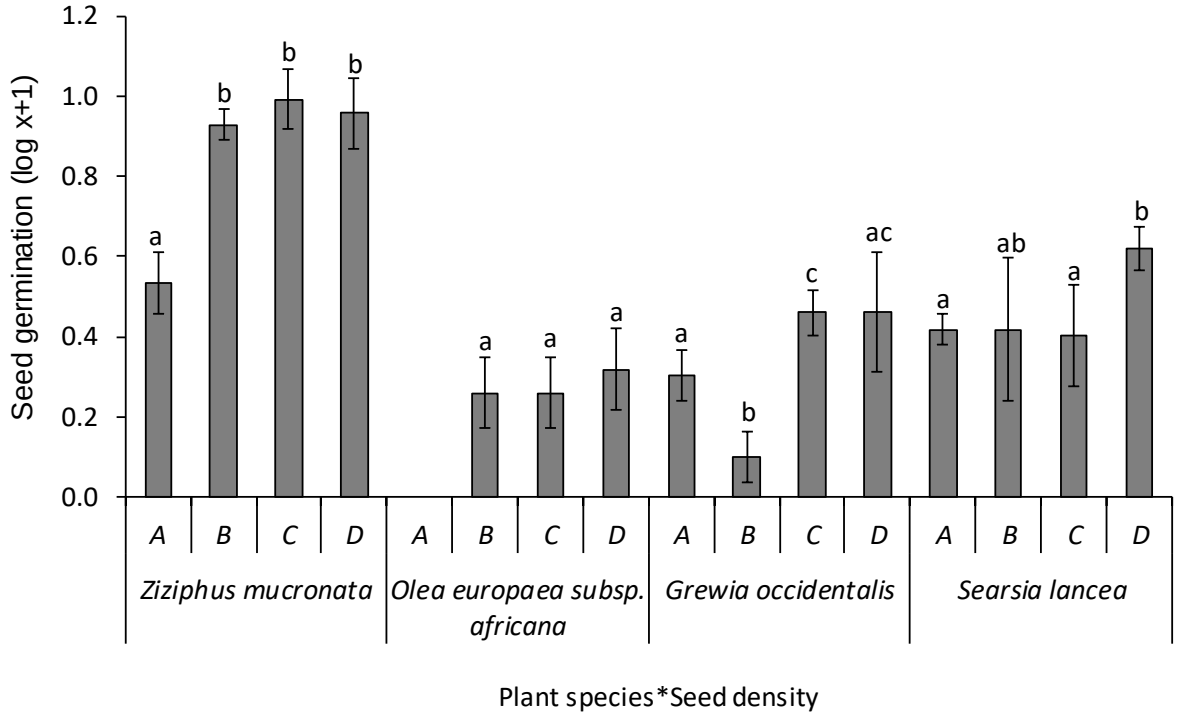

Figure 5.5: Mean seed germination for different plant species in competition level 3 between four tree/shrub species: *Z. mucronate, O. subsp. africana, G. accidentalis* and *S. lancea*. A, B, C, & D represented the seed densities of 20, 40, 60, and 80 seeds per tray. The error bars represent the standard error of sample mean with the letters above indicating significance differences per species.

iv) Level 4 competition: Five tree/shrub species (*Z. mucronata, G. occidentalis, S. lancea, E. rigida*, and *O. subsp. africana*)

The comparisons of the bird-ingested seed germination rates among five competing tree/shrub species – *Z. mucronata* (mean ± SE: 44.7 ± 8.1%; N = 24), *O. subsp. africana* (21.1 ± 9.6%), S. lancea (19.4 ± 5.6%), *G. occidentalis* (11.4 ± 3.7%), and *E. rigida* (0.0 ± 0.0%); Figure 3D – were highly significant ($F(4, 100) = 35.8$; $p < 0.001$; Figure 5.6). The Tukey HSD test showed that, while *Z. mucronata* was significantly high, those for the next three tree/shrub species were not significantly different from one another but were significantly greater than for *E. rigida*. There were no significant differences in the seed germination at different seed density levels ($F(3, 100) = 0.7$; $p = 0.568$; Figure 5.6). There were no significant interaction between the seed density levels and the tree/shrub species ($F(12, 100) = 1.6$; $p = 0.114$), while *Z. mucronata*, had the highest seed germination rate when

the total seed density was 75 (55.6 ± 14.9%), and partly when the density was 50 (53.3 ± 4.2%; Figure 5.6).

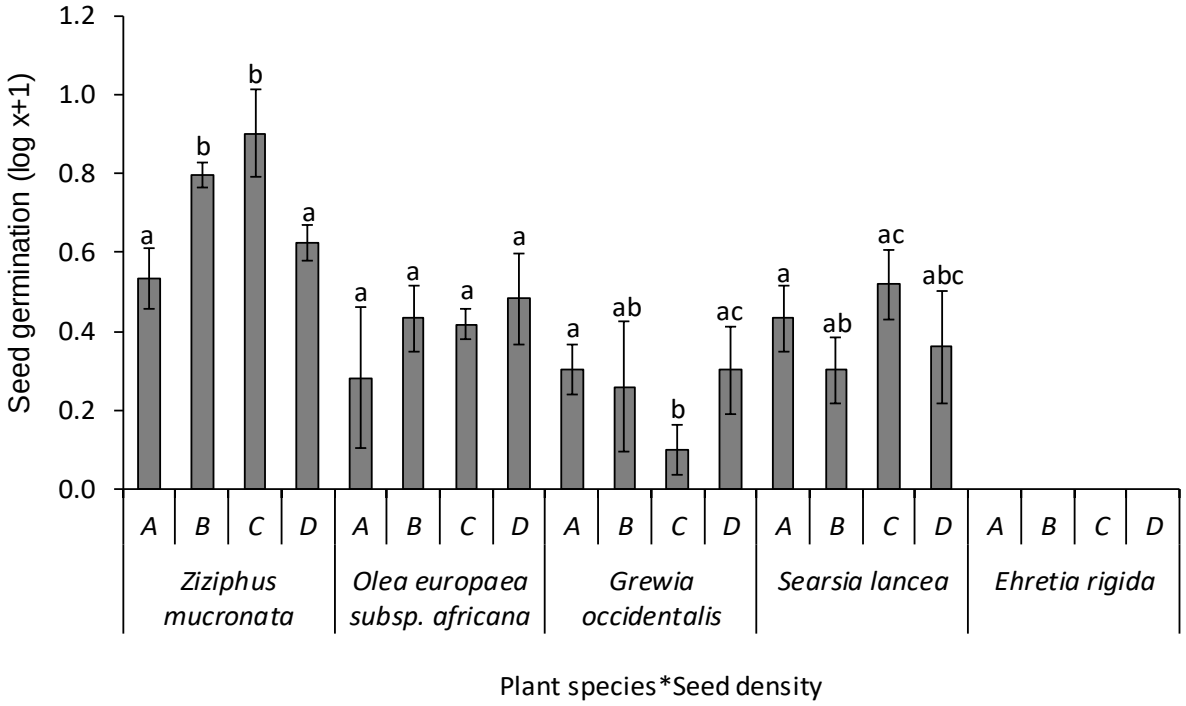

Figure 5.6: Mean seed germination for different plant species in competition level 4 between five tree/shrub species: *Z. mucronate, O. subsp. africana, G. accidentalis, S. lancea* and *E. rigida*. A, B, C, & D represented the seed densities of 25, 50, 75, and 100 seeds per tray. The error bars represent the standard error of sample mean with the letters above indicating significance differences per species.

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5.5.3. Germination pattern of Z. mucronata and O. subsp. africana across the seed density levels

Overall, *Z. mucronata* was the strongest competitor against the other tree/shrub species, with significantly higher seed germination rates recorded across the different levels of seed density (H (11, 96) = 30.5; p = 0.0013; Figure 5.7A). The multiple comparison test showed that *Z. mucronata's* seed germination was significantly (p = 0.0499) highest at the seed densities of 30 (mean rank: 67.8), 60 (61.8), 75 (63.8), and 80 (70.5), and these levels did not differ significantly (p > 0.05) among one other (Figure 5.7A).

There was a significant positive correlation between the germination of the bird-ingested seeds of *Z. mucronata* and the seed density levels per tray (Spearman rank order correlation: r = 0.268; N = 96; P = 0.008).

Additionally, *O. subsp. africana* also displayed the significant increase in the germination rate as the seed density per tray increase ($H(11, 96) = 26.2$; $p = 0.0061$; Figure 5.7B). The multiple comparisons tests showed that these differences were recorded only at the seed density level of 45 with mean rank 81.8 against the seed density level of 20 with mean rank 24.7 ($p = 0027$). Similarly, the seed germination rate showed a significant positive correlation to the seed density (Spearman rank order correlation: $r = 0.300$; $N = 96$; $P = 0.003$).

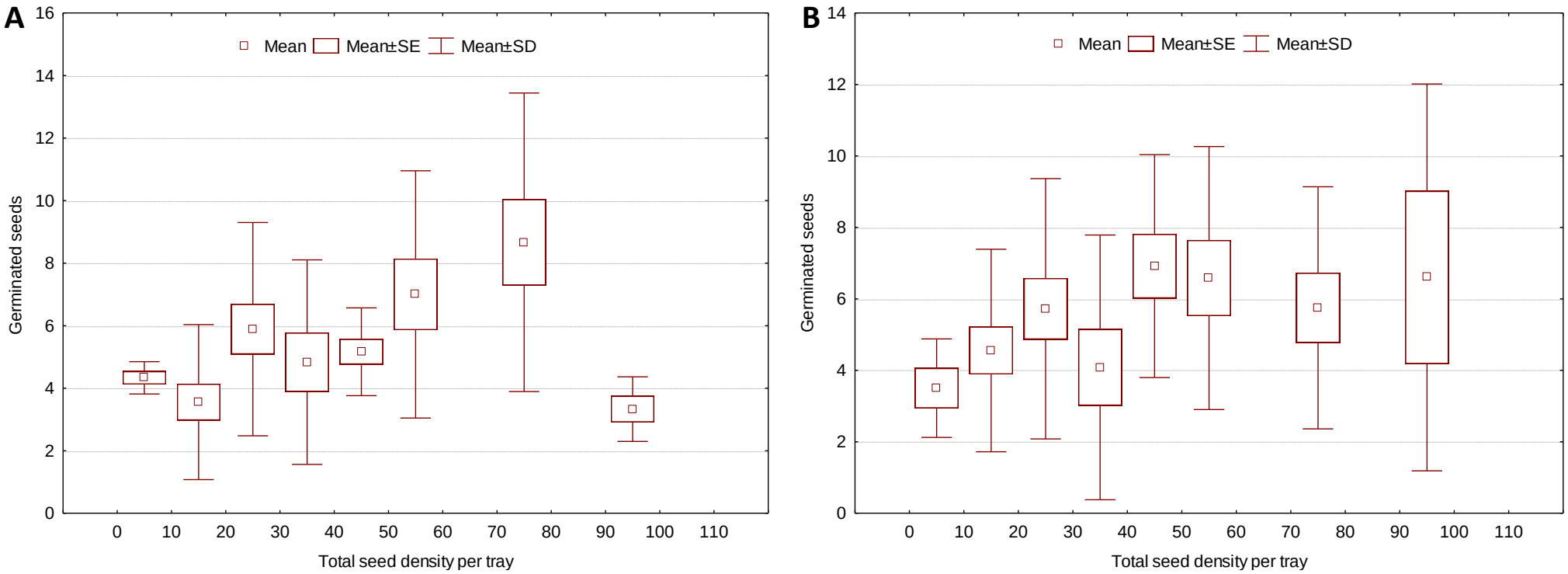

Figure 5.7: Overall variation in germination of bird-ingested seeds for *Z. mucronata* (A) and *O. subsp. africana* (B) across seed density levels (i.e., 10-100 seeds per tray) with different competing species.

5.6. Discussion

5.6.1. Germination trials: Bird-ingested vs depulped seeds

I tested the hypothesis that the passage of seeds through birds' gut improves germination and found – against the hypothesis – that not all bird-ingested seed germination was improved in four of the five tree/shrub species. Only *S. lancea* seeds had their germination improved by passage through birds' gut, probably due to scarification of the seeds' coat/endocarp in the avian digestive tract (Mokotjomela et al., 2015; Samuels and Levey, 2005; Traveset, 2001), suggesting that this tree/shrub species benefits from a higher effectiveness of seed dispersal than the others. In addition, the ingestion of *S. lancea* seeds by birds may release seed dormancy through the scarification associated with digestive processes (Mokotjomela et al., 2015; Traveset, 2001) and this also prevents the latent problems of either endogenous predation or disease that are reported in 60% of harvested seeds (Weiersbye and Witkowski, 2002), thereby suggesting that *S. lancea* has a soft seed cover with limited resistance to microbial attack. Indeed, given the current threats to the conservation of biodiversity, such as niche changes due to climate change and habitat fragmentation (Muller-Landau and Hardesty, 2005), the high observed effectiveness of seed germination may be essential to plant recruitment and to the highly recommended habitat restoration in critical biodiversity areas that are threatened by socio-ecological and socio-economic development projects (Martinez-Lopez et al., 2019; Mokotjomela and Nombewu, 2019; Ntloko et al., 2019).

The unchanged germination rates of the bird-ingested seeds of *O. subsp. africana, E. rigida*, and *Z. mucronata* are likely the result of the high resistance of their seed cover to treatment in the gut, as reported by Mokotjomela et al. (2015) for the seeds of *Acacia cyclops*, in combination with the limited retention times when the seeds are ingested by large vectors (Tsoar et al., 2011; Schurr et al., 2009), because large frugivorous birds are rare at the study site. The observed low germination of *O. subsp. africana* could be attributed to indehiscent, stony, and hard endocarp (Cuneo et al., 2010), which prevent germination by stopping moisture and oxygen from reaching the seed (Negash, 2010; Bekele, 2000). Also, *O. subsp. africana* seeds have a high seed dormancy that may persist for up to 35 months (Bekele, 2000), which indicates uncertainty about the seed's viability. The poor germination observed in *E. rigida* may be associated with an inherently low seed viability (~26%; William et al., 2014), and a low tolerance to moisture and humidity (Louw, 2012), whereas the study site has a humid microclimate due to the presence of a dam and wetland. This finding suggests that *E. rigida* is adaptated to arid environments, as indicated by William et al. (2014), and that it requires relatively low and intermittent moisture for successful seed germination (William et al., 2014). It is suggested that the unchanged but high seed germination in *Z. mucronata* is

due to it having a large seed size that, reportedly (Mandal et al., 2008), also enhanced germination in the seeds of *Hyptis suaveolens*. This explanation can also hold for *Z. mucronata*, since it was consistently found a significant positive correlation between seed size and germination rates. In addition, *Z. mucronata* has a high seed viability of up to 100% (Weiersbye and Witkowski, 2002) and a high physiological plasticity in a broad variety of environmental conditions (Refka et al., 2013; Zietsman and Botha, 1987). An unchanged germination of bird-ingested seeds has been previously reported in South Africa (Mokotjomela et al., 2015; Chama et al., 2013; Mokotjomela, 2012; Jordaan et al., 2011), which highlights, in this context, the role of birds in transporting seeds to new microsites. Wenny (2000) also found that both the seeds ingested by birds and those manually depulped germinated equally well (98%) for *Ocotea endresiana* (Lauraceae). However, I argue that these tree/shrub species could have evolved resistant seed covers in order to endure environmental uncertainties such as rodents' seed predation, diseases, and droughts (Pearson et al., 2014; Dardick and Callahan, 2014). Thus, further investigation that is beyond scope of this study might yield better insight into the roles of the reported characteristics in the life of seeds in the local context.

5.6.2. Interspecific competition for seed germination: seed density and species diversity

Germination is a key process for maintaining plants' genetic material in order to sustain species' population after their seeds are dispersed to new microsites (Carlo and Tewksbury, 2013; Hulme, 1998). When seeds of different plant species interact, their ability to affect and respond to competition determines the contribution of seed germination to individual plant species' population (Goets et al., 2018; Aschehoug et al., 2016; Johnson et al., 2008; Schupp, 1993). Part of this study investigated how bird-dispersed seeds handle competition mediated through seed density and species diversity in the microsite.

The finding shows that *Z. mucronata's* seed germination performance increased with seed density and species diversity may be due to its high behavioural plasticity (Refka et al., 2013; Zietsman and Botha, 1987), an attribute that has possibly allowed *Z. mucronata* to endure emerging physiological conditions when competing for survival with other species. Similar results were reported by Orrock and Christopher (2010), who observed an accelerated germination response of *Phytolacca americana* when seed density was increased. Indeed, dispersed seeds may have to overcome a variety of environmental pressures against germination to attain their subsequent plant recruitment (Orrock and Christopher, 2010; Wang and Smith, 2002). If the experimental germination trays represented a microsite that was conducive to dispersed seeds, as proposed by Howe (1986), then – in line with the enemy release hypothesis (i.e., for invader species), which postulates that new

environments have either few or less competitive neighbours (Le Roux et al., 2020), I argue that this could have enhanced *Z. mucronata's* seed germination. The study also highlights that the number of seeds (i.e., the amount of dispersal effectiveness, sensu, Schupp et al., 2010) in the microsite is important for the effective initiation of the recruitment process for each plant (Hulme, 1998; Howe, 1986) because, at a seed density of between 60 and 100, there was no significant increase in germination. Seeds' endocarp has some hormones that play a major role in maintaining seed dormancy and in protecting the embryo against pathogens by producing phenolic compounds (i.e., tannins) (Raviv et al., 2017). If the seeds of different species can sense each other prior to their emergence (Ward, 2016; Tielborger and Prasse, 2009), it is possible that the endocarp of the germinated seeds of *Z. muconata* released chemicals and/or hormones that eventually suppressed the germination of neighbouring seeds, while it enjoyed all the germination resources available at the microsite. The impact of the suggested chemical effect could be amplified by the large seed size of *Z. mucronata*.

The fact that *O. subsp. africana* displayed an increasing seed germination in the level two competition (i.e., a total seed density of 15-45; the competing species were *Z. mucronata* and *G. occidentalis*), suggests that there could have been a physiological enhancement from the seeds of other species, since Negash (2010) and Cuneo et al. (2010) found that the hard seed endocarp of *O. subsp. africana* thwarts the germination process. It has been consistently shown that dispersed seeds adjust their dormancy status in relation to the new conditions in the microsite, while also targeting the optimal conditions for germination (Footitt et al., 2018), and that they can sense the presence of seeds of other species (Tielborger and Prasse, 2009). Because the passage of seeds through birds' gut did not also improve the germination of *O. subsp. africana*, I argue that the disintegration of the endorcarps of the substantially germinated seeds of Z. mucronata might have resulted in new physiological effects that favoured seed germination in O. subsp. africana.

The poor seed germination of *G. occidantalis* and the failed germination of *E. rigida* (i.e., in competition Level 3: species: *Z. mucronata, O. subsp. africana, G. occidantalis*, and *S. lancea*; and Level 4: species: *Z. mucronata, O. subsp. africana, G. occidantalis, S. lancea*, and *E. rigida*) could be partly attributed to the adaptive mechanisms related to a need for the persistence of dispersed seeds through protection against microbes and physiological barriers to their germination (Dalling et al., 2011). Orthodox seeds tend dry up to preserve their viability (Hilhost and Toorop, 1997), and this requires some treatment to break their dormancy (Qasem, 2019; Rowarth et al., 2007). Bewley and Nonogaki (2017) distinguished between the sequence of the different physiological stages of the germination process, and they contended that, during the imbibition phase of germination in dry seeds (i.e., orthodox), germination may be prolonged if all the essential protein are not available to reinitiate the

metabolism in the dormant seeds. Moreover, it has been shown that dispersed seeds undergo a transition of dormancy that is mediated by the maternal plant to an independent seed-mediated dormancy, which is also aligned with the new conditions at the location where the seed is deposited (Finch-Savage and Footitt, 2017; Footitt et al., 2013). Thus, it is possible that the seed germination of the species in our study that were dispersed between March and July was retarded by the dormancy transition mentioned above. In this way, I show that the lack of the emergence of the seed in some species may not be necessarily because of competition, but also because of the factors were not controllable such fruiting maturing time and their consumption by birds although all seeds were sown at age before dormancy was changed.

Recalcitrant seeds must be sown immediately after harvesting or being dispersed (Barbedo et al., 2013), unlike orthodox seeds (*Z. mucronata, G. occidentalis, O. subsp. africana*, and *E. rigida*). I suggest that the poor seed germination performance in *S. lancea*, as a recalcitrant seeder, could be partly due to the loss of its viability through dehydration during storage, which corroborates a report that *S. lancea* seeds are sensitive to moisture and thermal stress (Nichols, 2005). Furthermore, this observation may be explained by the small seed size of *S. lancea*, which reportedly promotes persistence in the soil better than is the case with large seeds (Mole et al., 2000; Funes et al., 1999).

5.7. Concluding remarks

The germination trials with the bird-ingested seeds extracted from faecal samples showed that the foraging birds provide seed dispersal service in two different ways relating to quality and quantity of dispersal effectiveness (Kleyheeg et al., 2018; Mokotjomela et al., 2016; Schupp et al., 2010). These are the partly improved seed germination rate after passage through the gut; and solely, the transport of the seeds to new microsites. That *Z. mucronata* displayed highest germination rate of all shrubs yet birds did not change its seed germination, suggest the need for further studies to determine the avian frugivores that ingested seeds and compare their effectiveness in treating seeds. Mokotjomela et al. (2015, 2016) demonstrated different avian vector species have differential seed dispersal effectiveness (i.e., using germination component deemed most important for plant recruitment, Schupp, 1993; Schupp et al., 2010), and this finding complemented a study showing that not all seeds benefit improved seed germination from the ingestion by birds, but sometimes only transportation to new microsites (Chama et al. 2013).

Foraging birds disperse the ingested seeds away from their maternal plant to the new microsite where there is less intra- and inter-species competition for resources that might be limited where siblings' and congeners' populations are large (Howe, 1986). However, the results of this study suggest that seed competition for germination resources still occur. The

test experiments for seed competition during germination at the microsite, showed that out five species, only *Z. mucronata* and *O. subsp. africana* displayed significant change that I argue that is partly due to the reported behavioural plasticity in *Z. mucronata* and potential physiological enhancement of the germination medium for *O. subsp. africana* by the thriving *Z. mucronata*. Also, it is possible that differential seed survival prior emergence in the soil in this study favoured the larger seeded species over small seeded species (see Moles et al., 2003). The apparent increase in seed germination performance when seed density level increased in *Z. mucronata* and *O. subsp. africana* lead us to the conclusion that these two species are well adapted to the interspecific competition for germination resources underground. However, this finding needs further investigation since certain species may lengthen seed dormancy when sensing high conspecific seed density levels as a strategy to avoid competition (Footitt et al., 2013). As I cannot conclude that seed emergence of other species due to inability to compete, furthermore, studies on the physiological interactions of seeds underground are needed for elucidating on the germination behaviour of individual focal tree/shrub species. Finally, considering the high seed germination of the large seeded *Z. mucronata* in this study, it was suspected that *Z. mucronata* may account for the reported bush encroachment in the study site (Vukeya et al., 2020; Department of Environmental Affairs, 2019; Zietsman at al., 1989), thus I recommend adaptive conservation management plan should include monitoring and prioritised population control for this species.

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Chapter 6: General discussion and conclusion

The primary aim of this study was to determine long-term vegetation cover dynamics and potential environmental drivers on the FSNBG natural vegetation with major focus on plant-animal interactions. The mandate of SANBI is to champion the exploration, conservation, appreciation and enjoyment of South Africa's exceptionally rich biodiversity for all people (SANBI, 2019; Willis, 2015). In as much as SANBI mandate conservation research strategy emphasises sustainable biodiversity conservation, there are no studies that have quantitatively appraised the biodiversity conservation value and natural phenomena dynamics over time in different SANBI conservation gardens. SANBI gardens are established in areas with unique and threatened vegetation types (i.e., Critical Biodiversity Areas). Consequently, in this study, the appraisal of biodiversity value in FSNBG was achieved by comparing the vegetation cover change over ten-year interval for the period of 30 years and the potential drivers of the observed change in order to guide for adaptive management. In addition, biotic interaction between tree/shrub fruit and birds as model were used to study seed dispersal penology in the FSNBG.

With the use of remote sensing and geographical information system, the results revealed that vegetation cover of the study area showed a significant increase woody biomass over the 30-year period (1987-2017) in the study site. The sparse vegetation cover has decreased significantly over time while moderate and dense vegetation cover shown a significantly increased (25.11 ha and 8.64 ha) from 1987 to 2017 (i.e., Chapter 3). The increase of woody cover could be attributed from several factors including severity drought condition in Free State Province (van Rooyen, 2010; Botai et al., 2016) and the rise of CO_2 concentrations in the atmospheric induced by global climate changes (Ward, 2010), which enhance growth of woody species (Buitenwerf et al., 2012; Welz, 2013; Sintayehu, 2018), and suppress herbaceous plant cover (Kgosikoma and Mogotsi, 2013). Major conservation threats entailed biological invasions: 27 invasive alien plant species dominated by 13 woody plants and eight succulent species interspersed within different vegetation patches. Among vegetation types (Bloemfontein dry grassland, Winburg grassy shrubland and Riparian vegetation) preserved in FSNBG including national priority 'critical biodiversity vegetation' (Bloemfontein karroid shrubland), the riparian vegetation was the most invaded vegetation type, and Ink berry (*Cestrum laevigatum*) was found to be the most invasive species (i.e., Chapter 3). Alien and Invasive plants species are likely to transform native ecosystems by changing species composition, vegetation structure and ecosystem functioning, since they fragment natural areas, drive degradation and negatively impact biodiversity (e.g., disappearance of native species) (Mostert et al., 2017 Valery et al., 2008). Also, human settlement encroachment has substantially increased (i.e., covering ~ 18% of the buffer zone)

in the past 18 years (i.e., Chapter 3). The proportions of transformed area in the buffer area (i.e., anthropogenically altered landscape such as physical development of built infrastructure and plantation) has displayed a to be increasing over time. This may lead to reduce wildlife corridors that helps to increase population persistence by allowing continued exchange of genetic material between the connected population (Rosenberg et al., 1997). In this regard, I suggest urgent need for regular vegetation monitoring in the study site to manage biodiversity conservation threats.

Since the used of remote sensing and GIS imagery could not validate the existence of descriptive plant species of vegetation type (Hill et al., 2005). The collection of ground truth data enables calibration of remote sensing imagery data (Ducgin, 1987). The existence of CBA1 vegetation type 'Bloemfontein Karroid Shrubland' as national conservation priority preserved in FSNBG was validated with the descriptive plant species by Mucina and Rutherford (2006). The study confirmed that CBA1 vegetation type still exist with over minimum threshold of 60% habitat intactness (Mokotjomela and Nombewu, 2019; Kleynhans et al., 2008). The results of this study showed that small change in the CBA1 vegetation type have taken place but the basic ecosystem functions still predominantly unchanged (Kleynhans et al., 2008). However, the overall cover of the CBA1 vegetation type could still reduce splash erosion by intercepting rainfall by decreasing the overland flow and improving the infiltration of precipitation into the soil (Hesp, 1991). There were 55 plant species documented in the CBA1 vegetation. Most of the flagship plant species documented by Mucina and Rutherford (2006) (11 out of 12), still occurred in the CBA1 vegetation type which could partly support patch functionality. However, I infer that the ecological integrity of CBA1 vegetation type has changed since only 27 plant species out of 77 species documented in Mucina and Rutherford (2006) still exist in the CBA1 vegetation type (i.e., Chapter 3). The fundamental shift of CBA1 species diversity was attributed from invader plant species associated with extreme frost and drought in the Free State Province (see Tilman and Haddi, 1992; Basto et al., 2018; Abubakar, et al., 2020) and human mediated activities such as human settlement. In this regard, the observed change in ecological integrity of CBA1 vegetation type may led to an increase rate of species extinctions if it is not attended. This could suggest the agent's need for regular vegetation monitoring of CBA1 vegetation type and intervention to manage the prevailing conservation threats.

Seed dispersal is important for plant population maintenance (Kleyheeg et al., 2018; Mokotjomela et al., 2016; Schupp et al., 2010) and vertebrate including birds provide the much-needed long distance dispersal services for many plants (Nathan et al., 2003; Schurr et al., 2009; Mokotjomela et al., 2013; Mokotjomela 2012). To understand the seed dispersal phenology of tree/shrub in the vegetation of FSNBG, the study also investigated biotic interactions focusing on the plant fleshy fruit resources that are foraged by frugivorous birds

in FSNBG. A total of 22 frugivorous bird species from 12 families were responsible for fruits removal and seed dispersal of 14 tree/shrub fruits (10 native and four non-native species) (i.e., Chapter 4). Out of the 22 bird species, seven displayed a significantly high frequency of capture and were resident in foraging behaviour, while six species were migratory and colonial avian frugivores, including two alien bird species (*Sturnus vulgaris* and *Aeridotheres tristis*). In addition, the study results have shown statistically significant differences in number of bird-ingested seeds dispersed each month over a one-year period and 80% were dispersed between March and July (i.e., Chapter 4). The important fruit characteristics including fruits size, fruit colour and fruiting phenology (Gautier-Hion et al., 1985; Jordano, 2000; Gosper and Vivian-Smith, 2009; Galetti et al., 2011; Gosper et al., 2005; Mokotjomela et al., 2013) did not significantly influence fruits foraging choice by birds most probably due to limited diversity of fruiting species. However, birds showed preference for red fruits (*Z. mucronata, E. rigida,* and *V. rotundifolium*) and black fruits (*O. subsp. africana* and *D. lyciodes*) as reported in the previous studies (see Knight and Siegfried, 1983; Ally, 2010; Schaefer et al., 2014; Gagetti et al., 2015). Although several studies have positive correlation with fruits size (Gosper et al., 2005; Cazetta et al., 2008; Galetti et al., 2011), the study results showed no correlation of fruit size with the number of dispersed seeds. Among 14 tree/shrub fruits, both *Z. mucronata* and *O. subsp. africana's* fruits were most preferred by foraging frugivorous birds in the study site hence *O. subsp. africana* displayed longest fruiting period (i.e., 10 months) of which is likely to be potential keystone fruit resources (i.e., Chapter 4). In addition, it was also found that *E. rigida* and *S. lancea* seeds were dispersed between August and December of which may represent an evolutionary response to avoid interspecific competition for seed dispersal services while their fruiting pattern provides sustenance for resident frugivorous birds during periods of fruit scarcity. Overall, the study revealed two fruiting peaks on the FSNBG vegetation and differences in tree/shrubs fruiting phenology and fruit colour were found to be key factors to drives birds' seed dispersal phenology in the study site.

Seed dispersal quantity and quality contribute in shaping native plant communities (Kleyheeg et al., 2018; Mokotjomela et al., 2016; Schupp et al., 2010). Quality of seed dispersal is most important as it determine fate of seed (Schupp, 1993; Mokotjomela et al., 2016) and it is influenced by scarifying the seeds during ingestion that may improve the germination which is critical for dispersal effectiveness (Kleyheeg et al., 2018; Mokotjomela et al., 2016; Schupp et al., 2010; Schupp, 1993). During comparison of the germination trials of bird-ingested seeds and manual depulped seeds, *Z. mucronata* displayed highest germination rate of all tree/shrubs yet frugivorous birds did not improve its seed germination. Conversely, only *Searsia lancea* seeds had significantly high seed germination after passage through the bird gut probably due to scarification of the seeds' coat/endocarp in the avian

digestive tract (Mokotjomela et al., 2015), and the similar observation has been reported in Opuntia species in Free State province (i.e., Chapter 5) (Mokotjomela et al., 2021). In this regard, higher possible was seed dormancy was released by bird gut treatment to increase plant fitness during germination (Mokotjomela et al., 2021).

Foraging birds disperse the ingested seeds away from their maternal plant to the new microsite where there is less intra- and inter-species competition for resources that might be limited where siblings' and congeners' populations are large (Howe, 1986). The test experiments for seed competition during germination at the microsite, showed that out of five species, only *Z. mucronata* and *O. subsp. africana* displayed significant change that I argue that is partly due to the reported behavioural plasticity in *Z. mucronata* (Refka et al., 2013; Zietsman and Botha, 1987), and potential physiological enhancement of the germination medium for *O. subsp. africana* by the thriving *Z. mucronata*. The apparent increase in seed germination performance when seed density level increased in *Z. mucronata* and *O. subsp. africana* lead to the conclusion that these two species are well adapted to the interspecific competition for germination resources underground (i.e., Chapter 5). Since seed dispersal service (quality and quantity) is critical in shaping plant communities of grassland biome, the tree/shrubs species with high germination could account to observed bush encroachment in the study area (see chapter 3; Vukeya et al., 2020). Overall, I recommend that adaptive conservation management plan of FSNBG should include monitoring and prioritised bush encroachment control.

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