Effects of climate variability, land-use change and transformation on grassland plant diversity in South Africa

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DECLARATION

I, Marlize Muller, declare that the work presented in this PhD thesis is my own work. It is being submitted for the degree Doctor of Philosophy at the North-West University, Potchefstroom Campus. It has not been submitted for any degree or examination at any other university. All sources used or quoted have been acknowledged by complete reference.

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Dedicated to my family.

PREFACE AND ACKNOWLEDGEMENTS

> *“There lies all about us, if only we have eyes to see, a creation of such spectacular profusion, such spendthrift and extravagant richness, such intricate and absurd detail, as to make us drunk with astonished wonder.”*
>
> Michael Mayne

I am very privileged to have grown up on two nature reserves as my father is a nature conservator. On these reserves, my passion and love for nature grew and led me to pursue further studies in environmental sciences. Having this privilege has allowed me to learn many lessons I believe I would not have learned if I had grown up in a different setting. South Africa is very rich in terms of its fauna, flora, human races, cultures, geology, and biomes. Therefore, it is such a pleasure and privilege to be part of ecological studies in South Africa. I want to use the knowledge that I have obtained to have a positive impact on the environment and make a difference in our world. This study would not have been possible without several individuals and organisations, and I would like to express my sincere appreciation for their involvement and support in helping me with the completion of this thesis.

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ABSTRACT

Grasslands as open ecosystems are not successional stages or degraded forests but are ancient old-growth systems supporting a high diversity of fauna and flora. It is now known that forests and grasslands are two distinct ecosystem types that have existed for millennia. In South Africa, approximately 27.9% of the terrestrial land surface is covered by the Grassland Biome for which less than 25% of untransformed old-growth grassland are being moderately to well protected. This biome is one of the most threatened biomes in South Africa, with 40-60% of its area already irreversibly modified, whilst 60% of the remaining grassland is threatened. Grasslands are subjected to disturbances which can be grouped into two main disturbance types, namely endogenous and exogenous. Endogenous disturbances occur naturally, and grasslands evolved in the presence of these disturbances, including climate variability, fire, and herbivory. Exogenous disturbances are those that disrupt the soil surface and are usually of anthropogenic origin, including afforestation, agriculture, and urbanisation. The overarching aim of this study is to investigate, describe and critically evaluate the community composition and diversity responses of grassland ecosystems to direct and indirect anthropogenic disturbances. Two long-term datasets were used to investigate the effect of transformation on grasslands of South Africa. The first dataset was used to investigate land-use change effects on grasslands by comparing untransformed and transformed grasslands in terms of species diversity and composition. The second dataset was used to compare two adjacent grassland communities, of which one is encroached. These two communities were further investigated to determine the effect of a severe drought on species diversity and composition. Findings from the present study confirmed that exogenous disturbances transform grasslands by removing above- and belowground plant biomass, thus reducing the potential of perennial grassland plants to persist through resprouting. There is also an increase in exotic species which transform the community composition. Both exogenous and endogenous disturbances result in grasslands transforming to encroached sites. The origin of encroacher species can be savanna or forest as both are bordering grasslands. However, in this study, neither savanna or forest woody species seemed to dominate, and it is hypothesised that the ‘encroached community’ might be a transformed grassland, or alternatively a shrubland. Woody grasslands are somewhat less resistant and resilient to drought than an old-growth grassland. Woody grasslands could therefore be seen to be more vulnerable to drought, but the results are not definitive.

Key terms

Diversity, drought, encroachment, endogenous disturbance, exogenous disturbance, floristic composition, old-growth grassland, recovery, resilience, resistance, shrubland, species evenness, species richness, transformed grassland, and untransformed grassland.

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CHAPTER 1 INTRODUCTION

1.1 Background and rationale

Forests have long been considered to be the dominant climax vegetation on Earth, while non-forested open ecosystems such as tundra, grasslands, and savannas have been viewed as successional stages leading to this climax state of vegetation (Bond, 2019). This notion has been challenged by the fact that 58.5% of the planet's land area is covered by non-forested ecosystems (Dinerstein *et al.*, 2017). The impacts of global change on forest ecosystems have been well understood, documented, acknowledged, and contended (Liu *et al.*, 2022; Moreau *et al.*, 2022; Patacca *et al.*, 2023). In contrast, an understanding and protection of open ecosystems is far from being achieved (Bond, 2019; Bond & Parr, 2010). In the same vein, grasslands have been viewed as early successional stages on a trajectory to becoming a forest (Bond, 2019). Now we know, however, that forests and grasslands are two distinct ecosystem types that have existed for millennia (Bond, 2019). For these reasons, among others, open grassland ecosystems have not received the necessary attention.

Global tropical and sub-tropical savannas are often considered as grasslands due to their continuous layer of mostly C4 grasses and their dependence on natural disturbances, such as fire and herbivory (Veldman *et al.*, 2015b). For the purpose of this study and within the South African context, grasslands are separated from savannas and considered as ecosystems characterised by a continuous herbaceous vegetation layer dominated by graminoids (particularly the Poaceae), with less than 10% tree and shrub cover (Mucina & Rutherford, 2006; Veldman *et al.*, 2015b; White *et al.*, 2000). Grasslands cover 31-43% of the global land surface (Gibson, 2009; White *et al.*, 2000), support numerous endemic and threatened species and habitats (Carbutt *et al.*, 2017), and include 15% of the world's Centres of Plant Endemism (Egoh *et al.*, 2011; White *et al.*, 2000). The effects of land transformation and climate change on the rich diversity of grasslands remain poorly documented and not well understood.

If an open ecosystem is merely a successional stage brought about by anthropogenic disturbance, one would expect that it would be short-lived and patchy in extent, poor in plant and animal species, poor in endemics, and low in species turnover across physical habitat gradients (Bond, 2019). However, this is not true in all cases: natural and near-natural grasslands are known to host an exceptionally high diversity of animal and plant species, habitats, and communities (Bond & Parr, 2010; Carbutt *et al.*, 2017). Such hyper-diverse, primary grasslands are also referred to as 'old-growth' systems, since they have existed for millennia, and beside its high species richness, it hosts numerous endemic species and unique species compositions (Bond & Parr, 2010; Buisson *et al.*, 2019; Veldman *et al.*, 2015b). In South Africa, an approximate 27.9% of the terrestrial land surface is covered by the Grassland Biome (Mucina *et al.*, 2006) for which less than 25% of untransformed old-growth grassland are being moderately to well protected (Skowno *et al.*, 2019). This biome is one of the most threatened biomes in South Africa, with 40-60% of its area already irreversibly modified, whilst 60% of the remaining grassland is threatened (Cadman *et al.*, 2013; Little *et al.*, 2015).

Grasslands developed in the presence of disturbances, such as lightning-ignited fires, rainfall variability, and herbivory, and are therefore well-adapted to and dependent upon these natural disturbance regimes (Buisson *et al.*, 2019). Global environmental change alters rainfall patterns, grazing intensity, herbivore diversity, and natural fire regimes (Koerner & Collins, 2014). It is important to understand the response of grasslands to these changing drivers, as this helps predict future global patterns of grassland productivity and diversity (Fay *et al.*, 2003; Koerner & Collins, 2014). This is especially important for South Africa’s Grassland Biome. The remaining grasslands must be studied to understand how altered environmental conditions affect ecosystem diversity so as to enable improved conservation for them. The fact that grasslands provide essential ecosystem services (ESs), such as forage provision, soil formation, and climate regulation via carbon sequestration underscore the imperative nature of such studies (Cadman *et al.*, 2013; Everson & Everson, 2016; Guuroh *et al.*, 2018).

The Grassland Biome is among those in South Africa that requires highest priority for conservation, restoration, and research (DEA, 2013). These grasslands are threatened by various land-use changes, including afforestation, intensive agricultural practices, mining, and urbanisation. Activities associated with soil disturbance such as ploughing and surface mining are considered to be disturbances to which old-growth grasslands are intolerant (Buisson *et al.*, 2019). These disturbances remove above- and belowground plant biomass, thus reducing the potential of perennial grassland plants to persist through resprouting (Buisson *et al.*, 2019). The subsequent loss of diversity, structure, and functioning may greatly influence the ability of grasslands to deliver essential ESs. Although it is well accepted that land-use change threatens grassland structure and diversity (Egoh *et al.*, 2011; Herben *et al.*, 2018a; Klimešová *et al.*, 2017; Miller *et al.*, 2011; Veldman *et al.*, 2015b), disturbance effects have rarely been quantified across different land-use types.

Climate models predict that precipitation will become more variable in the near future within southern Africa, including the occurrence of more extreme and prolonged drought events due to atmospheric warming (IPCC, 2012, 2014). Even though climate is important in influencing ecological phenomena, climate does not always explain the absence of forests in cases where non-forested biomes are warm and wet enough to support them (Bond, 2019). Water availability, rather than temperature (Bond, 2019), is a limiting factor for plant growth in southern Africa and there is a tension between inputs (rainfall) and losses (evaporation, transpiration, and drainage) (Van den Hoof *et al.*, 2018). This is why the mesic eastern half of South Africa consists of a mosaic of grasslands, savannas, and forests (Bond, 2019). Other than temperature and rainfall, climate variables such as wind, solar radiation, relative humidity, and evaporation impact plant physiology in terms of their impact on water stress (Hoffman *et al.*, 2019). It is well known that increasing rainfall variability that is linked to global environmental change may impact grassland floristic composition and diversity (Bond & Parr, 2010). Even so, understanding of the way in which rainfall variability, specifically drought, will affect floristic composition and diversity in grassland ecosystems is poorly understood. Enhanced knowledge of floristic diversity is therefore necessary to understand the community and functional shifts related to management and climate change.

Grasslands are not only threatened by land-use change, agricultural expansion, and drought, but also by woody plant encroachment (Buisson *et al.*, 2019; Egoh *et al.*, 2011; Herben *et al.*, 2018b; Klimešová *et al.*, 2017; Miller *et al.*, 2011; Veldman *et al.*, 2015b). Encroachment is the increase in density, cover, and biomass of woody species (trees and/or shrubs) in grasslands (Van Auken, 2009), hence replacing grasses and forbs (Cadman *et al.*, 2013; O'Connor *et al.*, 2014). Negative effects of encroachment include erosion, decreased rangeland productivity, and loss of other ecosystem services (D'Odorico *et al.*, 2010).

The ability of an ecosystem to maintain a healthy and productive state, regardless of changes in climate or land-use, is of great interest for all involved in the evaluation and mitigation of global change (Ruppert *et al.*, 2015). This is referred to as ecosystem stability (Donohue *et al.*, 2013; Pimm, 1984). There are multiple ways in which ecosystem stability can be defined and estimated, including resistance (a system’s ability to withstand disturbance (Pimm, 1984)), recovery also known as legacy effect (a system’s potential to (or rate of) return to a previous state after a disturbance (Pimm, 1984)), and resilience (the amount of disturbance a system can absorb without changing the state (Gunderson, 2000), also refers to the capacity for recovery of function (Donohue *et al.*, 2013; Pimm, 1984). These may therefore be used to determine the effect of change to a system, specifically related to drought for this study.

1.2 Aims, questions, and objectives of the thesis

The overarching aim of this study was to investigate, describe and critically evaluate the community composition and diversity responses of grassland ecosystems to direct and indirect anthropogenic disturbances, including land-use change, climate variability in the form of a severe drought and woody encroachment.

To achieve this, the aim was sub-divided into two more focused sub-aims. The first of these sub-aims was to quantify the floristic loss engendered by direct anthropogenic disturbances, such as land-use change in the Grassland Biome of South Africa. With a view to this, existing floristic data sets that incorporated various land-use changes in grasslands across South Africa were analysed. The second sub-aim was to assess the way in which indirect anthropogenic disturbances such as woody encroachment and climate change (increase in occurrence of drought) affect grassland diversity. In this case, a long-term observational dataset collected by the South African Environmental Observation Network (SAEON) from a threatened grassland type in South Africa, namely the Woodbush Granite Grasslands (WGG), was resampled and analysed across different rainfall years. The WGG supports two alternative state communities: the present study will refer to these as ‘open’ and ‘closed’ communities, where the former comprises open grassland with low woody cover and the latter open grassland with a higher cover of woody species with an increase in shrub density. The WGG is critically threatened, and the remaining fragments are extremely small. An improved understanding of the way in which climate change affects its ecosystem structure will be of critical importance for the design of appropriate conservation practices centred on this grassland type.

Table 1-1 reflects the research questions, objectives, and hypotheses for each result chapter, while Figure 1-1 represents a visual representation on how the respective objectives are interconnected. The hypotheses will be presented in more detail within each of the respective chapters.

Table 1-1: The research questions, objectives, and hypotheses for this study.

Research questions	Objectives	Hypotheses
Q1: How do land-use change and -intensification affect floristic diversity in the Grassland Biome of South Africa? (Chapter 4)	O1a: To illustrate how plant community composition changes when old-growth grasslands are being transformed into alternative land-uses, such as afforestation, agriculture, mining, and urbanisation. O1b: To document plant diversity changes, as well as floristic gains and -losses after grassland transformation, and to summarise the observed changes in taxa (families, species (endemic, native, and introduced)), life forms, and growth forms.	H1: Considerable shifts in plant communities are expected due to the expected increase of exotic species and the decrease in forb species richness. The floristic diversity of transformed grasslands in South Africa is expected to be significantly lower compared to untransformed, old-growth grasslands due to the higher abundance of alien species and dominance of disturbance-tolerant life forms and growth forms in transformed grassland sites.
Q2: Are the observed changes in woody cover in parts of the threatened Woodbush Granite Grassland related to forest expansion or savanna encroachment, and what are the potential consequences of increased woody cover on floristic composition and diversity? (Chapter 5)	O2a: To compare woody cover and woody species abundance differences between the community with observed higher woody cover (that is 'closed' grassland community) and the 'open' community in the WGG. O2b: To determine whether the woody species present in the WGG represent savanna encroacher or forest precursor species. O2c: To evaluate the potential effects of increased woody cover on species composition, plant diversity and abundance of endemic, threatened, and exotic species.	H2: The closed plant community reflects a previously open system experiencing savanna encroachment. This increase in woody abundance is expected to have negative impacts on herbaceous richness and diversity, since the encroached grassland no longer maintains the old-growth grassland diversity.
Q3: Will a severe drought affect the floristic composition and diversity of an open and encroached grassland similarly? (Chapter 6)	O3a: To understand how an open and encroached grassland responds to a severe drought in terms of their floristic composition and diversity and relating this to ecosystem resistance, recovery, and resilience. O3b: To assess whether an encroached grassland will become more or less encroached due to a severe drought. O3c: To Investigate the effect of a severe drought on the woody species of an encroached grassland.	H3: Encroached grasslands are less resistant and resilient to drought than open grasslands, since they no longer support the old-growth grassland diversity with more resistance. The encroached community will have experienced a loss in species diversity, thus changing the community composition over time, meaning recovery the initial state will be more difficult.

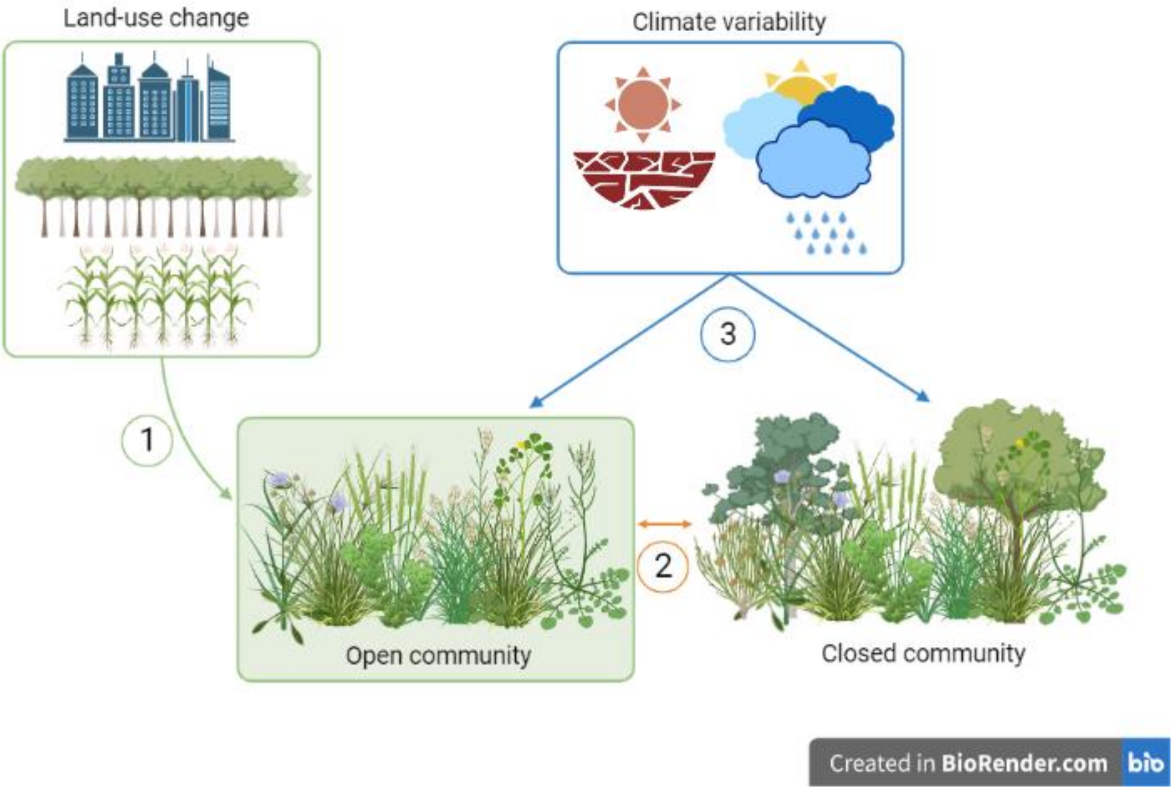

Figure 1-1: A visual representation of the research objectives: 1) the effects of land-use change on grassland diversity and composition; 2) the effect of increased woody covert on grassland diversity and composition; and 3) the effect of climate variability on an old-growth, open community and an old-growth community with increased woody cover (i.e. 'closed').

1.3 Structure of the thesis

This thesis conforms to the guidelines stipulated for a standard research thesis at the North-West University[1]. Chapters that represent results and discussion were structured to present a complete view of the research question addressed. For this reason, Chapters 4, 5, and 6 are presented in a format that will facilitate the submission of manuscripts for consideration of publication in scientific journals. Therefore, limited repetition of concepts and methods does occur throughout the thesis. Cited research is included as a single list of references at the end of the thesis.

Chapter 2: Literature review

A detailed overview of the relevant extant literature in terms of the present research title is provided in this chapter. It defines grassland ecosystems for this study, elaborates on vegetation structure, drivers, and dynamics of grasslands globally, and in South Africa, and highlights the

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[1] See section 6 of the Manual for M and PhD Studies

research gaps to be addressed, namely land-use change, woody encroachment, and rainfall variability.

Chapter 3: Study area

This chapter describes the study area. It provides information on the general ecology, including locality, climate, topography, geology, soil, and vegetation of the study sites.

Results and discussion:

Chapter 4: Effects of grassland transformation on floristic diversity of South African grasslands

This chapter addresses the first research question by providing descriptions of the community composition of untransformed and transformed grasslands by means of an assessment of species assemblages. The materials and methods for sampling and analyses used for this research question are described. Results on the floristic gains and -losses centred on transformed grasslands in terms of families, species (endemic, native, and introduced), life forms, and growth forms are also presented. The results presented in this chapter were published in *Bothalia (2021, 51(1): 147-155)*. Title: *A floristic assessment of grassland diversity loss in South Africa*. DOI: https://doi.org/10.38201/btha.abc.v51.i1.11

Results that are presented in Chapters 5 and 6 are narrowed down to one specific grassland type in South Africa that is currently under threat due to fragmentation and land conversion.

Chapter 5: Woody encroachment as a potential threat to grassland diversity

The second research question is addressed in this chapter. It describes the community composition of the fragmented WGG by assessing the potential effects of woody plant encroachment on species assemblages and diversity. The materials and methods for sampling and analyses used for this research question are described in this chapter.

Chapter 6: Effects of drought on grassland communities of the Woodbush Granite Grassland

The third research question is addressed in this chapter by describing the effect of a severe drought on community composition, resistance, resilience and the recovery by assessing and comparing species assemblages. Furthermore, the floristic gains and losses related to rainfall variability in the WGG are presented by means of the application of the temporal beta-diversity index. The chapter describes the materials and methods that were used for sampling and analyses.

Chapter 7: Discussion, synthesis of results, and conclusion

This chapter represents a summary of the results discussed in the preceding results chapters (Chapters 4-6). It synthesizes the relevance and implications of the research for local and larger-scale grassland conservation and management. It furthermore discusses the main findings and highlights the potential gaps for future research on this topic. Lastly, it presents possible management implications for grassland conservation.

CHAPTER 2 LITERATURE REVIEW

2.1 Grasslands as open ecosystems

Open ecosystems, which include grasslands, have long been considered to be of anthropogenic origin, and were regarded as degraded land or secondary successional stages caused by deforestation and burning of forested landscapes (Bond, 2019; Bond & Parr, 2010; Bredenkamp *et al.*, 2002). As such, grassland ecosystems are generally perceived to be secondary vegetation types created by human disturbances, while “natural” grasslands were perceived to be globally restricted to areas where the climate was unsuitable for forest plants to grow (Bond & Parr, 2010). According to these premises, successional grasslands will revert to forested systems unless they are maintained by fire or herbivory (Bond & Parr, 2010). Global ancient grasslands, also referred to as ‘old growth grasslands’ (Veldman *et al.*, 2015b) have been misunderstood and neglected in global conservation policies due to a weak understanding of its diversity and ecology (Bond, 2019).

One of the strongest reasons to doubt these views of anthropogenic origin about the extent of grasslands is their remarkable biodiversity generated and maintained in response to millions of years of exposure to natural disturbances (Bond, 2019; Bredenkamp *et al.*, 2002; Buisson *et al.*, 2019; Veldman *et al.*, 2015b). Some of the world’s richest biodiversity hotspots are open ecosystems that developed disturbance-tolerant flora with traits centred on their phylogenetic diversity and morphological traits (Bond, 2019; Bredenkamp *et al.*, 2002). The fact that open ecosystems are rich biodiversity hotspots implies that they have been stable and persistent long enough for speciation to have occurred, evidenced by species that date back millions of years (Bond, 2019; Bond & Parr, 2010). Even so, secondary grasslands created by deforestation and ploughing are widespread and are increasing due to the pressure of expanding human populations (Bond, 2019).

To enhance the understanding of the ecology of open ecosystems, it is important to distinguish between successional/secondary grasslands, that form after the removal of forest or after grassland plants were uprooted and left to restore naturally, and ancient grasslands, also known as old-growth grasslands, that have existed for millennia (Bond & Parr, 2010; Buisson *et al.*, 2019). Old-growth grasslands are ancient ecosystems of Cretaceous origin (~145 million years ago), characterised by high herbaceous species richness, high endemism, and unique species composition (Veldman *et al.*, 2015b).

2.1.1 Defining grassland ecosystems

Although it is reasonable to assume that grasses would predominate in grasslands, the name "grasslands" generally refers to a wider range of ecological vegetation types such as grassland versus forest, desert, tundra or wetland (Dixon *et al.*, 2014a). Although the idea still stresses the dominance of grasses or plants that resemble grasses (graminoids) and the absence of trees in that context, the entire range of growth forms may include grasses, other plants that resemble narrow-leaved grasses (that is non-woody graminoids), and even forbs (Dixon *et al.*, 2014a).

Grassland vegetation consists of graminoids, forbs defined as non-graminoid, herbaceous angiosperm species according to Bond and Parr (2010), and shrubs comprising a continuous herbaceous layer with very few trees (Veldman *et al.*, 2015b). According to Stavi (2019) grasslands are ecosystems with a tree density of 10 trees ha^{-1} or less. Grassland biomass is high, especially belowground, which turns them into complex and dynamic ecological systems (Carbutt *et al.*, 2017). Furthermore, open ecosystems which include grasslands, consist mainly of shade-intolerant plant species and they are disturbance-prone environments (Pausas & Bond, 2020; Wigley *et al.*, 2020).

In many cases, savannas and open-canopy grassy woodlands are defined in such a way as to include grasslands (Higgins & Scheiter. 2012; Nerlekar & Veldman, 2020). Grasslands are however distinguished from savannas, as they have lower average monthly temperatures during non-growing seasons (Bredenkamp *et al.*, 2002; Cowling *et al.*, 1997), fewer growth days, and lower summer heat units (Wakeling *et al.*, 2012). In South Africa, grasslands also occur at higher altitudes (Bredenkamp *et al.*, 2002). Grasslands furthermore are frost-prone ecosystems that experience occasional snowfall, whereas savannas are warmer with very little or no frost days during winter (Bredenkamp *et al.*, 2002).

There are similarities between the two biomes, with grasslands and savannas both dominated by C_4 grasses, burn regularly (more on fire in section 2.3.2.2) (Acocks, 1988), and have the same rainfall seasonality (Cowling *et al.*, 1997). However, savannas are characterised by the co-dominance of an almost continuous grass cover and discontinuous tree cover, while grasslands have sparse to no tree cover, whereas scattered shrubs may occur (Bond, 2019; Bond & Parr, 2010; Pillay & Ward, 2022). Plant growth, especially for tree species that occur across savanna and grassland biomes is lower in the cooler grasslands than in the warmer savannas (Wakeling *et al.*, 2012). This means that it takes longer for tree seedlings in grasslands to grow to a height above the flame and frost zone, so that there will be a lower probability of saplings escaping to adult tree height in the interval between fires and frost (Wakeling *et al.*, 2012). Given these considerations, the present project will employ the term “grassland(s)” to refer to treeless grasslands, hence excluding savannas and grassy woodlands.

Grasslands extend across a range of environmental conditions from the tropics to the arctic, from sea level to mountain tops, from arid to humid areas, from sites with long hydroperiods to well-drained sites, and from shallow, rocky to deep clay soils (Bond & Parr, 2010; Veldman *et al.*, 2015b). Grasslands of the northern hemisphere occur in the Eurasian steppes, Hungarian puszta, Mexican pastizales, and American prairies (Bredenkamp *et al.*, 2002; Mucina *et al.*, 2006). In the southern hemisphere, grasslands are particularly prominent and occur in the South American pampas, the Australian Alps, New Zealand, Oceania, Sub-Antarctic Islands, the Brazilian Cerrado, Madagascar, and southern Africa (Bond & Parr, 2010; Bredenkamp *et al.*, 2002; Brown & Makings, 2014; Dixon *et al.*, 2014a; Mucina *et al.*, 2006; Sage, 2004).

In South Africa, location of the study sites, grasslands can be divided into two types based on climate, namely temperate and tropical (Mucina & Rutherford, 2006). These two types of grasslands differ in terms of macroclimate, structure and, to some extent, in the composition of life forms and species (Mucina & Rutherford, 2006). In South Africa, temperate grasslands include upland vegetation types that make up the Grassland Biome (Cadman *et al.*, 2013). The tropical grasslands occur along the coastal belt of KwaZulu-Natal and the Eastern Cape that are part of the Indian Ocean Coastal Belt Biome (Cadman *et al.*, 2013). Both these grassland types, as well as the savannas of South Africa form part of the global tropical-grassy biomes (Parr *et al.*, 2014).

2.1.2 Plant diversity of grasslands

Open ecosystems that have persisted over space and time develop species that are endemic to open habitats (Bond, 2019; Clark *et al.*, 2023). However, for many regions globally, grassland diversity is poorly known, in contrast with woody flora, which are well known (Bond & Parr, 2010). This could be attributed to difficulty to collect and identify forbs, which are considered to contribute considerably to the overall plant species diversity in grassy ecosystems (Bond & Parr, 2010).

The grasslands of southern Africa are exceptionally rich in phytodiversity (Bredenkamp *et al.*, 2002; Huntley, 1994) which underscores their antiquity and primary status (Bredenkamp *et al.*, 2002). For example, the Highveld grasslands of South Africa host about 3 800 species in 112 000 km^2 (Cowling & Hilton-Taylor, 1994; Huntley, 1989).

2.1.2.1 Grass and forb species

The most dominant species in grasslands in terms of cover are grasses but, when it comes to the species composition or richness of grasslands, grasses represent only a sixth of the total flora, while herbaceous forbs are the life forms that contribute to the high diversity in these biomes despite the fact that they occur in low densities (Cadman *et al.*, 2013; Edwards *et al.*, 2010). Only about 600 of 11 000 grass species are documented as being ecologically dominant in grasslands (Edwards *et al.*, 2010). Therefore, the grass component attributes mostly to the biomass, while the forb component attributes mostly to the biodiversity pool (Carbutt *et al.*, 2011).

Forbs are of central importance for the conservation of biodiversity and the functioning of grasslands (Bråthen *et al.*, 2021). Grasslands have high forb family diversity, with 26 families found in a 0.1 ha plot, which is much higher than that found in similar-sized plots in the Fynbos Biome (Bond & Parr, 2010; Uys, 2006). Zaloumis (2013) found that resprouting forbs dominate grasslands in terms of the number of species. These forbs have very little aboveground biomass and are usually short and over-topped by surrounding grasses. The phenology of several forb species is fire-driven as some species will flower within days, or some within a few weeks after a fire. Such life-history patterns evolved to avoid competitive exclusion by grasses (Uys *et al.*, 2004; Zaloumis, 2013; Zaloumis & Bond, 2011).

Restored grasslands can give the illusion of containing the same species as a natural grassland due to their significant grass cover and biomass (Zaloumis & Bond, 2011). However, when their forb diversity is taken into account, it soon becomes clear that transformed grassland is species-poor (Siebert, 2011; Zaloumis & Bond, 2011). Uys (2006) reported that the floral richness of primary grassland ecosystems lies in the diversity of forb taxa.

2.1.2.2 Woody species

Large woody plants are mostly absent from grasslands, and only smaller to medium-sized shrubs occur sporadically throughout a grassland (Mucina *et al.*, 2006). The Grassland Biome of South Africa has remained treeless partly because the rates of woody growth have been too slow in terms of their fire-return periods to grow beyond the fire trap (Wakeling *et al.*, 2012). The fire trap refers to a 0-3 m zone above the soil surface that experiences fires frequently, resulting in the mortality of plants above-ground that are not adapted to survive the effects of fire (Wigley *et al.*, 2020). The competition for root space between grass and woody species may also play a role in excluding woody plants from grasslands (Tedder *et al.*, 2014). This could be caused by the fact that the competition slows down growth, which renders individuals more vulnerable to fire, frost, and herbivores (O'Connor *et al.*, 2014). Another factor that may be partly responsible for excluding woody vegetation is the occurrence of severe frost (Wakeling *et al.*, 2012), although frost alone does not account for the absence of trees in grasslands (Bond *et al.*, 2003; Botha *et al.*, 2020; Wakeling *et al.*, 2012). Frost could contribute to the exclusion of trees from high-elevation grasslands by causing a loss of biomass, which renders plants less likely to escape fires, but this does not occur in all grasslands (Wakeling *et al.*, 2012). Seedlings damaged by frost must resprout from ground level, thus increasing the time needed to grow to a height allowing them to escape the effects of further frost and fire (Wakeling *et al.*, 2012).

2.1.2.3 Non-native species

Non-native or alien species invasion has a negative effect on indigenous grass and forb diversity (Buisson *et al.*, 2019). Alien grass species also alter fire regimes, as they can lead to high biomass accumulation, thus increasing fire intensity (Buisson *et al.*, 2019). In general, these species have a large effect on the ecology of grasslands, since they can change species composition, and abundance (Parker *et al.*, 1999) and nutrient cycling and hydrology (Levine *et al.*, 2003), as well as ecosystem drivers such as fire, nutrient loss, altered local microclimate, and prevention of succession (D'Antonio & Vitousek, 1992).

2.1.3 Grasslands and the alternative stable state theory

Perceptions that climatic conditions govern vegetation distribution at a large scale have persisted for several years (Bond, 2019), despite evidence that similar climate conditions may support different ecosystem types. For example, under climatic conditions that are preferential for forest formation, savanna has occurred for thousands of years (Pausas & Bond, 2020). This suggests that climate is not the single factor that determines vegetation patterns (Cheng *et al.*, 2021). Furthermore, plantations in landscapes dominated by grasslands suggest that conditions are warm and wet enough to support forests and yet, forest trees have not been part of the landscape for millennia (Pausas & Bond, 2020). This infers that grasslands may occur across various climate regimes and are likely to be controlled by alternative drivers. Two such drivers are fire and herbivory. This is because fires and herbivores are the main biomass consumers of grasslands, and are essential for the maintenance of grassland ecology (Archibald & Hempson, 2016).

The alternative stable state (ASS) theory provides an alternative framework for explaining the distribution of vegetation and temporal changes (Beisner *et al.*, 2003; Pausas & Bond, 2020). The ASS theory suggests that ecosystems exist in multiple stable states, each comprising a different set of structures and functions under similar environmental conditions (Cheng *et al.*, 2021). Each stable state is resilient to external condition changes or disturbances to some extent, which ensures the long-term persistence of the state (Cheng *et al.*, 2021). Global vegetation models have predicted that, in terms of fire, multiple stable biome states are possible across Africa, whereas the potential for multiple stable states decreases with increases in atmospheric CO_2 that could enable the faster growth and survival of more tree species (Moncrieff *et al.*, 2015).

An ecosystem will function at an ASS after a disturbance threshold has been crossed, also referred to as the ‘tipping point’; (Van Nes *et al.*, 2016), which causes an abrupt and systemic transition from the initial state to the alternative state (Cheng *et al.*, 2021). This process is known as a “regime shift” or “critical transition” (Cheng *et al.*, 2021) and requires a driver to initiate the transition (D'Odorico *et al.*, 2012). Intermediate states are unstable opposed to the new, stable state that is defined by a new set of environmental functions (Pausas & Bond, 2020) and often of lower resilience (known as the hysteresis effect), which may complicate restoration (Scheffer & Carpenter, 2003). Also, removing the driver that caused the state change will not necessarily cause the system to switch back to the initial stage (Pausas & Bond, 2020). A state will however return to the original stable state after a small disturbance occurred due to the existence of a stabilising feedback processes (Pausas & Bond, 2020).

A concept that has been derived from the ASS is the alternative biome state (ABS), which is typically used to describe the ASS of terrestrial ecosystems at large spatial scales (Dantas *et al.*, 2016; Pausas & Bond, 2020). ABSs, therefore, refer to biome states that are structurally and functionally different but can persist over generations under relatively unchanging environmental conditions. Grasslands and Afromontane Forest display this tendency (Cheng *et al.*, 2021; Pausas & Bond, 2020).

Understanding the conditions under which shifts occur and in which they do not has been identified as a research challenge (Pausas & Bond, 2020). This concept may be applicable to the Woodbush Granite Grassland which is potentially undergoing change to an alternative stable state through and observed increased woody cover of indigenous species in some areas of the remaining grassland fragments.

2.2 The value of grasslands

Approximately one billion people around the world live in the world Grassland Biome, which provides many ecosystem services required to support livelihoods (Egoh *et al.*, 2011; Gao *et al.*, 2016). Ecosystem services (ESs) are the goods and services provided to humans through ecosystem processes (Díaz & Cabido, 2001; Millennium Ecosystem Assessment, 2005). ESs, therefore, collectively act as a bridge between biodiversity-ecosystem function and human well-being (Millennium Ecosystem Assessment, 2005; Zhao *et al.*, 2020). Ecosystem functioning or ecosystem process (Tilman, 2001), comprises the biogeochemical activities that occur within a system through a specific arrangement of biotic and abiotic components (Díaz & Cabido, 2001; Naeem & Wright, 2003). Ecosystem functioning is promoted by higher biodiversity, which also drives ecosystem services (Balvanera *et al.*, 2006; Duncan *et al.*, 2015; Quijas *et al.*, 2010).

Ecosystems are valuable, as they fulfil various functions and perform various services simultaneously, in other words, they are multifunctional (Li *et al.*, 2017). There is a misconception about the true value of grasslands. Grasslands have high economic value and support the wellbeing of humans by providing, among others, ecological infrastructure, carbon sinks, albedo surfaces, plant-based medicines, food plants, and grazing for livestock (Carbutt *et al.*, 2017; Díaz *et al.*, 2006; Mark *et al.*, 2013; O'Mara, 2012). Grasslands around the world are moreover major sources of good quality drinking water because of low run-off and high water infiltration rates (Neary, 2011). Water supply is one of the most valuable services provided by grasslands (Egoh *et al.*, 2011). Within the grasslands of South Africa, 38 river ecosystems have been identified as critical for freshwater ecosystem conservation (Egoh *et al.*, 2011; Nel *et al.*, 2007).

Grassland biodiversity enhances human well-being through the provisioning of basic materials, food- and medicinal plants for a healthy living. In South Africa, approximately 30% of all plants sold locally for use as traditional medicines originate from grassland ecosystems (Egoh *et al.*, 2011; Williams *et al.*, 2000).

Grasslands further play a role in reducing the impacts of climate change (Díaz *et al.*, 2006) through the mitigation of greenhouse gas emissions, as they store and sequester carbon, primarily belowground (O'Mara, 2012).

Grasslands maintain highly suitable conditions for human habitation; consequently, approximately 40% of South Africans live and work in grassland areas (Cadman *et al.*, 2013). The Grassland Biome is the most productive agricultural biome in South Africa and also holds large coal deposits and goldfields (Neke & Du Plessis, 2004). The aforementioned suitable conditions are also necessary for many of the land-uses upon which food production and other economic activities depend (Cadman *et al.*, 2013). Furthermore, grasslands globally house 11% of Endemic Bird Areas and 29% of ecoregions with outstanding biological distinctiveness (Egoh *et al.*, 2011; White *et al.*, 2000).

Carbon sequestration is one of the most important regulating services provided by grasslands (Zhao *et al.*, 2020). Tropical and subtropical old-growth grassy biomes (TGB), which include South African grasslands, have a high potential to store belowground carbon, even more so than forests (Dass *et al.*, 2018; Silveira *et al.*, 2020; Wiesmeier *et al.*, 2015). Furthermore, belowground carbon is noticeably stable in TGB and is largely unaffected by fire or grazing (Veldman *et al.*, 2019). Globally, grasslands store 10-30% of soil organic carbon (Follett & Reed, 2010). Grassland carbon stocks accumulate in four carbon pools, namely aboveground biomass, belowground biomass, soil, and dead organic matter (Zhao *et al.*, 2020). Ancient grasslands are especially important when it comes to storing carbon for long periods in fire- and drought-prone areas (Veldman *et al.*, 2015b).

Biodiversity-ecosystem service relationships can take various forms and shapes, display mixed relationships or be non-existent (Duncan *et al.*, 2015). This indicates the variability of the effect of biodiversity on ESs. For example, a study conducted by Cowles *et al.* (2016) corroborates that biodiversity has a strong and positive influence on above- and belowground biomass. This indicates that the conservation of biodiversity is vital for promoting above- and belowground ESs in grassland ecosystems (Cowles *et al.*, 2016). Experimental studies have found that, as the level of plant diversity increases, ecosystem functioning such as primary production also rises (Binder *et al.*, 2018; Chen *et al.*, 2017).

Globally, however, biodiversity and the ecosystem services of grasslands are being lost at an unprecedented rate due to disturbances that threaten ecosystem processes, including land-use change, biotic exchanges, and changes in climate (Chapin III *et al.*, 2000). This is especially true for the world's old-growth grasslands (Buisson *et al.*, 2019). The health and food security of humans is threatened by every grassland ecosystem that disappears due to degradation (Mittermeier *et al.*, 2011).

To ensure the supply of ecosystem goods and services, more, rather than less, species are needed in a system (Hooper *et al.*, 2005). Furthermore, having a range of species present that responds differently to environmental disturbances can stabilise ecosystem processes in response to these changes (Hooper *et al.*, 2005). Plants are at the first trophic level, and therefore play an important role in ecosystem functioning and consequently in the provisioning of ESs (Quijas *et al.*, 2010). For example, plants provide aboveground biomass, and the associated ecosystem service is the provisioning of fodder (Quijas *et al.*, 2010).

2.3 Grasslands and disturbances

Grasslands are threatened by agricultural conversion, altered fire regimes, invasive species, surface mining, urbanisation, overgrazing, loss of native animals, liming, nitrogen deposition, misinformed afforestation projects, elevated atmospheric carbon dioxide (CO_2) levels (Buisson *et al.*, 2019; Neke & Du Plessis, 2004; Veldman *et al.*, 2015b), and habitat transformation engendered by human development activities (Everson & Everson, 2016; Stavi, 2019). Furthermore, biodiversity is threatened at both extremes of a disturbance gradient, namely too much disturbance and lack of disturbance (Stankeva Terziyska *et al.*, 2020). It is estimated that temperate grasslands world-wide lost >70% of their natural extent by 1950 (Egoh *et al.*, 2011; Millennium Ecosystem Assessment, 2005). The Grassland Biome is the most threatened biome in South Africa (SA), given that 35% of the total habitat transformation in SA occurs here (Egoh *et al.*, 2011; Reyers *et al.*, 2005).

Old-growth grasslands have, however, evolved in the presence of disturbances including frequent fires, herbivory, soil disturbance by digging fauna, low-intensity grazing, shallow nutrient-poor soils, seasonal water deficits and seasonal flooding, and are therefore dependent on such disturbances (Buisson *et al.*, 2019; Silveira *et al.*, 2020; Veldman *et al.*, 2015b). Such disturbances are defined as endogenous and should not be confused with exogenous disturbances, which are mostly of anthropogenic origin, such as land-use change (see section 2.3.1) and cause degradation and shifts to alternative ecosystem states (Buisson *et al.*, 2019). Cause of degradation in grasslands can also occur as a result of poor management of endogenous disturbances, such as fire suppression or exclusion, overgrazing and poorly managed livestock, declines in native megafaunal herbivores, woody encroachment, invasive species, atmospheric nitrogen deposition, elevated atmospheric CO_2 concentrations, and soil disturbances (Veldman *et al.*, 2015b).

According to the Intermediate Disturbance Hypothesis (IDH), an optimal disturbance level allows for different ecological niches to be filled due to differing life-history characteristics and differing responses to disturbance (Russell *et al.*, 2019). This is so, because disturbance acts as a large-scale force that shapes plant strategies by removing plant biomass and injuring or breaking individual plants (Bellingham & Sparrow, 2000; Klimešová *et al.*, 2017). Disturbances differ in severity and frequency, that affect plant species in different ways and select for different plant strategies (Herben *et al.*, 2016; Klimešová *et al.*, 2017; Miller *et al.*, 2011). For example, plants from central Europe that have root sprouting ability occur in more severely disturbed habitats than those that do not have this ability (Klimešová *et al.*, 2017). This means that, in disturbed habitats, root sprouting is a good strategy given that it provides plants with a specific type of clonal growth (Klimešová *et al.*, 2017).

Grasslands can be divided into primary and secondary ones based on the extent of disturbance that they are subjected to (Bond, 2019; Cadman *et al.*, 2013). Primary grasslands might be modified by disturbances or management practices, although their overall essential ecological characteristics remain (Cadman *et al.*, 2013). Secondary grasslands have been altered from their original state to an alternate state as is the case in naturally restored cultivated areas and deforested plantations that have been left to recover naturally (Bond & Parr, 2010; Cadman *et al.*, 2013). These secondary grasslands differ from primary grasslands in terms of species composition, vegetation structure, ecological functioning, and the ecosystem services they provide (Cadman *et al.*, 2013).

2.3.1 Exogenous disturbances

Exogenous disturbances are primarily driven by allogenic factors (Burton *et al.*, 2020). Exogenous disturbances remove above- and belowground plant organs, thus reducing the potential for plants to persist through resprouting (Buisson *et al.*, 2019). Examples of exogenous disturbances include urbanisation, agriculture, afforestation and mining (Carbutt & Kirkman, 2022; Carbutt *et al.*, 2011). These disturbances influence soil conditions to such an extent that they can no longer support old-growth grassland species (Buisson *et al.*, 2019). It was found that, 20 years after cultivation practices occurred in grassland, secondary grasslands recovered only by 25% of the pre-disturbed species richness (Zaloumis, 2013). Exogenous disturbances may also lead to invasion by alien plants. This is attributed to alien and invasive plant species being rapid colonisers and establishing themselves readily in disturbed environments (Buisson *et al.*, 2019).

2.3.1.1 Land-use change

Land-use change has caused dramatic declines in the world’s old-growth grasslands (Buisson *et al.*, 2019). Globally, grasslands provide suitable conditions for a variety of land-uses due to the fertility of the soil and suitable climate (Bond, 2019). For these very reasons, it has been highly transformed and fragmented, especially since high-priority biodiversity sites often are located in production landscapes such as agriculture, dams, and mines (Carbutt *et al.*, 2011).

The land-use types mostly responsible for transformation in the Grassland Biome in South Africa are cultivation (21.00% of grasslands transformed), degraded lands (6.10%), forestry plantations (2.80%), urban and industrial areas (1.62%), and mines and quarries (0.26%) (Carbutt & Kirkman, 2022; Carbutt *et al.*, 2011). These different land-uses in addition to invasive non-native plants and insidious rural sprawl (Carbutt & Kirkman, 2022) are putting considerable pressure on the grasslands of South Africa (Cadman *et al.*, 2013).

In South Africa, the Grassland Biome is the most productive in terms of agriculture (Egoh *et al.*, 2011; Mentis & Huntley, 1982). Yet, these agricultural practices have a negative impact on grassland biodiversity and ecosystem services (Egoh *et al.*, 2011). Frequently cultivated crops and pastures support fewer indigenous plant species than long-lived pastures, as few grassland species are adapted to annual cultivation (O'Connor, 2005). Long-lived pastures, on the other hand, allow time for the colonization of certain grassland species (O'Connor, 2005).

Afforestation is a serious threat to grasslands, especially in the higher rainfall areas of the eastern plateau of South Africa where mainly *Pinus* spp. and *Eucalyptus* spp. are cultivated (Bond & Parr, 2010; Cowling *et al.*, 1997). It occurs within areas that contain the highest biodiversity, endemism, and rare plant species (Cowling *et al.*, 1997; Neke & Du Plessis, 2004), even though it occupies a relatively small total area of the biome (Bond & Parr, 2010). Grassland species are outcompeted by trees for light and water (Buisson *et al.*, 2019). Shade-intolerant grassland species disappear: however, some species may persist (O'Connor, 2005). There is also a chance that invasive alien plants from the timber plantations could migrate into surrounding grassland ecosystems (Cadman *et al.*, 2013).

Urbanisation also has a strong influence on the plant species composition of grasslands and increases the probability of their local extinction (Williams *et al.*, 2005). Urban expansion bolsters habitat fragmentation and biodiversity loss, especially if the expansion is not properly planned with biodiversity and ecosystems in mind (Cadman *et al.*, 2013).

2.3.2 Endogenous disturbances

Endogenous disturbances are caused by autogenic and primarily biotic factors (Burton *et al.*, 2020). Endogenous disturbances occur naturally and are part of the evolutionary history of grasslands (Buisson *et al.*, 2019), which include climate, fire, and herbivory. Endogenous disturbances maintain old-growth grassland plant diversity by suppressing competition between species, preventing woody encroachment, stimulating reproduction, and creating opportunities for recruitment (Buisson *et al.*, 2019).

Climate, fire, and grazing primarily influence and maintain vegetation dynamics in grassland systems (Koerner & Collins, 2014; Veldman *et al.*, 2015b). Old-growth grasslands require frequent fires and herbivory to persist, especially where conditions are suitable for forest development (Veldman *et al.*, 2015b). This is because fires and herbivores are the main biomass consumers of grasslands, and are essential for the maintenance of grassland ecology (Archibald & Hempson, 2016). Across a wide range of climatic and soil conditions frequent fires and herbivory limit the abundance of trees and shrubs, promote herbaceous productivity, consume dead plant material, return nutrients to the soil, stimulate reproduction, and maintain plant diversity (Veldman *et al.*, 2015b).

An increase in grazing intensity is one of the main drivers of grassland degradation (Van den Hoof *et al.*, 2018). High stocking densities and the sowing of alien grasses for grazing can irreparably change old-growth grasslands (Feller & Brown, 2000; Veldman *et al.*, 2015b). It has, however, been established that livestock grazing at appropriate densities and intervals may be compatible with or even beneficial for grassland systems (Veldman *et al.*, 2015b), as megaherbivores were common on most of the planet just a few thousand years ago (Bond, 2019).

2.3.2.1 Climate variability

Droughts have intensified since the early 1900s in terms of frequency, severity and extent (Masih *et al.*, 2014). South Africa has been witnessing an overall warming since 1960 in both summer and winter months (Gosling *et al.*, 2011). Precipitation is predicted to decrease over most of South Africa, especially in the west, with decreases of up to 20% (Engelbrecht & Engelbrecht, 2016; Gosling *et al.*, 2011). Furthermore, it has been projected that there would be further temperature increases up to around 4°C inland and about 3°C near the coast (Engelbrecht *et al.*, 2015; Gosling *et al.*, 2011).

Droughts in southern Africa are likely to increase and become more widespread and extreme (Masih *et al.*, 2014) due to greater evaporation in response to warmer atmospheric temperatures (Liu *et al.*, 2017). It affects humanity in various ways including loss of life, crop failure, food shortages, malnutrition, and health problems (Masih *et al.*, 2014). Furthermore, it causes environmental damage through land degradation, aridity, and desertification (Masih *et al.*, 2014). Drought stress causes a deficiency in the water supply to plant roots, which could result in physiological and biochemical changes in plants (Rao & Chaitanya, 2016).

Even though it is predicted that climate change will have many negative effects on grassland vegetation, resilience to change has been reported for grassland vegetation to a certain extent suggesting that negative effects of climate change are limited (Hoffman *et al.*, 2019; Masubelele *et al.*, 2014). For a system to maintain its function in response to an extreme drought, it either has to display resistance during the drought or high resilience after the drought (Wilcox *et al.*, 2020).

It has been found for high altitude grasslands of South Africa, that there is a shift between tallgrass and shortgrass systems due to interactions among climate, pastoralists and fire (Dabengwa *et al.*, 2022). Droughts thus pose severe threats to grazing systems and human livelihoods they support (Dabengwa *et al.*, 2022).

Grass, forb, and woody species generally respond differently to drought. In many grasslands, grasses tolerate drought better than forbs due to their C_4 photosynthetic pathways (Ward *et al.*, 1999), while forbs flourish occasionally due to their ability to avoid drought by making use of deep rooting systems (Nippert & Knapp, 2007). Woody plants are expected to increase in dominance and/or biomass in grassland ecosystems as the climate changes due to higher atmospheric CO_2 concentrations (Bond & Midgley, 2012; Higgins & Scheiter, 2012; Moncrieff *et al.*, 2015). In the presence of elevated rainfall and temperature, woody species will also benefit (O'Connor *et al.*, 2014).

Global environmental change is altering rainfall patterns, grazing, and fire regimes simultaneously. Therefore, it is important to consider all of these drivers to better understand grassland dynamics (Fay *et al.*, 2003; Koerner & Collins, 2014). Combined, these drivers (rainfall, fire, and herbivory) could significantly affect plant community composition and grassland ANPP (Koerner & Collins, 2014; Yarnell *et al.*, 2007). Fire frequency will be an important consideration for determining ecosystem resilience to extreme drought, as it will influence the proportion of grasses and forbs (Wilcox *et al.*, 2020).

2.3.2.2 Fire

Fire is a vital component for maintaining grasslands (Bond & Parr, 2010; Mucina *et al.*, 2006; Uys *et al.*, 2004), as it sustains biodiversity patterns and ecological processes (Archibald *et al.*, 2018; Cadman *et al.*, 2013). Fire is responsible for removing grass biomass (Bond & Parr, 2010) and restricting tree cover and woody plant encroachment by killing the stems of young trees, thus altering the rates of tree growth and survival of adult trees, which promote grasses (Buisson *et al.*, 2019; Parr *et al.*, 2014). Fire can be viewed as a no-preference herbivore that consumes complex organic compounds, including indigestible plants, and converts them to combustion by-products (Bond, 2019). Forbs in grassy systems are very resilient to frequent fires due to their belowground storage organs (Zaloumis & Bond, 2016).

Grassland fires are naturally started by thunderstorms during the transition from dry to wet seasons (Bond & Parr, 2010). Natural fire regimes have been altered by humans through the promotion of grazing, introduction of plants, suppression of fires, and modification of the season and number of ignitions, among others (Batista *et al.*, 2018). Unplanned and poorly timed fires could have detrimental effects on natural habitats, the functioning of an ecosystem, human lives, and properties (Batista *et al.*, 2018; Cadman *et al.*, 2013).

The exclusion of fire, on the other hand, could result in alternate-state grassland or transition to a different biome (Cadman *et al.*, 2013). By excluding fire from old-growth grassland communities, herbaceous species may be eliminated due to litter accumulation or competitive exclusion by other dominant plant species (Buisson *et al.*, 2019). When fires are excluded from a system for short periods, biomass increases which may lead to increased fire intensity when it burns again (Buisson *et al.*, 2019). Fire exclusion over longer periods results in forest expansion, the loss of herbaceous grassland species, and reduced flammability (Buisson *et al.*, 2019; Fidelis, 2020). Wakeling *et al.* (2012) demonstrated that, if fire is excluded from grasslands for long enough to allow tree saplings to grow, the grassland would eventually change to a forest. It has been argued that the exclusion of fire from a system could have more detrimental effects on an ecosystem than the fire event itself (Fidelis, 2020).

The likelihood of grassland loss increases as adjacent habitat is converted (Boakes *et al.*, 2010), for example, to plantations and becomes fragmented (Scholtz & Twidwell, 2022). Fire and fragmentation interact in three main ways, namely (1) fire either develops and connects habitat or destroys and fragments it; (2) after habitat is fragmented, fire in the landscape is then altered because people suppress or ignite fires, or there is increased edge flammability or increased obstruction to fire spread; and (3) fire and fragmentation interact to affect species richness, abundance and extinction risk (Driscoll *et al.*, 2021). A change in fire regime could ultimately lead to changes in habitat such as grasslands becoming encroached by woody species (Stevens *et al.*, 2017).

2.3.3 Woody Plant Encroachment

Woody plant, shrub or bush encroachment is the increase in density or cover of woody species (trees and/ or shrubs) in grasslands (Van Auken, 2000), hence replacing grasses and forbs (Cadman *et al.*, 2013; O'Connor *et al.*, 2014). Shrub encroachment starts with the deposition of seeds within a grassland community (Archer *et al.*, 2017). For this reason, woody species that disperse their seeds by wind or birds would likely be the first to colonise a new area, although some woody species are also distributed by ungulates (Archer *et al.*, 2017). Once these species have established in grasslands, their seed is further distributed by animals or self-distributed (Archer *et al.*, 2017).

Shrub encroachment is promoted by severe grazing by means of the reduction of the fuel load or the reduction of grass competition (O'Connor *et al.*, 2014). A reduction in fuel load will affect the intensity and frequency of fires. In the 1960s and 1970s, an increase in bush encroachment in southern Africa occurred due to severe drought and a peak in national cattle numbers (O'Connor *et al.*, 2014). Drought combined with heavy grazing results in the reduction of perennial grass species (O'Connor, 1985; O'Connor *et al.*, 2014; Pfeiffer *et al.*, 2019).

Fire suppression leads to an increase in woody cover in grasslands (Stevens *et al.*, 2017). Consequently, a loss of species and ecosystem services is incurred (Abreu *et al.*, 2017; Fidelis, 2020). It has been recorded that the exclusion of fires in a moist grassland resulted in the establishment of a scrub forest (O'Connor *et al.*, 2014). Furthermore, higher levels of CO_2 results in higher root biomass, which enables woody plants to rapidly regrow following the loss of aboveground biomass caused by disturbances such as fire (O'Connor *et al.*, 2014).

Shrub encroachment could have negative effects including increased erosion, decreased rangeland productivity, and loss of other ecosystem services (D'Odorico *et al.*, 2010). Some known causes of shrub encroachment include climate change, overgrazing, CO_2 enrichment, nitrogen deposition, changes in fire intensity or frequency, and combinations of these factors (Belayneh & Tessema, 2017; Cadman *et al.*, 2013; Van Auken, 2000; Venter *et al.*, 2018). Therefore, shrub encroachment itself is often a product of exogenous disturbance but, in a number of cases, it is caused by alterations of endogenous disturbances (Archer *et al.*, 2017).

There are two kinds of explanation for shrub encroachment, namely bottom-up and top-down controls (Cipriotti *et al.*, 2014). Bottom-up controls are resources, such as water and nitrogen, that act as limiting factors, since grass and shrub species have different root characteristics that allow them to use soil resources differently. Included in the bottom-up control are the two-layer and resource-pool hypotheses (Germino & Reinhardt, 2014). The two-layer hypothesis states that grass species use shallow soil water, whereas woody species make use of the deeper soil water[2]. In its turn, the resource-pool hypothesis states that shallow soil water promotes the growth of all plant species, whereas deeper soil water supports the physiological activities and survival of deep-rooted. Top-down controls involve disturbances such as fire, grazing, and drought that limit shrub growth.

2.3.4 Forest expansion

Across the world, mosaics of grassland and forest with clear transitions between them are a common occurrence (Bond & Parr, 2010; Parr *et al.*, 2014). In some cases, however, these forests cross over into the grasslands. Forest expansion is the increase of forest species where they did not historically occur (Veldman *et al.*, 2015a). Forest expansion is of concern since the conversion of grasslands to forests comes at a high cost to biodiversity (Bremer & Farley, 2010) and ecosystem services (Jackson *et al.*, 2005). Dense tree cover drastically restricts the productivity and richness of light-demanding herbaceous plants while decreasing habitat for animals

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[2] The vertical distribution of soil water can be divided into two layers, namely an active layer (0-2 m) and a relatively stable layer (2-21 m) (Wang *et al.*, 2015).

acclimated to open habitats, making dense tree cover inherently incompatible with grasslands (Veldman *et al.*, 2015b). Furthermore, trees require more water and soil nutrients than grasses and forbs, and possess notably distinct strategies for allocating carbon above and below ground (Jackson *et al.*, 2007). Expanding forests have the potential to significantly impact hydrology, limit spoil-carbon storage, and modify nutrient cycles (Veldman *et al.*, 2015a).

Many of the world's open ecosystems occur where the climate can theoretically support forests (Bond, 2019), therefore fire exclusion is a major contributor to forest expansion (Stevens *et al.*, 2017; Veldman *et al.*, 2015a). Forests typically have closed canopies and relatively sparse ground cover, which does not provide sufficient flammable biomass to carry fires (Gillson, 2015; Hoffmann *et al.*, 2012). Another factor that limits the expansion of forest into grassland is the occurrence of frost (Botha *et al.*, 2020). African trees are of tropical or subtropical origin and are generally frost sensitive (Bredenkamp *et al.*, 2002). Frost prevents tree recruitment by killing saplings or keeping them in a "frost trap" by repeated annual top kill (Finckh *et al.*, 2016; Wakeling *et al.*, 2012; Whitecross *et al.*, 2012).

2.4 Ecosystem stability

The ability of an ecosystem to maintain a healthy and productive state, regardless of changes in climate or land-use, is of great interest for all stakeholders (landowners, decision makers, and scientists) involved in the evaluation and mitigation of global change (Ruppert *et al.*, 2015). This ability is referred to as ecosystem stability (Donohue *et al.*, 2013; Pimm, 1984). There are multiple ways in which ecosystem stability can be defined and estimated, including resistance, recovery, and resilience (Donohue *et al.*, 2013). Both the environmental conditions of today and the legacies of historical events influence the patterns and functions of ecosystems (Johnstone *et al.*, 2016; Ogle *et al.*, 2015; Sala *et al.*, 2012).

Resistance refers to a system's ability to withstand disturbance (Hodgson *et al.*, 2015; Pimm, 1984). Resilience refers to the amount of disturbance a system can absorb without changing the state (Gunderson, 2000; Hodgson *et al.*, 2015), also the capacity for recovery of function (Pimm, 1984). Recovery or legacy effect is the processes that returns the system to an equilibrium state (Hodgson *et al.*, 2015). Legacies can also be defined as the change in resources or ecosystem properties that continue to affect an ecosystem after a disturbance event (Griffin-Nolan *et al.*, 2018; Yahdjian & Sala, 2006). Legacies affect both the resistance to current environmental conditions and are influenced by the resistance and resilience of past events (Hoover *et al.*, 2021).

The long-term resilience of ecosystem functions, processes, and services depends on high levels of biodiversity (Oliver *et al.*, 2015). Biodiversity provides resilience, or an "insurance" role: greater diversity buffers systems against change (Mace *et al.*, 2012). The traits of the dominant plant species also strongly influence the resilience and resistance of an ecosystem (Díaz & Cabido, 2001). For example, communities dominated by plant species that grow fast, often have high resilience and low resistance (Aerts, 1995; Lepš *et al.*, 1982; MacGillivray *et al.*, 1995).

Resistance and resilience can be evaluated as rate functions and as the degree of recovery, depending on the type of disturbance (Lake, 2013). It is also important to note that both an original and degraded state of an ecosystem have resistance and resilience. The resistance and resilience may be viewed as being positive in that they serve to maintain the original state, while that of the degraded state may be viewed as negative in that it will serve to limit the effects of recovery (Lake, 2013).

2.5 Conservation and restoration of grassland ecosystems

The grasslands of South Africa are a conservation priority, as the restoration of these old-growth systems can be very limited once they have been destroyed by exogenous disturbances that lead to a loss in belowground biomass (Buisson *et al.*, 2019; Ferraro *et al.*, 2021; Nerlekar & Veldman, 2020; Silveira *et al.*, 2020; Veldman *et al.*, 2015b; Zaloumis & Bond, 2011). This loss can be further accelerated by the increasing pressure of climate change and human-related activities (Bond, 2019; Zaloumis & Bond, 2011).

It is important to know and understand the ecosystem that is to be preserved and the species it consists of in order to conserve biodiversity and the ecosystem services they provide (Andrade *et al.*, 2019). This is especially true for grasslands, as they are in many cases degraded systems originating from the clearing of forests (Silveira *et al.*, 2020). The conservation of old-growth grasslands must therefore be conceptualized in terms of their biodiversity, which is not always evident to the untrained eye (Veldman *et al.*, 2015b). Furthermore, information regarding the presence of rare, threatened, or alien plant species is important for the evaluation of conservation value and consequent decisions about management and land-use change (Andrade *et al.*, 2019).

Some tropical grasslands have been recognised for their biodiversity, and are therefore conserved, while others receive little conservation attention (Buisson *et al.*, 2019; Strassburg *et al.*, 2017). For instance, the Montane Grasslands of southern Africa are under-recognised (Buisson *et al.*, 2019; Dixon *et al.*, 2014b; Mittermeier *et al.*, 2011). This should be challenged so that existing old-growth grasslands receive attention and are conserved rather than trying to restore secondary grasslands (Buisson *et al.*, 2019), given that the former support higher floral and faunal biodiversity (Stavi, 2019).

To recover abandoned agricultural land, a fire program has to be implemented, or restoration will not occur (Buisson *et al.*, 2019; Silveira *et al.*, 2020). Fire can be used to remove fire-sensitive invasive species, but it can also promote fire-tolerant invasive species or open up sites in which invasive species can establish themselves (Buisson *et al.*, 2019). One should, therefore, consider the fire ecology of invasive species carefully when one plans to use fire as a restoration tool (Buisson *et al.*, 2019). In areas where fire cannot be used to manage grasslands, grazing by livestock may be a substitute, as they will control the biomass of palatable plants and prevent woody encroachment (Buisson *et al.*, 2019). This will, however, require a good understanding of the ecology of the system and how it will respond to such grazing (Buisson *et al.*, 2019).

It is critical to make a distinction between reforestation and afforestation (Silveira *et al.*, 2020). Some believe, potentially ignorantly, that the restoration of grasslands should involve the planting of trees as suggested by the Bonn Challenge "Atlas of forest and landscape restoration opportunities" (Silveira *et al.*, 2020; Temperton *et al.*, 2019) and the trillion trees campaign supported by the World Economic Forum (Bastin *et al.*, 2019). Planting trees where they did not historically occur has been shown to decrease plant and animal life and ecosystem services (Jackson *et al.*, 2005; Veldman *et al.*, 2015a; Veldman *et al.*, 2019). It is especially concerning that current large-scale tree planting programmes have not been properly planned: they do not completely consider long-term changes, prevent the escape of alien species into open ecosystems, or cope with intense fires fuelled by trees (Bond, 2022).

The transformation of an old-growth grassland to an agricultural field can occur within one day, while the restoration of the field may take up to a century or more, and that is when conditions are favourable (Veldman *et al.*, 2015b). In the presence of invasive species and altered biophysical conditions, transformed grasslands may never return to an old-growth state (Stromberg & Griffin, 1996; Veldman *et al.*, 2015b). These alien invasive species are serious threats to old-growth grassland biodiversity and a major challenge for restoration (Veldman *et al.*, 2015b).

Even though colonization of native grassland plant species is possible after grasslands have been transformed, such plant communities remain different in terms of species- and functional trait composition, even after 40 years since the disturbance event (Muller *et al.*, 2021; Zaloumis, 2013). Even if grasslands can recover from disturbances by way of succession, it will take decades, or even centuries to return to a natural state (Silveira *et al.*, 2020; Zaloumis, 2013). Furthermore, if we want to preserve the provisioning of ecosystems by natural systems, there is a need to focus on the preservation and restoration of their biotic integrity in terms of species composition, relative abundance, functional organisation, and species numbers, rather than focusing only on increasing the number of species present (Díaz *et al.*, 2006).

Grasslands have been ignored in a conservation context for a long time and have suffered transformation and degradation as a consequence (Matsika, 2007; Van Oudtshoorn *et al.*, 2011). Conservation of species richness deserves the highest priority in ecological agendas, because the connections between biodiversity and ecosystem functioning and services are reasons enough to protect the biotic integrity of existing and restored grassland ecosystems (Díaz & Cabido, 2001; Díaz *et al.*, 2006).

2.6 Summary

The grasslands of South Africa are ancient, old-growth ecosystems that developed in the presence of climate variability, fire, and herbivory. These act as drivers of grassland dynamics and need to be better understood to promote grassland conservation. Old-growth grasslands are threatened by exogenous disturbances including land-use change, cultivation, afforestation, and urbanisation.

Grasslands contain a wealth of biodiversity and functional traits that are vital for maintaining ecosystem functions which, in turn, provide various ESs. Consequently, this thesis will aim to investigate, describe, and critically evaluate the responses of grassland ecosystems to land-use change, rainfall variability, as well as woody encroachment.

CHAPTER 3 STUDY AREA

3.1 General ecology of grasslands in South Africa

The vegetation of South Africa (SA) is classified into nine biomes (Mucina & Rutherford, 2006), of which the Grassland Biome covers the second highest land surface area (27.9%; Figure 3-1). The largest biome in SA is the Savanna Biome (32.5%), which is also considered to be one of the grassy biomes of SA (Mucina & Rutherford, 2006). The Grassland Biome of SA borders warm, moist savannas to the north and east and warm to hot, dry savannas to the northwest, while the south and southwest borders stretch along cool, dry semi-desert Karoo scrub (Bredenkamp *et al.*, 2002). The extent of the Grassland Biome in SA is defined on the basis of vegetation structure (Section 3.1.1) and environmental factors, such as annual mean summer rainfall and minimum temperatures in winter (Section 3.1.2) (Mucina *et al.*, 2006).

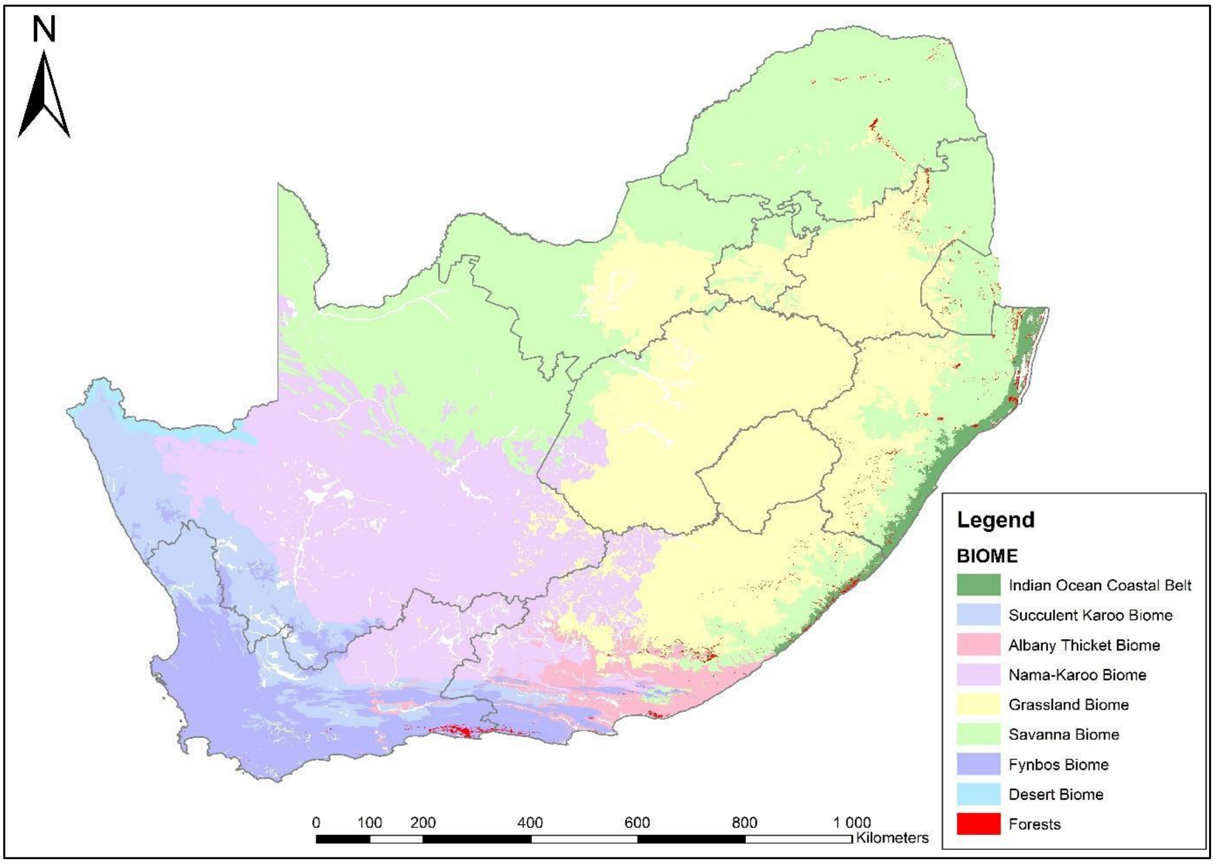


Figure 3-1: A vegetation map of South Africa illustrating the distribution of its nine vegetation biomes (map created by Wynand Muller).

3.1.1 Vegetation structure of the Grassland Biome

Grasslands consist of graminoids (especially Poaceae), and forbs, which form a relatively continuous herbaceous layer, and some shrubs (Mucina *et al.*, 2006; Veldman *et al.*, 2015b). Grasslands have sparse to no tree cover but as indicated, scattered shrubs may occur (Bond, 2019; Bond & Parr, 2010; Veldman *et al.*, 2015b). The interaction between cold temperatures and fire keeps the grasslands treeless (Wakeling *et al.*, 2012).

3.1.2 Climate of the Grassland Biome

The major grassland types of southern Africa are distributed along an altitudinal temperature and rainfall gradient, as seen in Figure 3-2 (Bredenkamp *et al.*, 2002). Grasslands occur where summer rainfall and winter drought are found (Mucina & Rutherford, 2006). Rainfall in the South African grasslands ranges from 400 to >1200 mm per year (Cowling *et al.*, 1997; Mucina & Rutherford, 2006). A distinction can be made between arid and moist grassland in terms of a mean annual precipitation (MAP) threshold of 500-700 mm (Mucina *et al.*, 2006), as in Figure 3-2.

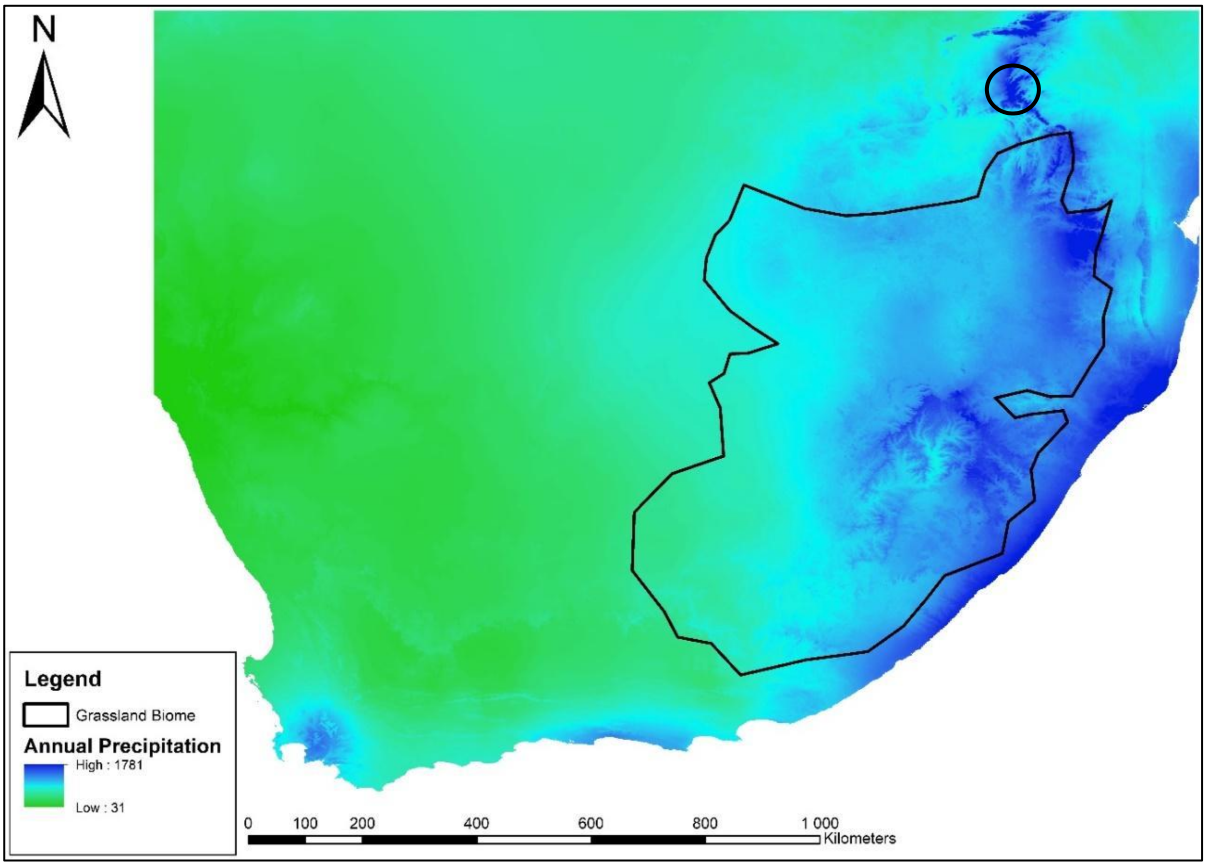


Figure 3-2: A map showing the mean annual precipitation (MAP, mm) for southern Africa, with the approximate position of the Grassland Biome indicated (Bioclim 12) (map created by Wynand Muller).

Elevation (Figure 3-3) has a strong influence on climate variability in the Grassland Biome. An increase in altitude corresponds with a decrease in temperature and increase in rainfall (Mucina & Rutherford, 2006). At high altitudes (>2000 m.a.s.l.) with low temperatures and high rainfall (>800 mm MAP), grasslands occur that mainly comprise C_3 grasses and trees are generally absent (Bredenkamp *et al.*, 2002). At mid-altitudes (1500 – 2000 m.a.s.l.) and the attendant warmer temperatures and lower rainfall (500 – 800 mm MAP), C_4 grasslands occur, while (sub)tropical trees are absent due to frost (Bredenkamp *et al.*, 2002) and frequent fires (Bond, 2019).

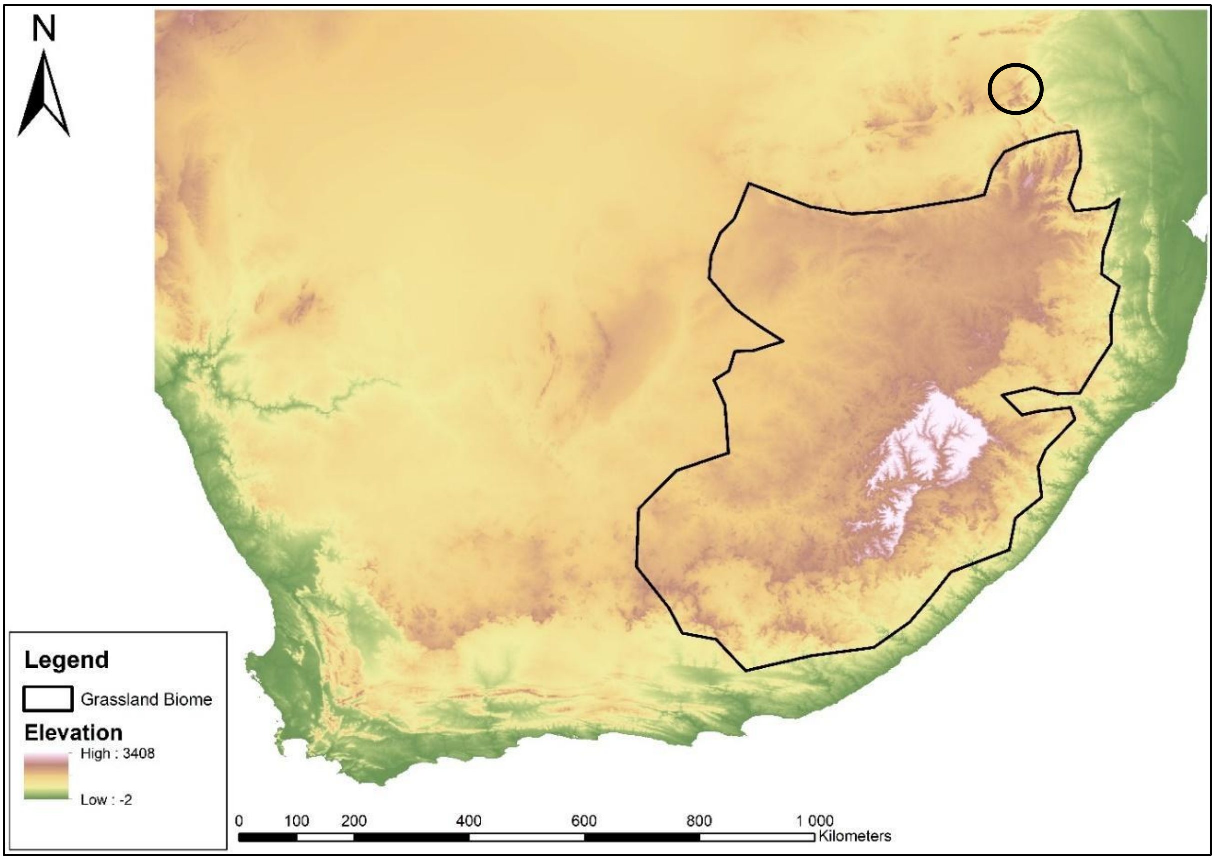


Figure 3-3: A map showing the elevation (m.a.s.l.) for southern Africa, with the approximate position of the Grassland Biome indicated (map created by Wynand Muller).

The high elevation of this biome in the interior of the African continent, engenders large intra-annual temperature fluctuations and a high occurrence of frost (Figure 3-4) (Mucina *et al.*, 2006). In the winter months, the climate in the biome is extremely cold and dry: June to August are usually the coldest months, during which months frost is a rare to common occurrence (Mucina *et al.*, 2006).

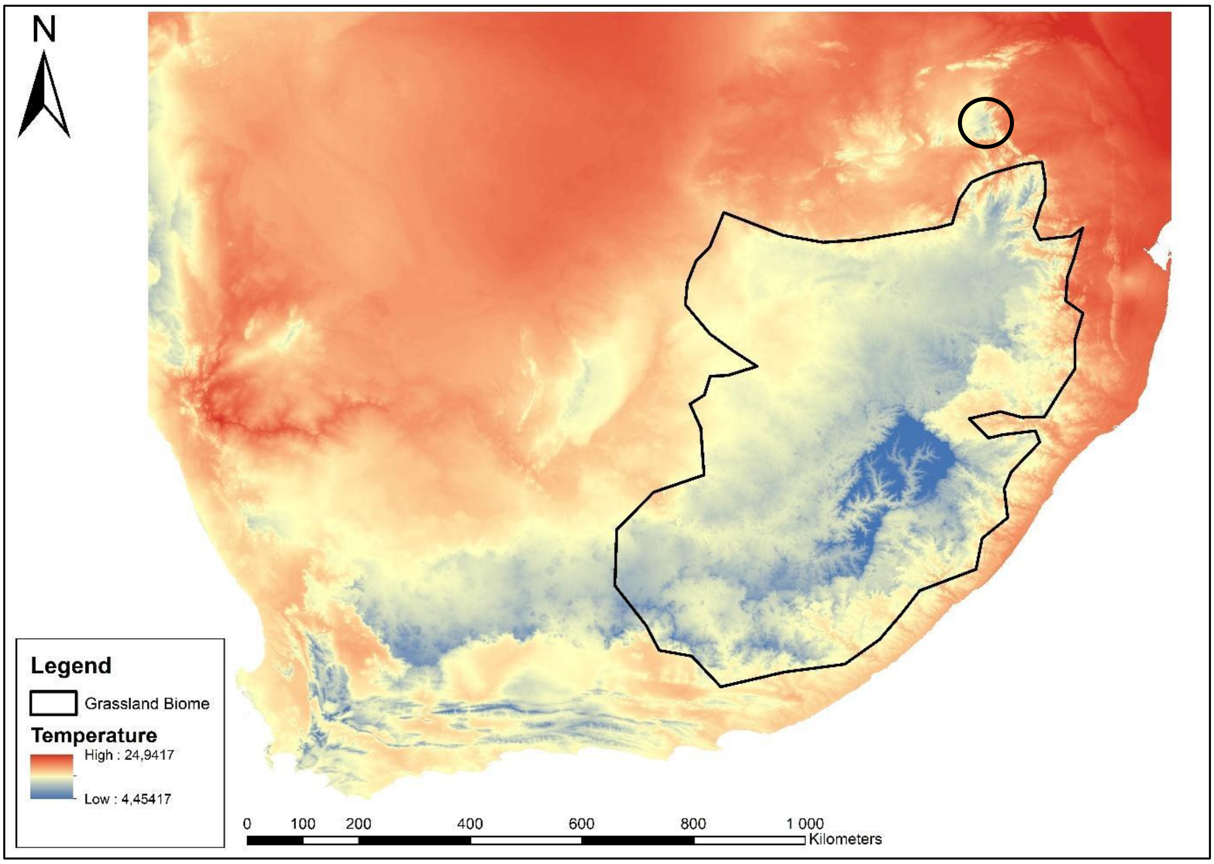


Figure 3-4: A map showing the annual mean temperature (°C) for southern Africa, with the approximate position of the Grassland Biome indicated (Bioclim 01) (map created by Wynand Muller).

3.1.3 Fire and herbivores as extra determinants of the distribution of the Grassland Biome

Climate sets the limit for plant growth, while fire and herbivores determine vegetation patterns (Cowling *et al.*, 1997). Frequent fires and herbivory are essential for old-growth grasslands to persist, especially in areas where precipitation and soil nutrients are sufficient for forests to develop (Bond & Keeley, 2005; Veldman *et al.*, 2015b). If fires and herbivory are excluded from grassland, rapid transition to forest or shrubland physiognomy might be caused, which will lead to losses of forb and grass diversity (Veldman *et al.*, 2015b).

Fire in grasslands promotes the growth of shade-intolerant understorey plants by removing aboveground biomass, as has been occurring for most of the history of terrestrial plant life (Bond, 2019). Fires often occur in late winter from July to September (Mucina & Rutherford, 2006). Lightning is a common occurrence in the biome, making the likelihood of lightning-induced fire relatively high (Mucina *et al.*, 2006).

Grasslands grazed by ungulates developed 20 to 30 million years ago (Jacobs *et al.*, 1999; Veldman *et al.*, 2015b). Herbivory increases plant diversity due to increases in richness and evenness of forb species in particular (Koerner & Collins, 2014). It has been found that livestock grazing at appropriate densities and intervals can be beneficial to grassland biodiversity in some systems (Veldman *et al.*, 2015b).

Several further factors that maintain biodiversity in grasslands are frequent fires, herbivory by native megafauna, soil disturbance by burrowing animals, low-intensity domestic livestock grazing, shallow soils, nutrient poor soils, seasonal water deficit, seasonal flooding, and high (toxic) concentrations of soil metals (Veldman *et al.*, 2015b).

3.2 The bioregions of the Grassland Biome

The Grassland Biome consists of four bioregions (Figure 3-5), namely the Drakensberg Grassland (Gd), Dry Highveld Grassland (Gh), Mesic Highveld Grassland (Gm), and Sub-Escarpment Grassland (Gs) (Mucina & Rutherford, 2006). Within these four bioregions, a total of 72 nationally recognised grassland vegetation types or units occur. These vegetation units differ from each other in terms of species composition, the latter as influenced by climate (including temperature, frost and precipitation), topography and geology (Mucina *et al.*, 2006).

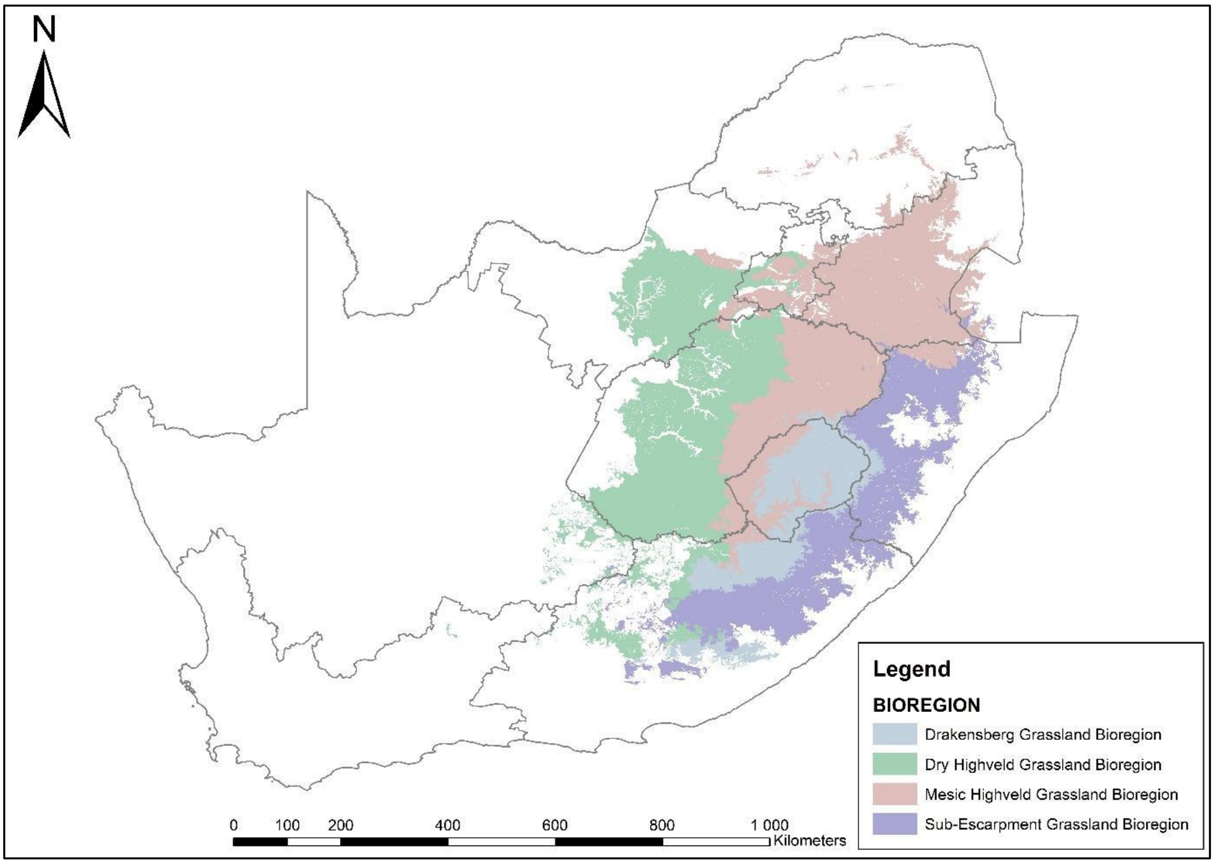


Figure 3-5: The four bioregions of the Grassland Biome of South Africa (map created by Wynand Muller).

In the year 2000, almost 30% of the biome had been permanently transformed (Mucina *et al.*, 2006). By 2018, it was determined that 59% of the biome remained in natural extent (Skowno *et al.*, 2021). Significant parts of the remaining grasslands may be of a secondary nature or may be degraded by gradual processes such as shrub encroachment (Mucina *et al.*, 2006). A further concern is that the untransformed grasslands are highly fragmented, given that some fragments consist of only a few hectares (Mucina *et al.*, 2006).

3.3 The Drakensberg Grassland Bioregion

The Drakensberg grasslands are located primarily in the Great Escarpment of the Drakensberg region in South Africa and Lesotho (Mucina *et al.*, 2006). These grasslands are some of the highest elevation regions of southern Africa, with steep topographies (Mucina *et al.*, 2006). Precipitation is frequent with high rainfall and mist as well as snow and frost at high elevations (Mucina *et al.*, 2006). The Drakensberg Grassland Bioregion consists of ten vegetation units (Mucina *et al.*, 2006).

3.4 The Dry Highveld Grassland Bioregion

Highveld grasslands are found in the central plateau of South Africa (Mucina *et al.*, 2006). The topography is generally flat with some small mountains (Mucina *et al.*, 2006). Annual rainfall, which forms an east-to-west gradient of decreasing moisture, is one of the main environmental factors that controls vegetation patterns (Mucina *et al.*, 2006). MAP in this area is 600 mm (Mucina *et al.*, 2006). The Dry Highveld Grassland Bioregion consists of 15 vegetation units (Mucina *et al.*, 2006).

3.5 The Sub-Escarpment Grassland Bioregion

Sub-Escarpment grasslands are located at the foothills of the Drakensberg and Northern Escarpment (Mucina *et al.*, 2006). This area consists of a diversity of land forms, including hills and flat plains, as determined by the rate at which the landscape ascends and the degree to which the landscape has been shaped by geomorphological forces (Mucina *et al.*, 2006). A strong rainfall gradient from northeast to southwest is extant (Mucina *et al.*, 2006). The Sub-Escarpment Grassland Bioregion consists of 18 vegetation units (Mucina *et al.*, 2006).

3.6 The Mesic Highveld Grassland Bioregion

The Mesic Highveld Grassland Bioregion is the largest of the four and consists of the highest number of vegetation types (Mucina & Rutherford, 2006). This bioregion is mainly found in the higher-precipitation areas of the Highveld and extends northwards along the eastern escarpment (Mucina & Rutherford, 2006). It also enjoys the highest mean altitude (m.a.s.l.) of all bioregions (Mucina & Rutherford, 2006). The Mesic Grassland Bioregion has a Mean Annual Precipitation (MAP) of 726 mm, Mean Annual Temperature (MAT) of 14.7°C, and Mean Annual Frost of 36 days (MAF; <0°C) (Mucina *et al.*, 2006).

The Mesic Highveld Grassland Bioregion consists of 29 vegetation units that are distinguished on the basis of geology and other substrate properties, as well as elevation, topography and rainfall

(Mucina *et al.*, 2006). One of these vegetation units is the critically endangered Woodbush Granite Grassland (WGG) (Mucina *et al.*, 2006).

3.7 Woodbush Granite Grassland

The WGG is a critically endangered ecosystem with less than 6% of this unit still in a natural state (Mucina *et al.*, 2006; Niemandt & Greve, 2016). The largest intact WGG fragment covers 192 ha and is located next to Haenertsburg, a small village located on the northern Drakensberg Escarpment in Limpopo, followed by a 60-ha fragment next to the Ebenezer Dam in close proximity to the village (Dzerefos & Witkowski, 2016; Dzerefos *et al.*, 2017). The original extent of the WGG (Figure 3-6), that is, prior to land-use change, was approximately 340 km^2 (Mucina *et al.*, 2006).

The WGG is threatened by silviculture (69%), cultivation, and urban development (Mucina *et al.*, 2006; Niemandt & Greve, 2016). This unit is also threatened by bush encroachment by scrub forest and sour bushveld, which is worsened by the exclusion of fire (Mucina *et al.*, 2006).

Of the largest remaining fragment of WGG next to Haenertsburg, 66% has been declared as a nature reserve in 2016 and is known as the Haenertsburg Nature Reserve (HNR) (Limpopo Provincial Government, 2016).

3.7.1 Locality

The WGG is located in the Limpopo Province of South Africa, within the Greater Tzaneen Local Municipality (GTM) which is part of the Mopani District Municipality (Figure 3-6). It is roughly centred around Haenertsburg village (33°56’49’’ S 29°56’54’’ E) and occurs in the foothills of the Northern Drakensberg Escarpment and is part of the Wolkberg Centre of Endemism (Niemandt & Greve, 2016; Van Wyk & Smith, 2001).

The WGG borders Mamabolo Mountain Bushveld, Ohrigstad Mountain Bushveld, and Tzaneen Sour Bushveld savanna vegetation units as seen in Figure 3-7 (Mucina & Rutherford, 2006), these are anticipated to expand into adjacent grassland areas as a result of climate change (that is, drier, warmer climate and increased atmospheric CO_2) (Bond & Midgley, 2000; Bond & Midgley, 2001; Clarke *et al.*, 2013).

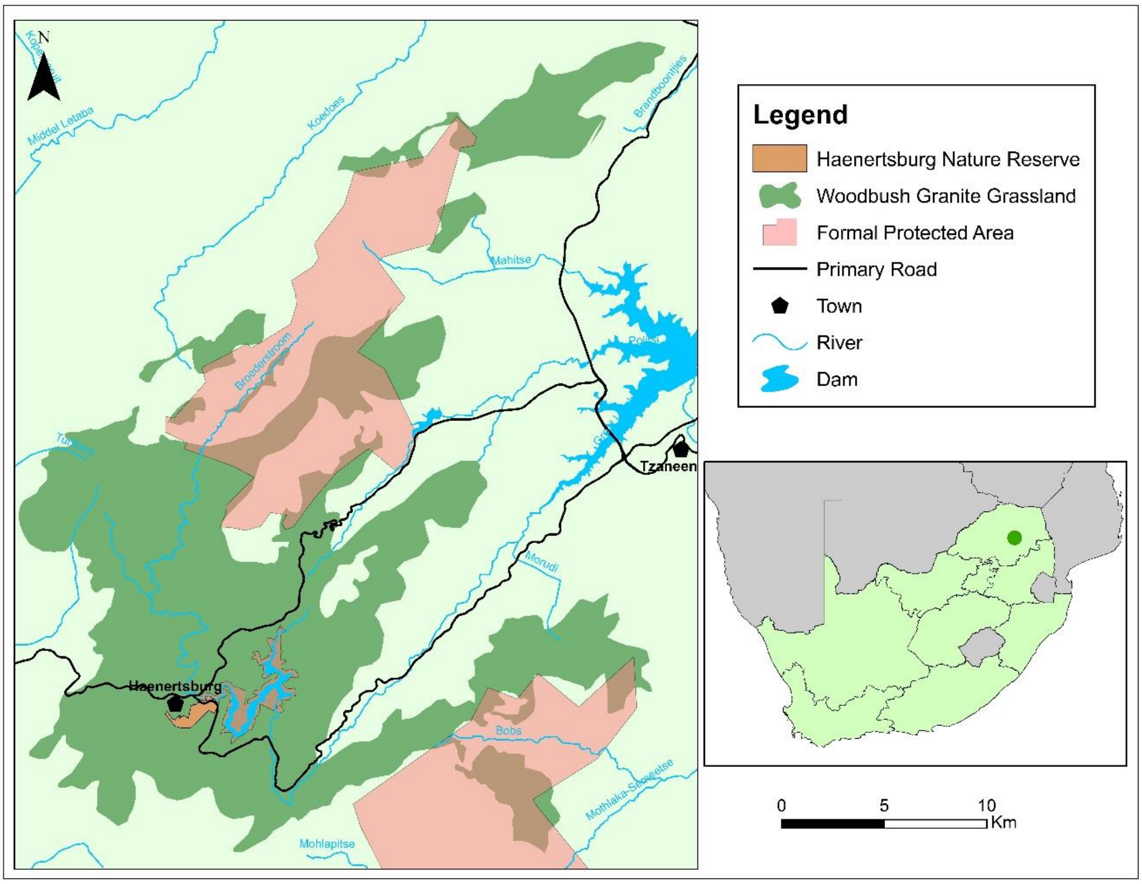


Figure 3-6: Original extent of Woodbush Granite Grassland of South Africa. Currently, only small remnant fragments of natural grassland remain (map created by Wynand Muller).

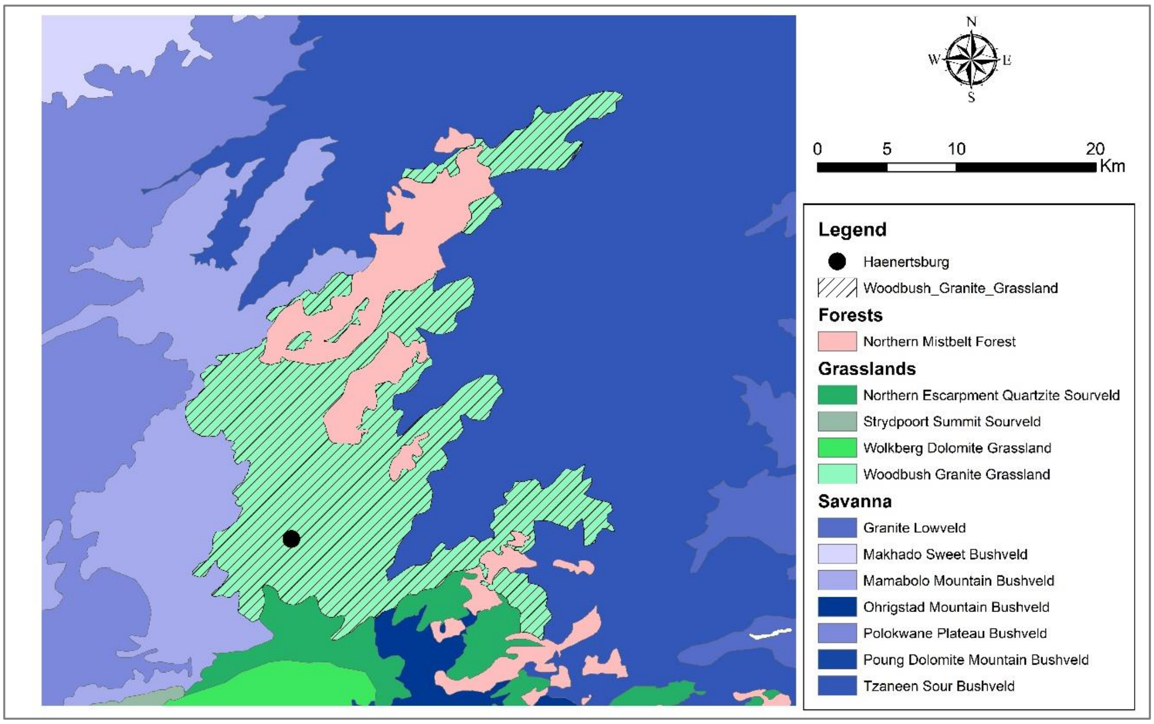


Figure 3-7: Woodbush Granite Grassland and bordering vegetation units, including Northern Escarpment Quartzite Sourveld Grassland, Mamabolo Mountain Bushveld, Ohrigstad Mountain Bushveld, and Tzaneen Sour Bushveld savannas, as well as Northern Mistbelt Forest (map created by Wynand Muller).

3.7.2 Climate

3.7.2.1 Precipitation

This WGG vegetation unit receives summer rainfall that peaks in January, with a MAP of 700 mm in the east to 1500 mm in the west and an average annual rainfall of 1166 mm (Mucina *et al.*, 2006). The long-term (1903-2019) average annual rainfall for Haenertsburg is 827 mm (Figure 3-8). Daily rainfall ranges from 7.5 to 80.8 mm and daily average maximum relative humidity ranges from 58.7 to 71.5% (Tshikhudo *et al.*, 2019). Mist is a common occurrence, which causes an orographic effect on the escarpment. Frost is not common in winter, with a mean of only seven frost days (<0°C) (Mucina *et al.*, 2006).

The present project has established that the average rainfall for the Haenertsburg area is 827 mm, in comparison to the entire WGG average rainfall of 1166 mm (Mucina *et al.*, 2006). The average obtained for the Haenertsburg area was calculated by using the total rainfall as recorded for the past 117 years (Figure 3-8).

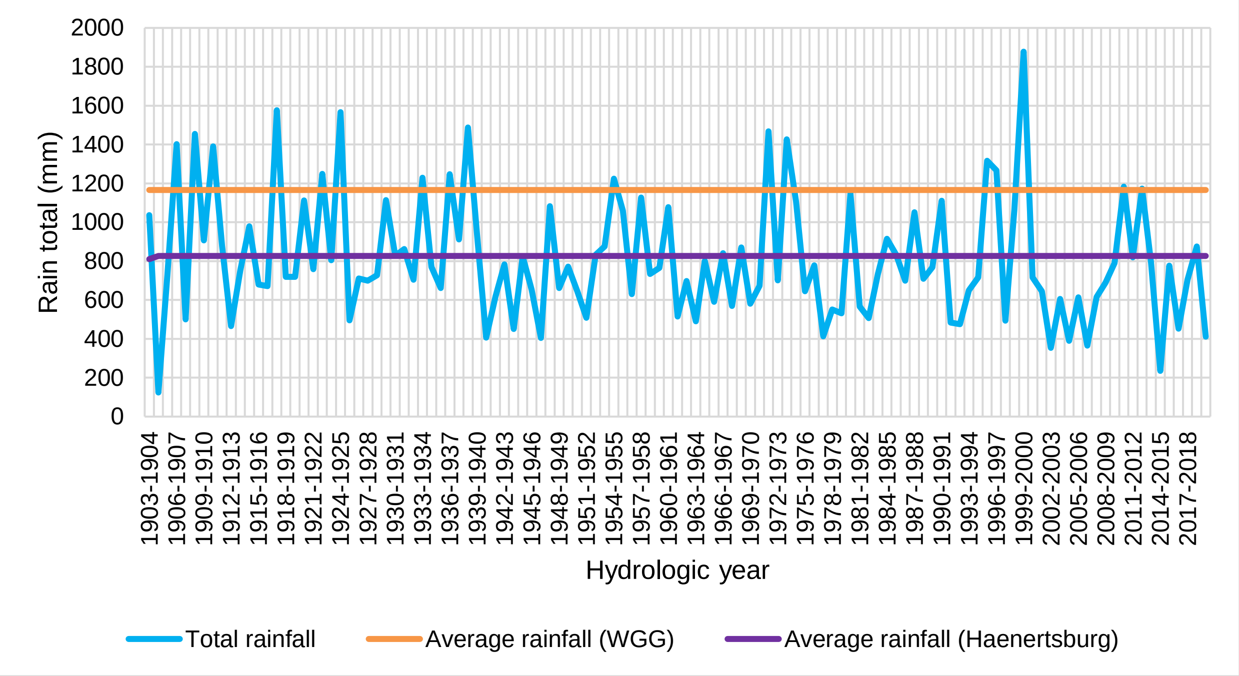


Figure 3-8: Long-term annual rainfall record for the hydrological years 1903 to 2019 for Haenertsburg village, along with means for Haenertsburg village (purple line, 827 mm, 1903-2019) and WGG more broadly (orange line, 1166 mm, from Mucina *et al*., 2006).

According to the data provided by the South African Weather Service (SAWS) and data obtained from the SAEON weather station in Haenertsburg, precipitation in the 2015 hydrological year was well below the long-term site mean (Figure 3-8). It was subsequently classified as an extremely dry year according to the Standardised Precipitation Index (SPI; Figure 3-9). This was in fact designated as one of ten “extremely dry” events between 1903 and 2021.

Drought intensity was quantified using SPI, *a* well-supported precipitation index (Vicente-Serrano *et al.*, 2012). SPI values were calculated based on annual precipitation sums representing Haenertsburg's hydrological years from 1904 to 2022. SPI values were assigned to drought intensity classes based on the classification of the US National Drought Mitigation Center (http://droughtmonitor.unl.edu), and adding the class of 'normal precipitation' (SPI of -1 to +1) (Ruppert et al., 2015). The SPI is a preferred method because of its solid theoretical development, robustness, and versatility in drought analyses (Redmond, 2002).

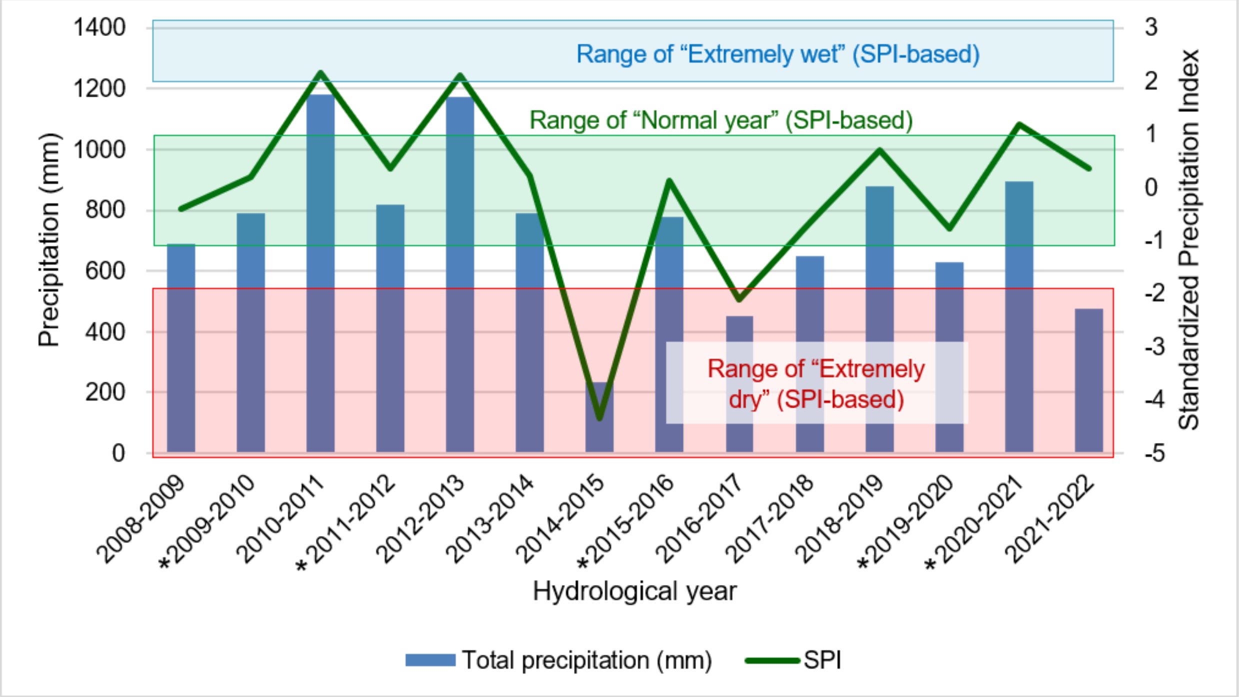


Figure 3-9: The total precipitation (mm) and Standardised Precipitation Index (SPI) for hydrological years since 2009, when sampling in the WGG was initiated. Sampled years are indicated by an asterisk.

3.7.2.2 Temperature

The mean annual temperature for the WGG is 16.6°C (Mucina *et al.*, 2006). The average maximum summer temperature is 22.4°C and ranges from 27.5 to 31.4°C (Tshikhudo *et al.*, 2019), and the average minimum summer temperature is 13.2°C (Mucina *et al.*, 2006). The average maximum winter temperature is 20.1°C and the average minimum 6.9°C (Mucina *et al.*, 2006).

3.7.3 Topography

The WGG is situated on a mountainous plateau covered by grassland with low-shrub cover on the south- and east-facing slopes. It occurs at an altitude of 1080-1800 m (Mucina *et al.*, 2006).

3.7.4 Geology and soil

The WGG occurs on Archaean granite, gneiss and greenstone basement, specifically Turfloop granite (Randian) and remnants of Goudplaats gneiss (Swazian), occasionally dolerite dykes or sills and quartz veins also occur (Mucina *et al.*, 2006).

The dominant land type is Ab, with Hutton (Glenrosa and Shortlands) soils (Mucina *et al.*, 2006). The granitic soil of the WGG tends to be deep and is therefore favoured for planting crops or plantations (Dzerefos, 2004).

3.7.5 Vegetation

The total plant species richness in the WGG is estimated at 661, of which 36 are considered threatened (Dzerefos *et al.*, 2017; Niemandt & Greve, 2016). Endemic species include *Aloe lettyae* Reynolds, *Wahlenbergia brachiata* (Adamson) Lammers, *Chlorophytum radula* (Baker) Nordal, *Kniphofia crassifolia* Baker, *Inezia speciosa* Brusse and *Indigofera rehmannii* Baker f. (Mucina *et al.*, 2006).

The WGG comprises *Loudetia simplex-Diheteropogon filifolius* grassland with diagnostic grass species being *Ctenium concinnum* Nees, *Koeleria capensis* (Steud.) Nees, *Rendlia altera* (Rendle) Chiov., *Alloteropsis semialata* (R.Br.) Hitchc. and *Loudetia simplex* (Nees) C.E.Hubb. (Cowling *et al.*, 1997). Conspicuous or diagnostic forbs include *Eriosema angustifolium* Burtt Davy, *Vernonia centaureoides* Klatt, *Helichrysum platypterum* DC., *H. mariepscopicum* Hilliard, *Rabdosiella calycina* (Benth.) Codd, *Craterocapse tarsodes* Hilliard & B.L.Burtt and *Wahlenbergia squamifolia* Brehmer (Cowling *et al.*, 1997).

Dominant plant species (in terms of occurrence) sampled in the Haenertsburg Nature Reserve in 2020 include the grasses *Cymbopogon nardus* (L.) Rendle, *Hyparrhenia filipendula* (Hochst.) Stapf, and *Setaria sphacelata* (Schumach.) Moss. Dominant forbs include *Berkheya setifera* DC., *Crabbea hirsuta* Harv., *Eriosema cordatum* E.Mey., *Helichrysum nudifolium* (L.) Less. var. *oxyphyllum* (DC.) Beentje, *Helichrysum platypterum* DC., *Hypericum aethiopicum* Thunb., *Pentanisia angustifolia* (Hochst.) Hochst., *Phyllanthus parvulus* Sond., *Senecio venosus* Harv., *Tragia rupestris* Sond. and *Hilliardiella oligocephala* (DC.) H.Rob. Dominant woody species include *Pseudarthria hookeri* Wight & Arn. var. *hookeri* and *Tylosema fassoglense* (Schweinf.) Torre & Hillc.

Alien invasive species have established in disturbed areas and along river courses, including *Acacia mearnsii* De Wild., *Acacia melanoxylon* R.Br., *Pinus* spp. *Rubus cuneifolius* Pursh and *Solanum mauritianum* Scop. The Formosa Lily (*Lilium formosanum* Wallace) is classified as a Category 1 invader and poses a threat to native grassland species (Lennox & Dzerefos, 2013).

The HNR contains a section of grassland that has become encroached by shrubs (giving rise to a ‘closed’ community) while the adjacent part of the grassland is more open and ‘natural’. From 1983 to 2003, annual burns were carried out in both sections (section 3.7.8). From 2004 to the present, no fixed fire programmes were run in either area. Both areas burned eight times between 2004-2021, with the fire return internal ranging from 1 to 5 years. It is therefore probable that the transition from annual burns to more irregular burns in 2004 permitted the encroachment of shrubs into at least part of the grassland. Figure 3-10 presents a set of satellite images to display the changes observed in the grassland. Google Earth Pro 7.3.4.8248 Pro images for the years 2001, 2009, 2017, 2019, and 2021 were used. Unfortunately, there were no images available for 2002-2008. This is particularly unfortunate in the case of 2004, when the encroachment likely intensified.

Increases of woody plants can occur over decades (Archer *et al.*, 2017). It has been found that such increases are caused either by exotic species or by native ones that have increased in abundance within their historic ranges or expanded their geographic range (Archer *et al.*, 2017). The encroachment of woody species threatens the intactness of grassland ecosystems especially by threatening endemic plants and animals (Archer *et al.*, 2017). It has been found that an increase in MAP results in higher potential for landscapes to support woody cover increases (Archer *et al.*, 2017). Woody encroachment has been associated with degradation due to intensive grazing by cattle and sheep (Archer *et al.*, 2017). Grazing reduces the density and continuity of fine fuels which, in turn, reduces fire frequency and intensity, thus facilitating woody species establishment (Archer *et al.*, 2017). When woody species germinate near to mature grasses, they are potentially faced with intense competition for light, water, and soil nutrients (Archer *et al.*, 2017). Furthermore, grazing reduces grass biomass and therefore reduces the ability of grass species to competitively suppress woody seedlings (Archer *et al.*, 2017).

Figure 3-10: Satellite images of the WGG adjacent to the town of Haenertsburg indicating vegetation change, specifically woody cover in the area marked by red in comparison to the unchanged, open part of the grassland indicated in blue. Images were acquired for the years indicated on the pictures by making use of Google Earth Pro 7.3.4.8248. (http://www.google.com/earth/index.html; Date of access: 4 Apr. 2022).

3.7.6 Fauna

It is estimated that 237 bird species (eight of which are threatened), 62 mammal species (19 of which are threatened), 38 reptile species (seven of which are threatened), 11 amphibian species (one of which is threatened), and an unknown number of invertebrate species (four of which are threatened) occur in the WGG (Niemandt & Greve, 2016). The critically endangered Wolkberg Zulu Butterfly (*Alaena margaritacea*) is endemic to the WGG (Lennox & Dzerefos, 2013).

3.7.7 Historical land-use of the WGG

3.7.7.1 Grazing

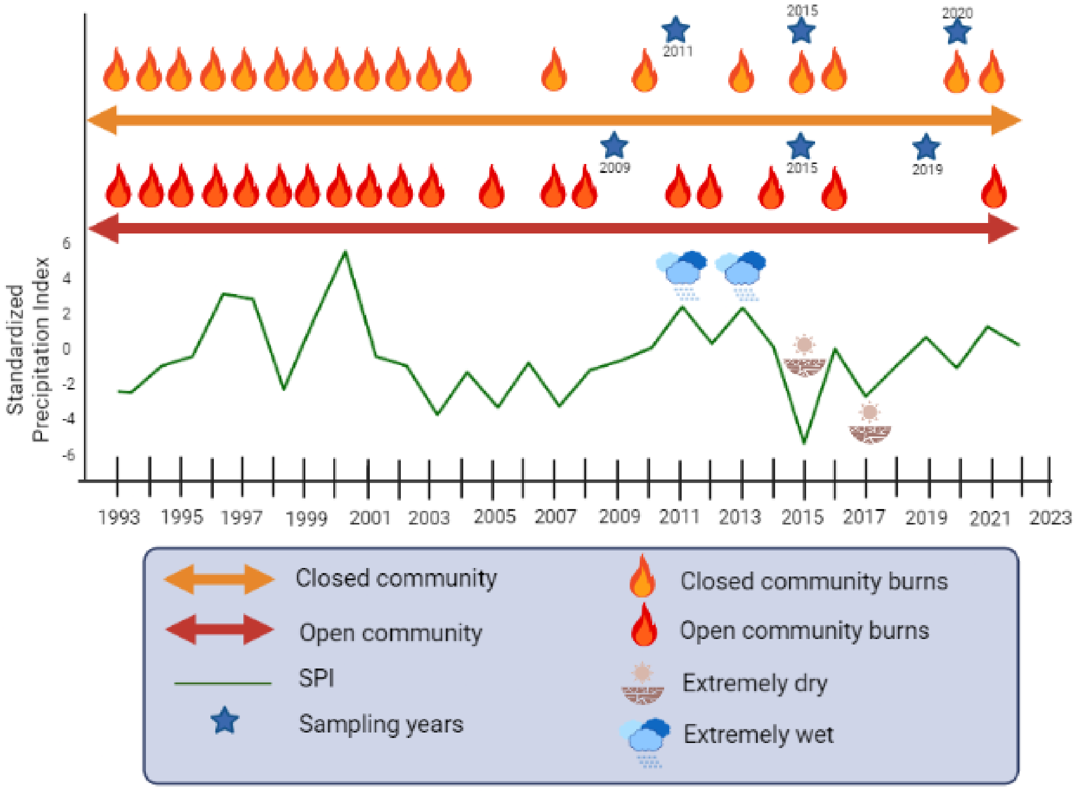
Grazing in the WGG has occurred at various intensities ranging from none to the use of the grassland as pastures for grass-fed dairy cattle (Mutileni, 2016). In 1887, Haenertsburg was established by gold diggers whose horses and cattle grazed in the grassland (Dzerefos, 2004). Ongoing uncontrolled cattle grazing has contributed to the degradation of the WGG (Dzerefos, 2004; Niemandt, 2015). Grazer numbers have, however, declined since then, which could have contributed to encroachment (Zehnder *et al.*, 2020). Livestock grazing and natural herbivory do not have the same effect on grasslands (Little *et al.*, 2013), as livestock grazing is often of an extreme intensity that can lead to detrimental effects when the area is overgrazed (Niemandt, 2015). Increases in heavy grazing at regional scale reduce the competitive effect of grasses, since livestock mostly feed on herbaceous plants, while woody species are defended by chemical and physical traits. With a decrease in herbaceous biomass, woody species growth is supported by the increase in available resources (Coetzee *et al.*, 2008; Estell *et al.*, 2012; O'Connor *et al.*, 2014). Grazing abandonment could also lead to encroachment (Guardiola *et al.*, 2013; Kinnebrew *et al.*, 2020), while reduced browser numbers have a similar impact due to the reduced pressure on established plants and seedling regeneration (O'Connor *et al.*, 2014).

3.7.7.2 Fire management

Fire, which is an important driver of grassland vegetation dynamics, has been implemented annually between 1983 to 2003 in both communities (Table 3-1 & Figure 3-11). Since 2003, the open and closed communities were both burned on eight occasions. Consequently, fire is not considered to be a driver for the encroachment observed.

Table 3-1: The fire history of the Woodbush Granite Grassland sites at Haenertsburg.

Year	Fire history
Pre 1983	Unknown
1983-2003	Annual burn at all sites
2004	Implementation of reduced fire as per concern over fire-sensitive species
2004-2014	The open community burned in 2005, 2007, 2008, 2011, 2012, 2014 The closed community burned in 2004, 2007, 2010, 2013, 201
2014	The decision was taken to implement an adaptive strategy as per concern over woody thickening
2015	Closed community burned
2016	Nature reserve declared, open and closed communities burned
2017-2019	No burns implemented
2020-2021	The open community burned in 2021 The closed community burned in 2020 and 2021



Created in BioRender.com bio

Figure 3-11: Timeline of the open and closed communities showing occasions when they experienced fire, as well as extremely wet and extreme dry years (as based on Standardised Precipitation Index), including years when they were sampled.

3.7.8 Sampling sites

The South African Environmental Observation Network (SAEON) was established in 2002 to deliver long-term reliable data for scientific research which would then be used for informed decision making about managing the ecosystem. The SAEON Ndlovu Node focuses on the understanding of the environmental changes observed in the savanna biome as well as in the montane grasslands of South Africa. In montane grasslands, monitoring involves shrub cover and plant diversity and the distribution of key species predicted to migrate in response to climate change.

The SAEON Ndlovu Node established 92 permanent vegetation monitoring plots in the WGG. These plots were monitored periodically since 2009, and baseline vegetation data were collected to compile a floristic dataset. The sampling and data collection conform to the SAEON sampling protocol for long-term grassland monitoring. A total of 40 plots in the open community and 33 plots in the closed community were used in the present study (Figure 3-12). Floristic sampling was done for the open community in 2009, 2012, 2015 and 2019. The same was compiled for the closed community in 2011, 2015, and 2020. The difference in the onset of monitoring (that is, 2009 vs 2011) was to standardize the reference sites: sampling in other words commenced in the first year after fire. Changes in plant community metrics were quantified during the time period that preceded the 2013-2016 drought (‘pre’; 2009/2011) as well as during (2015) and after (‘post’; 2019/2020) the drought. Detailed sampling protocols are described in the experimental design sections of Chapters 5 and 6.

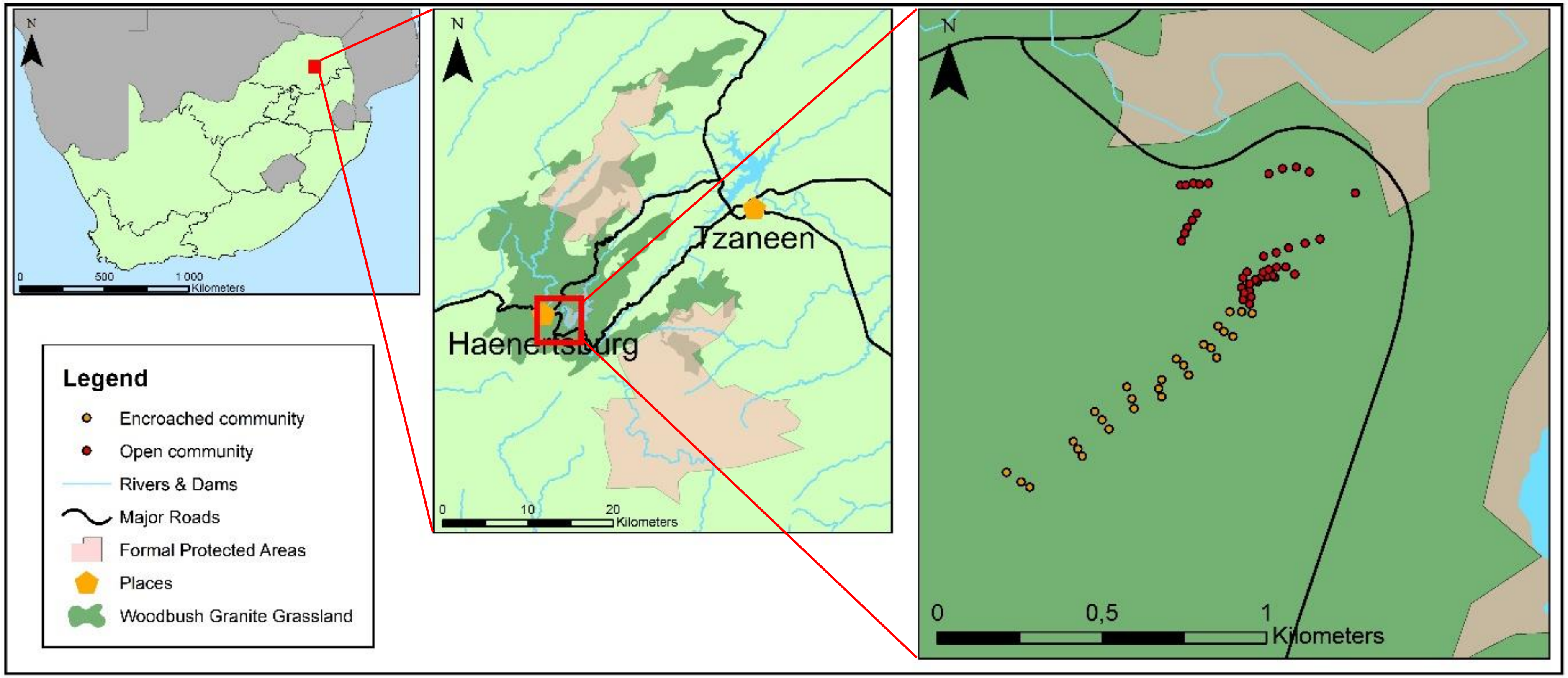


Figure 3-12: The extent of Woodbush Granite Grassland and sampling sites (dots) in the Haenertsburg Nature reserve in the Limpopo province of South Africa. "Formal Protected Areas" refers to areas formally declared areas, and does not reflect the current conservation status (map created by Wynand Muller).

CHAPTER 4 EFFECTS OF GRASSLAND TRANSFORMATION ON FLORISTIC DIVERSITY OF SOUTH AFRICAN GRASSLANDS

Part of the results presented in this chapter was published in a special issue of *Bothalia*: *African Biodiversity & Conservation*.

Title: A floristic assessment of grassland diversity loss in South Africa.

Authors: M. Muller, S.J. Siebert, B.R. Ntloko, and F. Siebert.

Year: 2021

Volume, number, and pages: 51(1): 147-155

DOI: https://doi.org/10.38201/btha.abc.v51.i1.11

4.1 Introduction

Habitat transformation through land-use change threatens the persistence of many grassland ecosystems worldwide (Bond, 2016). Contrary to what is often assumed, grasslands are hyper-diverse ancient ecosystems, habitats, and communities, that support a considerable number of endemic and threatened species (Carbutt *et al.*, 2017). Habitat transformation threatens the integrity of these systems in terms of soil disturbance and the removal of plant biomass and species, and the effect is widely recognised and measurable (Herben *et al.*, 2016; Klimešová *et al.*, 2017; Miller *et al.*, 2011). The poor understanding of forb dynamics in grasslands necessitates a closer look at floristic change when it comes to the question as to whether land-use change leads to species losses or gains in transformed grassland (Veldman *et al.*, 2015b).

The Grassland Biome covers approximately one-third of South Africa's land surface (Cadman *et al.*, 2013). It is one of the most at-risk South African biomes, of which 40-60% of it has been irreversibly modified, whereas less than 3% of grasslands are formally protected (Cadman *et al.*, 2013; Little *et al.*, 2015), as depicted in Figure 4-1. The intactness of unprotected South African grasslands is threatened as there is an increase in the intensity of agriculture and afforestation (Botha *et al.*, 2017; O'Connor & Kuyler, 2009) and habitat transformation due to urban and industrial development activities (Everson & Everson, 2016; O'Connor & Kuyler, 2009; Siebert *et al.*, 2001). Changes in composition, structure, and functioning of these grasslands influence the ability to deliver fresh water, soil formation, climate regulation, and reduction of disaster risk (Carbutt *et al.*, 2017; Mark *et al.*, 2013). In addition, these changes cause a probable loss of biodiversity and grassland production (Everson & Everson, 2016).

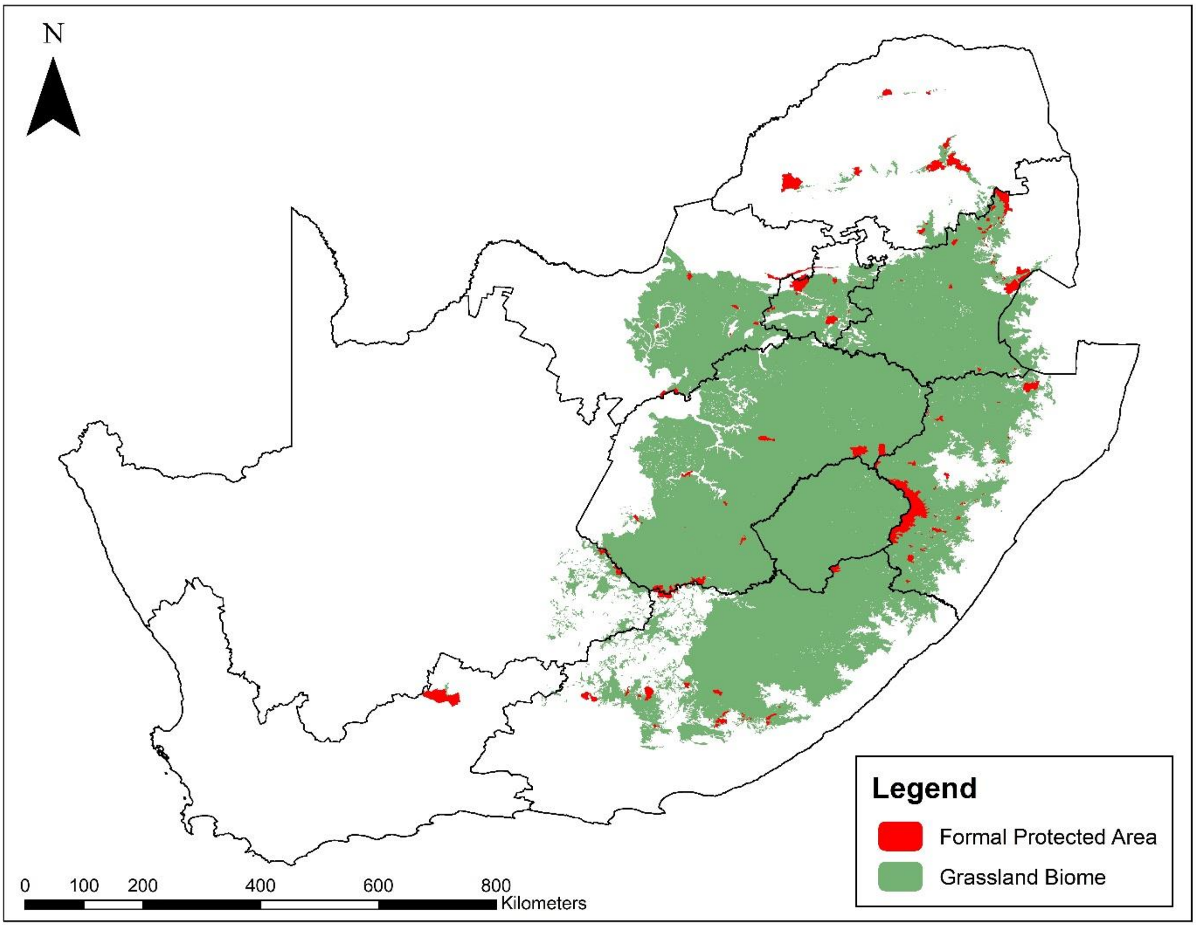


Figure 4-1: The areas of the Grassland Biome that are formally protected. Map produced by Wynand Muller.

O'Connor and Kuyler (2009) meticulously investigated the impact of land-use on the biodiversity integrity of moist grasslands in South Africa and highlight the loss of useful plants in terms of an ecosystem services perspective. The present study focuses on biodiversity intactness in that it specifically considers the loss of native floristic diversity. It emphasises the indigenous forb component, which is fast moving up the research agenda according to Siebert and Dreber (2019).

The aim of the project that resulted in the present chapter was, therefore, to assess and describe the community composition of old-growth and transformed grasslands and determine floristic gains and -losses in terms of families, species (endemic, native, and introduced), life forms, and growth forms, as caused by disturbances. This was done to address the question of the ways in which floristic diversity changes across different pathways of land-use intensification in South Africa's Grassland Biome.

It was hypothesised that the floristic diversity of grasslands in South Africa would decrease when subjected to land-use changes, that include agriculture and urbanisation (Cadman *et al.*, 2013; Carbutt *et al.*, 2011; Cowling *et al.*, 1997; Egoh *et al.*, 2011; O'Connor, 2005; Williams *et al.*, 2005). It was expected further that significantly lower floristic diversity would be present in transformed grassland, in contrast to adjacent untransformed grasslands. Therefore, it was expected that considerable shifts would have occurred in plant communities due to an increase in exotic species and loss of forb species.

4.2 Material and methods

4.2.1 Study area

Eighteen study sites were selected in four bioregions of the Grassland Biome as well as a tropical grassland bioregion of the Indian Ocean Coastal Belt Biome of South Africa (Figure 4-2). Grasslands can occur in patches, known as satellite grasslands, within other biomes (Bredenkamp *et al.*, 2002; Cowling *et al.*, 1997). The chosen grasslands occurred at altitudes ranging between 30 and 3 100 m.a.s.l., and ten of these were located between 1 000 and 1 800 m.a.s.l.

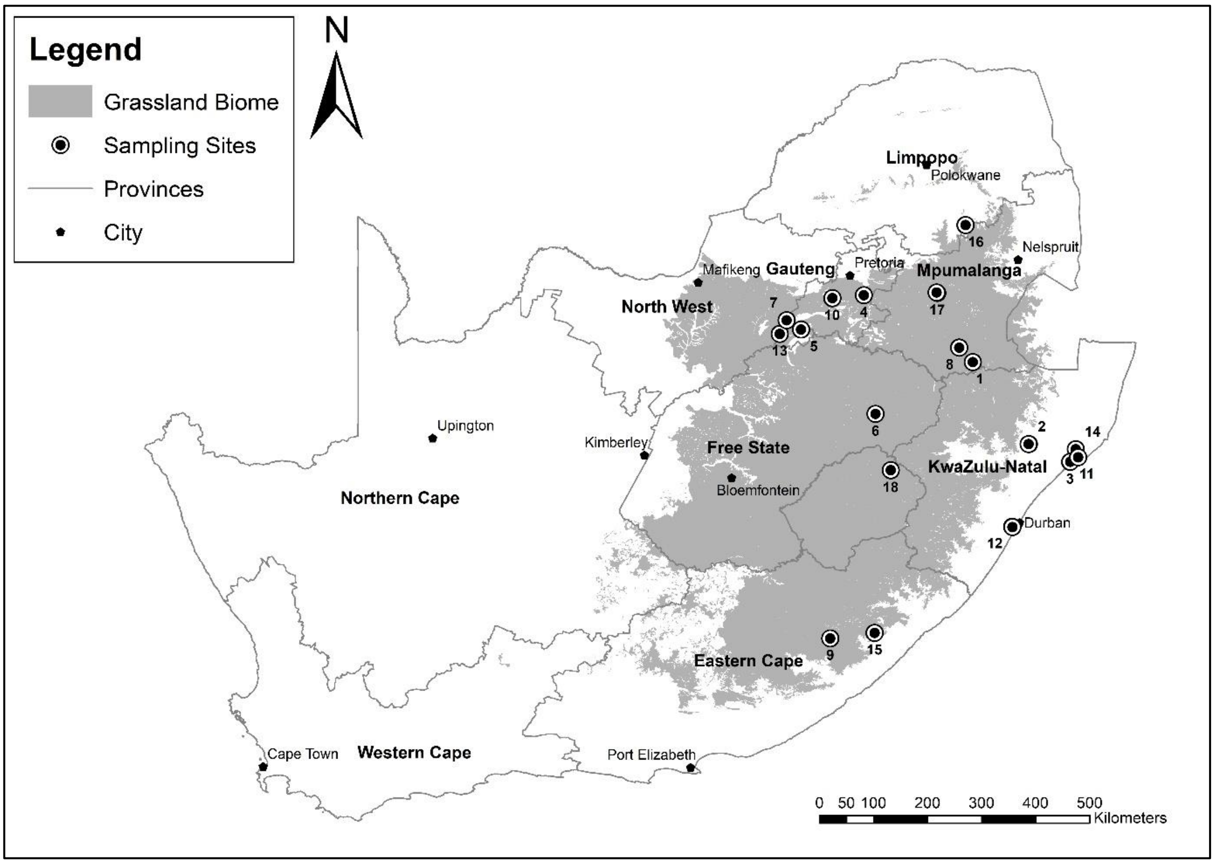


Figure 4-2: Location of the 18 study sites studied in the present project as found within the Grassland Biome of South Africa. Land-use transformation at localities are as follows: Agriculture, afforestation 1, 2, 3; field margins 4, 5, 6; old fields 7, 8, 9; urban, green space 10, 11, 12; peri-urban 13, 14, 15; mine rehabilitation 16, 17, 18. Map produced by Wynand Muller.

The mean annual temperature for the grassland sites ranges from 10 to 21 °C, and the overall mean is 16.3 °C (median 15.9 °C). June to August are the coldest months within which mean frost days per annum vary between 0 and 96, with a mean of 25 (median 28) across all study sites (Mucina *et al.*, 2006). All sites experience summer rainfall ranging from 600 to 1 000 mm per year and a mean of 761 mm (median 717 mm) across sites. Twelve sites receive less than 800 mm per annum.

4.2.2 Land-use classification

Two dominant land-use types in the Grassland Biome were included in this study, namely agriculture and urbanisation (Neke & Du Plessis, 2004). In the agricultural land-use type, surveys included field margins, old/ abandoned fields, and afforestation. Afforestation as a form of land-use was represented by commercial plantations and grouped under agriculture due to the small sample size. The urban land-use type included urban open space, peri-urban areas, and rehabilitated mine tailings.

4.2.3 Field surveys

Floristic data were gathered at 18 sites from 2002 to 2019. At each site, sampling was conducted in four plots in untransformed grassland, each paired with a plot in an adjacent transformed land-use plot, tallying eight plots per site, that were no more than 150-250 m apart. All 144 plots were surveyed in late spring or early to mid-summer. Each 100 m^2 plot was divided into 25 subplots of 4 m^2 each to record species occurrence and abundance. Species were identified in the field or photos were taken for later identification. Floristic data from the subplots were combined to compile a total inventory for each 100 m^2 plot.

Plant species nomenclature and classification followed Ranwashe (2019). Naturalised and invasive categories were determined according to the guidelines of the Department of Environmental Affairs (2016). Life and growth forms of plant species were determined in accordance with Germishuizen and Meyer (2003). Categories of threat were defined in terms of the *Red List of South African Plants* (South African National Biodiversity Institute, 2017).

Species abundance (N) was calculated as the total number of individuals in any given sample. Species richness (S) represented the total number of species within each 100 m^2 plot. Alpha-diversity was expressed by using the Shannon–Wiener diversity index (H'), which condenses species richness and evenness into a single value. The Pielou (J') index was applied to plot data to calculate evenness. All of these values were calculated by using Primer 2007.

4.2.4 Data preparation analysis

A data matrix was compiled and consolidated in Excel (Windows, 2016).

Non-Metric Multi-Dimensional Scaling (NMDS) analysis in Primer 2007 was used to explore changes in species composition between transformed and untransformed grasslands. With a view to determining whether significant differences in plant assemblages existed, permutational multivariate analysis of variance (PERMANOVA) was performed by using species abundance data. Analyses were conducted with 999 permutations by using Bray–Curtis similarity and Type III sums of squares after a square root transformation of species data so as to reduce the influence of common species. To account for location variability in the paired nested sampling design, each plot was treated as a random variable nested within a transformation type, that is, an urban or agricultural one, the latter which were treated as the fixed factor. Pair-wise test results were employed to indicate the strength of the difference between transformed and untransformed plots.

Similarity percentage analysis (SIMPER) was applied to determine which forb and grass species contributed the most to differences between transformed and untransformed grasslands. Simple paired t-tests were applied to test for significant differences between untransformed and transformed plots in terms of selected diversity measures. Percentage decrease in richness and abundance of species per growth/life form, threat status, endemism, and alien taxa were calculated with regard to the statistical means.

4.3 Results

4.3.1 Floristics

Overall, 1 146 plant species were recorded, of which 144 were non-native. The untransformed grassland contained 962 species, which included 35 naturalised and 15 invasive taxa (5%), 175 South African endemics (18%), and 20 threatened species (2%). The transformed grasslands harboured 582 species, including 92 naturalised and 46 invasive taxa (24%), 47 South African endemics (8%), and six threatened species (1%).

The most prominent families in the localities were the Asteraceae, Poaceae, Fabaceae, and Cyperaceae, in order of most species diverse (Table 4-1), whereas the Poaceae was most abundant (Table 4-2). Transformation was less favourable to the Asteraceae and Fabaceae and more beneficial to the Cyperaceae and the Poaceae especially.

Habitat transformation affected the species per family. The geophytic Hyacinthaceae and Iridaceae showed the largest species losses (18 and 19 species respectively) when grassland was transformed and the weedy Amaranthaceae and Solanaceae benefited in terms of species additions (nine and five species respectively; see Table 4-1). Changes in the frequency of species were found to be even more pronounced (Table 4-2). Five of the top ten families that showed high frequencies of occurrence in untransformed grassland were reduced by 73% in transformed grassland. These were replaced by five families which, in turn, showed a 75% increase in transformed grassland (Table 4-2).

Table 4-1: Top ten families of untransformed and transformed grasslands based on the proportion of each family's part of the total species pools of 962 and 582 respectively. Superscripts indicate up or down movement in ranking in transformed grassland.

Family	Proportion of all species (%)		Shift in ranking
	Untransformed	Transformed	
Asteraceae	20,4	19,6	1/1
Poaceae	12,9	17,1	2/2
Fabaceae	10,2	10,1	3/3
Cyperaceae	3,6	3,1	4/4
Apocynaceae	3,2	1,7	5/10 ^{v5}
Malvaceae	2,5	2,9	6/5 $^{\wedge 1}$
Scrophulariaceae	2,4	1,5	7/11 ^{v4}
Iridaceae	2,3	0,7	8/23 ^{v15}
Hyacinthaceae	2,2	0,5	9/31 ^{v22}
Lamiaceae	2,1	1,9	10/7 $^{\wedge 3}$
Amaranthaceae	0,5	2,4	30/6 $^{\wedge 24}$
Solanaceae	0,6	1,8	36/9 $^{\wedge 27}$

Table 4-2: Top ten families of untransformed and transformed grasslands based on the proportion of each family's part of the total recorded individuals of 20 640 and 12 042 respectively. Superscripts indicate up or down movement in ranking in transformed grassland.

Family	Proportion of all individuals (%)		Shift in ranking
	Untransformed	Transformed	
Poaceae	40,2	43,9	1/1
Asteraceae	18,3	15,2	2/2
Fabaceae	8,4	7,3	3/3
Cyperaceae	2,9	3,1	4/4
Rubiaceae	2,3	2,2	5/6 ^{v1}
Acanthaceae	2,0	0,5	6/19 ^{v13}
Malvaceae	1,6	1,3	7/12 ^{v5}
Hyacinthaceae	1,5	0,1	8/47 ^{v39}
Commelinaceae	1,2	1,2	9/13 ^{v4}
Lamiaceae	1,2	0,3	10/28 ^{v18}
Verbenaceae	0,6 (22)	2,6	22/5 $^{\wedge 17}$
Amaranthaceae	0,2 (47)	2,1	47/7 $^{\wedge 40}$
Brassicaceae	0,1 (50)	1,9	50/8 $^{\wedge 42}$
Solanaceae	0,3 (33)	1,8	33/9 $^{\wedge 24}$
Myrtaceae	0,25 (36)	1,7	36/10 $^{\wedge 26}$

4.3.2 Composition

Changes in the species number and frequency of families were expected to affect the composition of the transformed grassland. The results from a NMDS showed clustering, which evidenced that untransformed and transformed grasslands are separate assemblages (Figure 4-3). Results obtained from pair-wise tests in PERMANOVA indicated a significant difference in floristic composition between transformed and untransformed grasslands in both urban (df = 70, t = 2.17, $p = 0.001$) and agricultural (df = 70, t = 2.88, $p = 0.001$) transformation types (Figure 4-3). Bray

Curtis similarity measures in the PERMANOVA design reported a low 6.96% and 5.7% similarity in species composition between transformed and untransformed agricultural and urban grasslands respectively. This implies that transformation changed species composition in grasslands by ~90% on average.

Figure 4-3: Non-Metric Multidimensional Scaling (NMDS) ordination for transformed and untransformed grasslands.

The fifteen most common grass species explained 21.26% of the dissimilarity between transformed and untransformed grasslands, including *Cynodon dactylon* and *Hyparrhenia hirta* which weighted towards the former and *Digitaria eriantha* and *Themeda triandra* towards the latter (Table 4-3). Comparatively, the first 15 forbs species only contributed 8.52% to the dissimilarity, and *Cyperus esculentus* and *Richardia brasiliensis* weighted towards transformed and *Helichrysum rugulosum* and *Scabiosa columbaria* towards untransformed grassland (Table 4-3).

4.3.3 Diversity

Changes in the species composition were expected to influence species richness and diversity in transformed grassland. A simple paired t-test showed significantly lower diversity for all measures, that is, J’, H’, S and N, in the transformed grassland ($p<0.001$, Table 4-4). Species richness decreased by nearly 50%.

Table 4-3: Similarity percentage analyses (SIMPER) of grass species comprising >0.6% to compositional differences between transformed and untransformed grasslands. Bold values indicate the highest mean abundance, while the asterisk (*) denotes alien taxa. Superscripts: G, grass; F, forb.

Species	**Av. dis.**	**Cont. %**	**Cum. %**	**Mean abundance**	
				Transf.	**Untransf.**
Themeda triandra Forssk.^{G}	2.92	3.067	3.07	1.32	**13**
Cynodon dactylon (L.) Pers. ^{G}	1.853	1.947	5.01	**7.85**	2.21
Eragrostis curvula (Schrad.) Nees ^{G}	1.768	1.858	6.87	**5.63**	4.85
Eragrostis plana Nees ^{G}	1.731	1.818	8.69	**5.13**	4.32
Hyparrhenia hirta (L.) Stapf ^{G}	1.457	1.531	10.22	**5.35**	2.63
Digitaria eriantha Steud. ^{G}	1.326	1.393	11.61	2.43	**4.93**
Setaria sphacelata (Schumach.) Moss ^{G}	1.179	1.239	12.85	1.28	**4.89**
Aristida junciformis Trin. & Rupr. ^{G}	1.148	1.206	14.06	0.74	**5.24**
Sporobolus africanus (Poir.) Robyns & Tournay ^{G}	1.145	1.203	15.26	**4.79**	0.94
Eragrostis chloromelas Steud. ^{G}	1.117	1.173	16.44	1.1	**4.56**
Pennisetum clandestinum Hochst. ex Chiov. ^{G} *	1.115	1.172	17.61	**4.65**	0
Heteropogon contortus (L.) Roem. & Schult. ^{G}	1.109	1.165	18.77	0.17	**5.07**
Tristachya leucothrix Trin. Ex Nees ^{G}	1.014	1.066	19.84	0.9	**4.31**
Richardia brasiliensis Gomes ^{F} *	0.755	0.793	20.63	**3.07**	0.35
Helichrysum rugulosum Less. ^{F}	0.752	0.79	21.42	0.56	**3.25**
Eragrostis racemosa (Thunb.) Steud. ^{G}	0.709	0.745	22.17	0.13	**3.1**
Cyperus esculentus L. ^{F} *	0.695	0.73	22.89	**3.1**	0.04
Helichrysum nudifolium (L.) Less. ^{F}	0.653	0.686	23.58	0.38	**2.86**
Eragrostis capensis (Thunb.) Trin. ^{G}	0.648	0.681	24.27	0.33	**2.85**
Sisymbrium turczaninowii Sond. ^{F}	0.637	0.67	24.94	**2.61**	0.15
Nidorella podocephala (DC.) Goldblatt & Manning ^{F}	0.62	0.652	25.59	0.93	**2.17**
Hilliardiella oligocephala (DC.) H.Rob. ^{F}	0.516	0.542	26.13	0.17	**2.26**
Tagetes minuta L. ^{F} *	0.501	0.526	26.66	**2.18**	0.07
Scabiosa columbaria L. ^{F}	0.487	0.511	27.17	0.38	**2.07**
Conyza bonariensis (L.) Cronquist ^{F} *	0.484	0.509	27.68	**1.75**	0.4
Commelina africana L. ^{F}	0.442	0.464	28.14	0.49	**1.78**
Felicia muricata (Thunb.) Nees ^{F}	0.412	0.433	28.57	0.25	**1.61**
Plantago lanceolata L. ^{F}	0.397	0.417	28.99	**1.57**	0.42
Bidens pilosa L. ^{F} *	0.393	0.412	29.41	**1.68**	0.03
Zornia capensis Pers. ^{F}	0.372	0.391	29.79	0.18	**1.6**

Av. dis.: Average dissimilarity; Cont. %: contribution %; Cum. %: cumulative contribution %; Transf.: transformed; Untransf.: untransformed. Distance/similarity measure: Bray-Curtis.

Table 4-4: Mean values (±SD) of selected diversity measures, growth forms, and alien, threatened, and endemic species per plot. The percentage decrease is given in brackets. T-test results are reported for diversity measures, with significance set at p<0.05.

Measure	Untransformed		Transformed		df	t	p
Pielou's evenness (J')	**0.903** ±0.05		0.849 ±0.08	(6%)	142	4.92	<0.001*
Shannon Diversity Index (H')	**3.595** ±0.2		2.823 ±0.4*	(21.5%)	142	13.93	<0.001*
Total species (S)	**54.7** ±10.2		28.5 ±7*	(47.9%)	142	17.9	<0.001*
Alien	0.9 ±1.1*	(87%)	**6.9** ±4.4				
Invasive	0.5 ±0.9*	(82.8%)	**2.9** ±3.2				
Threatened	**0.5** ±0.7		0.1 ±0.4*	(80%)			
Endemic	**6.7** ±3.2		1.3 ±1.4*	(80.6%)			
Annual	4.7 ±3.4*	(39%)	**7.7** ±5.4				
Perennial	**49.9** ±9.3		20.8 ±8.3*	(58.3%)			
Grass	**13.4** ±5.1		7.9 ±3.6*	(40.3%)			
Geophyte	**5.1** ±2.5		0.9 ±0.9*	(80.4%)			
Forb	**23.5** ±5.2		12.3 ±4.4*	(47.7%)			
Parasitic	**5.8** ±2.8		0.1 ±0.3*	(98.3%)			
Creeper	**1.6** ±1.3		0.9 ±1.2	(43.8%)			
Dwarf shrub	**6** ±2.7		1.6 ±1.3*	(73.3%)			
Succulent	**5.8** ±2.8		0.3 ±0.6*	(94.8%)			
Suffrutex	**5.8** ±2.8		0.1 ±0.4*	(98.3%)			
Tree/shrub	**5.9** ±2.7		4.2 ±6.2	(28.8%)			
Total individuals (N)	**286.7** ±64		167.3 ±40.8*	(41.6%)	142	13.36	<0.001*
Alien	3.6 ±6.9*	(90.4%)	**37.4** ±26.2				
Invasive	1.6 ±3.4*	(92.3%)	**20.8** ±22.9				
Threatened	**1.7** ±3.1		0.3 ±0.9*	(82.4%)			
Endemic	**29.2** ±17.1		7.4 ±15.1*	(74.7%)			
Annual	19.4 ±16.2*	(52.8%)	**41.1** ±36.3				
Perennial	**267.3** ±63.5		126.1 ±52.5*	(52.8%)			
Grass	**115.3** ±48.7		73.4 ±35.3*	(36.4%)			
Geophytic	**17.6** ±11.8		3.2 ±4.6*	(81.8%)			
Forb	**104.2** ±36.2		58.6 ±29.1*	(43.8%)			
Parasitic	**23.7** ±14.4		0.2 ±0.6*	(99.2%)			
Creeper	**5.3** ±6.4		3.1 ±4.1	(41.5%)			
Dwarf shrub	**24.8** ±14.1		5.9 ±7.8*	(76.2%)			
Succulent	**23.7** ±14.4		1.1 ±2.6*	(95.4%)			
Suffrutex	**23.8** ±14.4		0.2 ±1.7*	(99.2%)			
Tree/shrub	**24.1** ±14.1		21.5 ±27.6	(10.8%)			

4.3.4 Conservation status

The lower evenness of transformed grassland indicated the uneven proportional contribution of individuals between species and was indicative of the fact that some species become more dominant and others more marginal. Dominance shifts can be ascribed to increased numbers of alien, invasive, and annual species in the transformed grassland. The former two were >80% lower in untransformed grassland (Table 4-4). Threatened and endemic species richness and abundance decreased by >80% in transformed grassland (Table 4-4).

4.3.5 Growth forms

When alien species displace extant ones, including endemics, certain growth forms were found to become more or less prominent. Species losses (Table 4-4) were recorded for species that had underground storage organs and bud-banks (USOs), such as geophytes (>80%), parasitic plants, and suffrutices (>90%). Succulents were treated separately from the dwarf shrub growth form and also decreased by over 90% in transformed grasslands (Table 4-4).

4.4 Discussion

Asteraceae and Fabaceae (Figure 4-4) were the most dominant forb families in both transformed and untransformed grassland. This trend has been reported in extant studies (Botha *et al.*, 2017; Zaloumis & Bond, 2011). It can be deduced that regenerative traits cause resilience to disturbance, including wind dispersal and seed dormancy for rapid colonisation in the Asteraceae, ballochory and/ or endozoochory as seed dispersal traits in general, and resource acquisition traits that increase resprouting capacity in the Fabaceae. Many Asteraceae species are also resprouters that enjoy thickened belowground organs (Filartiga *et al.*, 2017). However, the Amaranthaceae, Brassicaceae, Solanaceae, and Verbenaceae benefit from the transformation of grasslands, and their numbers and dominance increase along with the introduction of weedy, mostly alien, species. These groups are renowned for their ability to colonise frequently transformed human-made habitats (Pysek *et al.*, 1995). In contrast, certain families, such as the geophytic Hyacinthaceae and Iridaceae (Figure 4-5), were extensively disadvantaged by habitat transformation, as they are sensitive to soil disturbance since their bud-banks are found belowground (Fidelis *et al.*, 2014). The general trend is therefore one of species loss and displacement by new flora, mostly annuals, with colonising traits that are better suited to a transformed environment, such as creepers, clonal plants, and those with fruit or seed adapted for exozoochorous or anemochorous dispersal (Botha *et al.*, 2017; Zaloumis, 2013). Species lost from the system usually have longer lifespans, poorer dispersal abilities, a greater extent of specialised resource use, and lower reproductive rates among others (Díaz *et al.*, 2006).

Figure 4-4: (A) Geoxylic suffrutex in the Fabaceae (Erythrina zeyheri Harv.) and (B) an herbaceous, hemi-cryptophyte in the Asteraceae (Gazania krebsiana Less.) families.

Figure 4-5: Species of the Iridaceae that were disadvantaged by habitat transformation. Bulbous cryptophytes (A) *Gladiolus crassifolius* Baker. and (B) *Hesperantha coccinea* (Backh. & Harv.) Goldblatt & J.C.Manning.

Poaceae were abundant in transformed grasslands. South African grasslands do not seem to follow natural succession when they are left to recover: the grassy component does establish itself, but the plant community is not representative of natural vegetation (Van Oudtshoorn *et al.*, 2011; Zaloumis & Bond, 2011). Figure 4-6 depicts this phenomenon, where the transformed grassland is dominated by Poaceae and looks healthy, whereas closer inspection soon reveals hardly any herbaceous forbs.

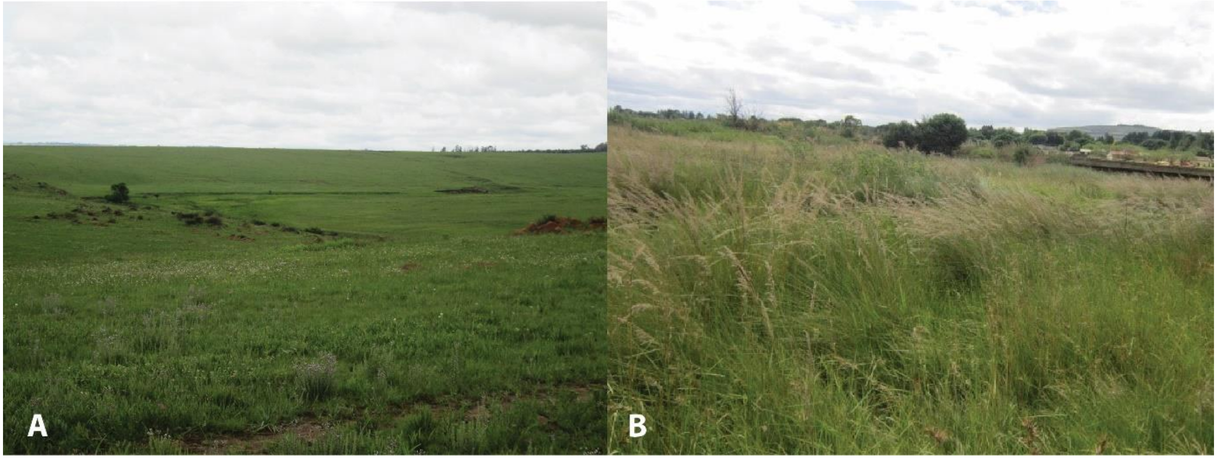


Figure 4-6: An untransformed grassland (A) and transformed grassland (B) in South Africa.

Many species of ancient grasslands are not tolerant of anthropogenic disturbance (Siebert, 2011). In the current study, common native species disappeared completely where grasslands were transformed, such as the grasses *Alloteropsis semialata* (R.Br.) Hitchc. and *Schizachyrium sanguineum* (Retz.) Alston. Both these species generally occur in open grassland or savanna, and they are deemed to be Increaser I species (Van Oudtshoorn, 2012). Forbs that disappeared after transformation included the following: *Gerbera ambigua* (Cass.) Sch.Bip. and *Haplocarpha scaposa* Harv. (both with perennial root stocks); the geophytes *Ledebouria luteola* Jessop (underground bulbs) and *Hypoxis argentea* Harv. ex Baker. (with a belowground tuberous rootstock); the dwarf shrubs *Athrixia phylicoides* DC. and *Tephrosia capensis* (Jacq.) Pers.; and the suffrutices *Elephantorrhiza elephantina* (Burch.) Skeels and *Ziziphus zeyheriana* Sond. (each with a woody belowground rootstock). Other species, such as the palatable and productive grass (Decreaser), *Themeda triandra*, which is considered to be a keystone species and indicator of undisturbed grassland (Snyman *et al.*, 2013), were severely reduced. It is however important to note that, if an area had not experienced any disturbance, a decrease in species richness occurred nontheless, comprising mostly forb species, while an increase was found among grass and shrub species (Fidelis *et al.*, 2012; Overbeck *et al.*, 2005). Grasslands should therefore be exposed to naturally occurring endogenous disturbances (Buisson *et al.*, 2019).

Many new and mostly alien species had entered the transformed system. These can be considered as indicators of disturbance (Morris & Scott-Shaw, 2019), such as the prostrate and grazing resistant *Richardia brasiliensis* Gomes. This present project confirmed the results of O'Connor (2005), namely that a substantial decrease in *T. triandra* and increase in *R. brasiliensis* are indicative of transformed grassland. *R. brasiliensis* is a perennial species native to South America, but it also occurs in disturbed areas of South Africa, the USA, Mexico, Indonesia, and Hawaii (Chandran & Singh, 2003). *It* outcompetes native species for space, light, water, and nutrients (Wells *et al.*, 1986), as its thickened rootstock (Chandran & Singh, 2003) provides it with the ability to survive and colonise transformed grasslands.

As indicated, untransformed grasslands have a greater variety of plant species diversity (27% higher) and richness (92% higher) than transformed grasslands (Nerlekar & Veldman, 2020; O'Connor, 2005; Siebert, 2011) and contain greater numbers of long-lived perennial forbs with under-ground storage organs (Veldman *et al.*, 2015b). At a local scale, old-growth grasslands present a much higher species richness than secondary or deforested grasslands (Buisson *et al.*, 2019; Veldman, 2016; Veldman & Putz, 2011; Veldman *et al.*, 2015b; Zaloumis & Bond, 2016).

Species loss is specific to certain growth forms, especially those with belowground organs that are adapted to survive harsh winter conditions, drought, and fire (Bond, 2016; Bond & Parr, 2010). The loss of these and other foundation species open up niches for colonisation by alien species (Prevéy *et al.*, 2010). This is problematic, as the loss of native species hampers the grassland ecosystem from fulfilling all its functions (Zavaleta *et al.*, 2010). Overall, the situation in transformed grassland is not only one of species depletion but also one of an increase in woody growth-form dominance, which is predicted to become more pronounced in South African grasslands by 2030 (Gibson *et al.*, 2018).

Importantly, due to significant grass cover and biomass, restored grasslands can give the illusion of containing the same species as natural ones (Zaloumis & Bond, 2011). However, a focus on forb diversity soon shows that transformed grassland is species poor (Zaloumis & Bond, 2011). Uys (2006) indicates that the floral richness of grassland ecosystems lies in the diversity of its forb taxa. Zaloumis and Bond (2016) compare the composition of grasslands that had been afforested with conifers 20 to 40 years prior to their study with adjacent grasslands that had no history of afforestation. The secondary grasslands presented a completely different grass species composition, and even looked different in the dry season when compared to natural grasslands having a white and reddish hue. The forb composition also differs among the grasslands: natural grasslands have three to five times more forb species than derived ones (Zaloumis & Bond, 2016). The forbs of the natural grasslands are dominated by long-lived perennials with large belowground storage organs that allow them to rapidly grow and resprout after fire before being suppressed by grass species (Veldman *et al.*, 2015b; Zaloumis & Bond, 2016). Figure 4-7 depicts examples of these species. Natural grasslands also contain geoxylic suffrutices that are absent in many secondary ones (Zaloumis & Bond, 2016).

Figure 4-7: Grassland species with belowground organs. (A) *Agapanthus inapertus* P.Beauv. (Agapanthaceae; tuberous belowground structure); (B) *Crinum bulbispermum* (Burm.f.) Milne-Redh. & Schweick. (Amaryllidaceae; bulbous belowground structure); (C) *Eulophia welwitschii* (Rchb.f.) Rolfe (Orchidaceae; belowground corms); (D) *Eulophia ovalis* Lindl. var. *ovalis* (Orchidaceae; belowground rhizome); (E) *Cyrtanthus tuckii* Baker var. *transvaalensis* I.Verd. (Amaryllidaceae; belowground bulbs).

4.5 Summary

Major plant families remain floristically dominant after transformation, but a negative impact occurs on phylogenetic diversity, whereas the abundance of the Poaceae and Cyperaceae are promoted by disturbance. Non-typical grassland families with a wide array of disturbance-tolerant traits show an increase in phylogenetic diversity upon disturbance, mainly as a consequence of the colonisation by alien weedy species.

Species composition of grassland shifts due to disturbance, as indicated by species that are better adapted for entering the system or existing pre-adapted ones that become more dominant due to competition release and/ or altered microclimates and soils (Pysek *et al.*, 1995). This is evidenced by the proportional increase of grass species concomitant with a large reduction in forb species with USOs (Fidelis *et al.*, 2014). The present study found no evidence for extensive woody encroachment in transformed areas.

This study set out to assess species loss due to transformation. The present data conclusively establish that it severely impacts species richness and diversity. These changes are of concern, as grasslands have high economic value and support the wellbeing of humans by providing, among others, ecological infrastructure, carbon sinks, albedo surfaces, plant-based medicines, food plants and grazing for livestock (Bengtsson *et al.*, 2019; Carbutt *et al.*, 2017; Mark *et al.*, 2013), as denoted in preceding chapters. Further studies are needed to determine whether these floristics shifts can still maintain and provide the ecosystem services that are expected from grasslands in South Africa.

CHAPTER 5 WOODY ENCROACHMENT AS A POTENTIAL THREAT TO GRASSLAND DIVERSITY

5.1 Introduction

Grasslands cover 31-43% of the global land surface (Gibson, 2009; White *et al.*, 2000) and are known for hosting an exceptionally high diversity of animal and plant species, habitats, and communities (Bond & Parr, 2010; Carbutt *et al.*, 2017). Grasslands refer to herbaceous vegetation that is dominated by graminoids (particularly the Poaceae), with less than 10% tree and shrub cover (Mucina & Rutherford, 2006; Veldman *et al.*, 2015b; White *et al.*, 2000). Grasslands extend across a range of environmental conditions, from the tropics to the arctic, from sea level to mountain tops, from arid to humid areas, from sites with long hydroperiods to well-drained sites, and from shallow, rocky to deep, clay soils (Bond & Parr, 2010; Veldman *et al.*, 2015b).

Grasslands have been neglected in research as they have been viewed as early successional stages on a trajectory to becoming climax forests (Bond, 2019). Succession in the southern hemisphere is however not linear and grasslands can exist as multiple alternate stable vegetation states (Dantas *et al.*, 2016; Pausas & Bond, 2020; Sankaran *et al.*, 2005; Staver *et al.*, 2011). Disturbances, including aboveground consumers (fires and herbivores) and climate (particularly seasonal drought and cold winters), play a large role in determining grassland distribution and vegetation patterns (Bond, 2019; Pausas & Bond, 2020). The degree to which these disturbances are present determines the vegetation type, be it biome, structure, or state. Fires and herbivory keep grassy systems such as grasslands and savannas open (Buisson *et al.*, 2019) while, in the absence of these disturbances, these systems tend to change into closed-canopy forest (Pausas & Bond, 2020). Another factor maintaining forest-grassland-savanna mosaics is frost and freezing night temperatures, which kill tree seedlings (Joshi *et al.*, 2020). Where there is a decrease or absence of these stressors, one would expect a grassland to become more wooded. In contrast, if the stressors are increased in intensity and/ or frequency or are restored where previously present, the system has the potential to become more open again.

If these disturbances are removed or altered in grassy ecosystems the vegetation pattern shifts will be facilitated from open to closed systems (Higgins & Scheiter, 2012; Moncrieff *et al.*, 2015; Parr *et al.*, 2014; Pausas & Bond, 2020). The system will then progress into an alternative state characterised by different species and communities than the original open system. This occurs when a threshold of change has been crossed. Thresholds of change are those points at which even a slight modification of the biophysical or environmental conditions (such as grazing and fire) can cause an ecosystem to shift into a different ecological state (Andersen *et al.*, 2009).

These alternative states (Box 5-1) are not permanent and can switch from one to another (Bond, 2019). When one state is shifted to another and the new state is stable, the intermediate situations are by unstable definition unstable (Pausas & Bond, 2020). The dependence of grasslands on frequent disturbance makes them especially vulnerable to biome switches driven by rapid change (comprising just a few years) in woody cover (Parr *et al.*, 2014). Savanna or forest pioneer species begin to enter the grassland ecotone by means of seed dispersal, usually by animals. These pioneer species have traits such as high growth rate, high seed production, and fire tolerance that enable them to survive in the grassland system (Parr *et al.*, 2014). They increase to such an extent that they can facilitate the establishment of other savanna or forest species (Pausas & Bond, 2020). It is not yet fully understood what occurs in a system undergoing a transition from grassland to forest or savanna in light of climate change (Parr *et al.*, 2014).

Box 5-1: Alternative Stable States

It has long been believed that climatic conditions govern the ecosystem that occurs in a specific area (Bond, 2019). But it has been found that the same type of climatic conditions can support the occurrence of different ecosystems. For example, it is known that under climatic conditions that are preferential for forest formation, savanna has occurred for thousands of years (Pausas & Bond, 2020). This suggests that climate is not the only factor that determines vegetation patterns (Cheng *et al.*, 2021). Global vegetation models have predicted that with fire as a factor, there are multiple stable biome states possible across Africa, but the potential for multiple stable states decreases with increases in atmospheric CO_2 that could enable the faster growth and survival of more tree species (Moncrieff *et al.*, 2015).

The Alternative Stable State (ASS) theory provides a new framework to explain the distribution of vegetation and temporal changes (Beisner *et al.*, 2003; Pausas & Bond, 2020). The ASS theory suggests that ecosystems exist in multiple stable states, each with a different set of structures and functions, under similar environmental conditions (Cheng *et al.*, 2021). Each stable state is resilient to external condition changes or disturbances to some extent, which ensures the long-term persistence of the state (Cheng *et al.*, 2021).

On the escarpment of the Limpopo Province of South Africa, a mosaic of grassland, savanna, and forest occurs. One of the grassland vegetation types in this mosaic, as mentioned, is the threatened Woodbush Granite Grassland (WGG), which hosts extremely high levels of biodiversity. The largest remaining fragment of WGG is contained in the Haenertsburg Nature Reserve (HNR) and is approximately 192 ha in size. Within this WGG fragment, two distinct plant communities have been observed in recent decades. Despite similar micro-environmental conditions and equal exposure to historical rainfall events (Figure 5-2) and fire regimes (Figure 5-3), one community presents a grassland with an open non-woody physiognomy, while the other has a much higher density of woody, primarily shrub-like vegetation. Henceforth, the two communities are referred to as ‘open’ and ‘closed’, respectively (Figure 5-1; Box 5-2). Both communities experienced the drought that occurred between 2013 and 2015, and both burned eight times between 2003-2019. Before 2003 both areas burned annually for at least 20 years in succession.

Figure 5-1: The open (A) and closed (B) communities of the Haenertsburg Nature Reserve.

Box 5-2: Encroachment, woody thickening, densification, regrowth, or woody invasion

Encroachment can be defined as an increase in the density, cover, and biomass of indigenous woody species (Van Auken, 2009). The term ‘encroachment’ is synonymous with woody thickening, densification, regrowth, thicketisation, woody weed invasion, xerification, shrub invasion, bush encroachment, and brush encroachment (Belayneh & Tessema, 2017; Eldridge *et al.*, 2011).

Woody thickening refers to the increase in the density and cover of indigenous shrubs (Eldridge & Soliveres, 2015).

Densification refers to the encroachment by woody species (Graham *et al.*, 2020).

Regrowth refers to indigenous shrubs and trees that re-establish in an area that was previously cleared for agricultural purposes. Regrowth could therefore be considered to be natural regeneration of indigenous woody species (Eldridge *et al.*, 2003).

Woody weed invasion refers to plant species that form dense impenetrable thickets (scrub) (Ayers *et al.*, 2000).

Bush encroachment refers to the conversion of open ecosystems to dense, tree or shrub-dominated thickets with little grass cover (Meik *et al.*, 2002).

Brush encroachment refers to the increase in density of indigenous woody species that have been present in a system at lower densities for thousands of years (Van Auken, 2000).

Since all the terms are synonymous, the term ‘encroachment’ will be used in this study to refer to the increase in cover and abundance of woody species in a grassland system.

The cause of the difference in physiognomy between the two communities is unknown, since this section of the WGG fragment experienced similar fire, herbivory, and climatic conditions over at least 40 years. Presumed causes of the observed state changes within the WGG include local drivers such as micro-climate changes caused by forestry plantations surrounding the WGG, previous human settlements, and agricultural expansion during the past century.

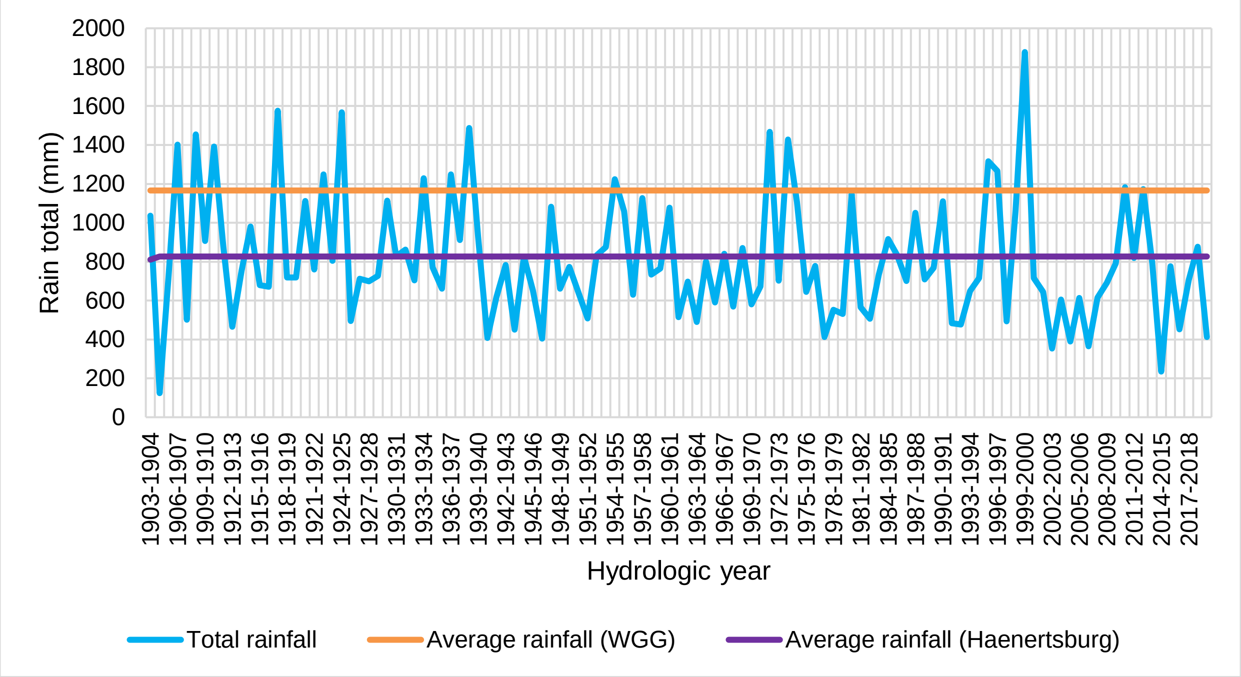


Figure 5-2: Long-term annual rainfall record (1903-2019) for Haenertsburg village, along with means for Haenertsburg village (purple line, 827 mm, 1903-2019) and WGG more broadly (orange line, 1166 mm, from Mucina et al., 2006).

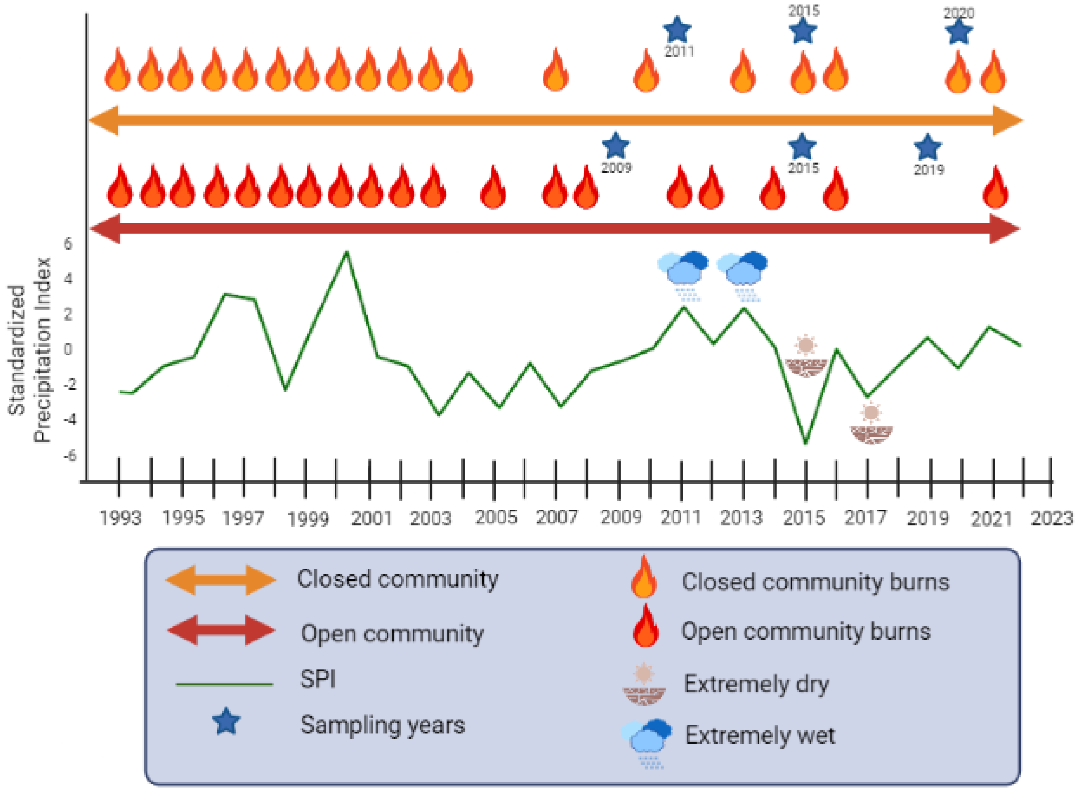


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Figure 5-3: A timeline of the open and closed communities showing when they experienced fire, extremely wet years, and extreme dry years (as based on Standardised Precipitation Index) and when they were sampled.

The aim of this study was therefore to determine whether the apparent transition of parts of the Critically Endangered WGG to a more closed physiognomy is caused by forest expansion or savanna encroachment, so as to evaluate the potential consequences of this for floristic composition and diversity. This aim gave rise to the following objectives, namely to:

a. Compare the current (2019/2020) woody cover and woody species abundance differences between the community with observed higher woody cover (that is 'closed' grassland community) and the 'open' grassland community in the WGG.
b. Assess the current (2019/2020) ecology of all the woody species recorded in the WGG to determine whether they represent savanna encroacher or forest precursor species, and whether an increase in their abundance and cover could be detected from 2011 to 2020 in the closed community.
c. Evaluate the potential effects of increased woody cover on species composition, plant diversity and abundance of endemic, threatened, and exotic species (2019/2020).

It was hypothesised that the closed plant community of the WGG reflected a previously open system that had experienced woody thickening as a result of savanna encroachment. There is a visual increase in the occurrence and cover of woody species despite there being regular fires in the area (Figure 5-3). Savanna woody species are also adapted to fire through its resprouting ability (Acocks, 1988; Clarke *et al.*, 2013), whereas forest species are generally negatively affected by fire and frost (Botha *et al.*, 2020; Joshi *et al.*, 2020). One would therefore expect that the regular fire and frost events would maintain the grassland-forest boundary, but that fire adapted savanna might cross the grassland-savanna boundary. Fire may not be sufficient to stop recruitment of woody species in regions where frost has decreased in frequency, as seen in Africa (Midgley & Lötze, 2008; Rigby & Porporato, 2008), and a consequent increase in woody cover may be expected (Botha *et al.*, 2020).

The increase in woody abundance is usually expected to have negative impacts on herbaceous richness and diversity engendered by shading and litter accumulation, as well as competition with shade-adapted species (Abreu *et al.*, 2017; Parr *et al.*, 2012). The reason is that an increase in woody species leads to the replacement of forbs and graminoids by litter, shrubs, and further woody species (Pinheiro *et al.*, 2016). Grassland plant species diversity, including endemic and threatened species, is furthermore expected to decrease, since the integrity of grassy ecosystem plant diversity and function depend on canopy openness (Bond, 2016; Lehmann & Parr, 2016). Extant literature also indicates an increase in exotic species in encroached sites, since many of the exotics are pioneer species that colonise newly formed habitats (Buisson *et al.*, 2019). These expectations and indications were examined in the current study.

5.2 Materials and methods

This section describes the survey methods that were followed to collect floristic data and the statistical analyses related to the objectives of this chapter. Consider that the two plant communities studied in this chapter will be referred to as ‘open’ and ‘closed’.

Box 5-3: Definitions

Open community	A grassland with none or very few woody plants, typically with less than 10% tree and shrub cover (Mucina & Rutherford, 2006; Veldman *et al.*, 2015b; White *et al.*, 2000).
Closed community	A grassland community type with a semi-closed woody canopy and a discontinuous grass layer.
Long-lived forbs	‘Mound-forming’ species that have the potential to dominate the herbaceous layer and many of them have been described as both herb (forb) and shrublet/shrub in the literature. Examples include *Afroaster comptonii, Cyphia elata, Indigofera hedyantha* and *Pteridium aquilinum.* This group was included under woody species for analyses.
Woody species	Includes shrubs and long-lived mound forming forbs that have the potential to dominate the herbaceous layer. The term ‘woody species’ will be used in this chapter, although not all of these species have the typical shrub or tree physiognomy.
Herbaceous	The non-woody vegetation of the WGG.
Encroachment	The increase in the cover and abundance of indigenous woody shrub species in grassland.
Abundance	Refers to the cover of a particular plant species within a given area (Legendre, 2019).
Occurrence	Refers to the presence or absence of a particular plant species within a given area (Legendre, 2019).

5.2.1 Study site, experimental design, and sampling

Results obtained in this chapter originated from data that were sampled in the HNR within the WGG. Two grassland communities with apparent differences in woody plant cover (that is the open community, and the closed community respectively – see Box 5-3) were floristically investigated and compared. This included examining the effect of encroachment on grassland species composition and diversity. Permanent plots for long-term field observations were established in both communities: 40 plots were established in the open community and 33 in the closed community. Sampling was done during 2009/2011 and 2019/2020, towards the end of the wet growing season, in February, when percentage cover values were likely to be at or approaching maximum.

Floristic surveys were conducted in the permanent plots with a size of 4 x 4 m, which were divided into 16 subplots with a size of 1 x 1 m each (Figure 5-4). In the four corner subplots (A-D) of the 4 x 4 m plot, the percentage aerial cover of each grass, forb, and woody species was recorded.

The cover of each species present in a plot was estimated because of difficulty in differentiating between different individuals in dense herbaceous vegetation types and given that many of the grassland species have clonal growth by rhizomes or stolons or may sprout from underground organs. For the purpose of this study, species cover was used as a proxy for species abundance (Andrade *et al.*, 2019). The percentage cover of bare soil and litter was also recorded.

Each 4 x 4 m plot was marked out with metal rods. A metal rod was planted in each corner, as well as a further one diagonally across from each corner of the plot to mark the inside corner of subplots A to D (Figure 5-4). The outside corner of subplot A was marked with a longer metal rod to be more easily seen. The GPS reading was taken at subplot A located in the north-east corner of the larger plot. Subplots, B, C, and D were in the south-east, south-west and north-west corners of the plot, respectively.

Surveys started in subplot A by placing a 1 x 1 m quadrat made from white PVC pipes over the rod markers. All the forb and grass species within the subplot were identified. Subsequently, a similar procedure was followed for subplots B, C, and D. After herbaceous vegetation sampling in the four sub-plots had been completed, the remaining 12 subplots were searched for woody individuals, and the percentage cover recorded per species per subplot. The woody species included the long-lived forbs, shrubs, and trees.

Plant species were categorised as native (excluding endemics), endemic, naturalised (excluding invasives), or invasive alien. The threat status of all species was determined according to *the Red List of South African Plants* (http://redlist.sanbi.org/). The life-form (that is, grass, forb, or woody) of each plant species was assigned, as well as the life history such as perennial or annual (Germishuizen & Meyer, 2003). The life-forms were further divided into geophytes, parasites, creepers, shrubs, succulents and suffrutices (Germishuizen & Meyer, 2003; Kremer-Köhne, 2021).

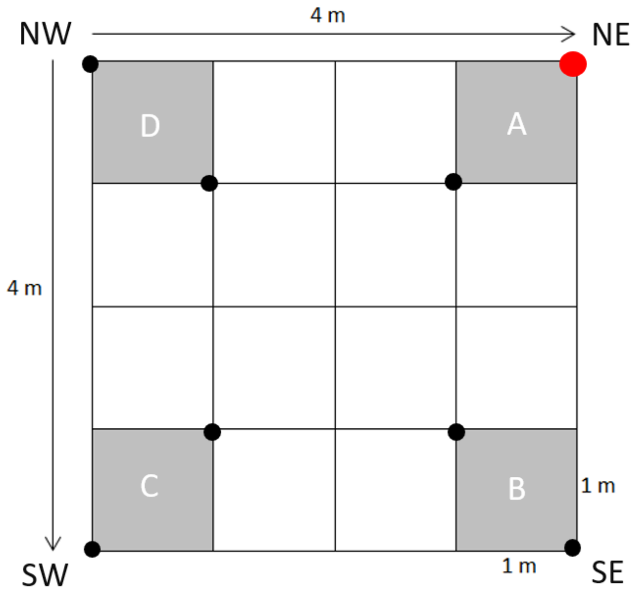


Figure 5-4: An illustration of the 4 x 4 m plots and the 16 subplots. The red dot marks the location of the tallest metal rod located at the outside corner of subplot A. The black dots mark the location of the shorter metal rods used to facilitate plot and subplot orientation.

5.2.2 Data preparation and analyses

A species-plot matrix was compiled and consolidated in Microsoft Excel 2016. The data matrix consisted of floristic data in terms of species presence or absence and cover for the herbaceous and woody species layer for each permanent plot across the sampling years. Data were formatted to be easily imported into PRIMER 6 and R software.

5.2.2.1 Changes in woody species and cover in the open versus closed communities

To determine if the WGG was experiencing encroachment by woody species, the open and closed communities were compared to each other in terms of their woody species occurrence (presence/absence) and abundance. Woody species selected for analyses were either character species (e.g., species restricted to the closed community), or diagnostic (that is abundant) of the closed community. For each of the pre-selected species, a relative occurrence was calculated in the form of the number of subplots in which a particular species occurred in a total of all 528 subplots (that is, 16 subplots of woody data x 33 closed community plots).

Making use of a central plant database of the South African Biodiversity Institute (SANBI), which was limited to the Savanna and Grassland biomes of South Africa, each woody species was assigned to a preferred habitat type withing the two biomes in terms of habitat descriptions provided by the respective plant collectors of the woody species. A table was constructed in which all habitat descriptions per woody species were summarised. Five main groups, as based on habitat descriptions, were identified, comprising (i) grassland species (that is, habitat descriptions that included ‘open grassland types’), (ii) savanna species (that is, habitat descriptions that included ‘savanna’, ‘bushveld’, and ‘thornveld’), (iii) woodland species (that is, habitat descriptions that included ‘woodland’ and ‘thicket’), (iv) forest species (that is, habitat descriptions that included ‘forest’ and ‘forest margins’), and (v) other species that were recorded across biomes (such as ‘fynbos’, ‘Karroo’, ‘sandveld’, ‘Nama karoo’, ‘desert’, and ‘Afro-alpine’). A proportion for each group was calculated as follows: the number of species belonging to each of these habitat groups was divided by the total number of species. This was done to calculate the proportion of species per habitat type.

The woody species were grouped into their growth form: shrubs, that are species that are mostly found in shrub form according to Kremer-Köhne (2021), Van Wyk and Gericke (2017), and Germishuizen and Meyer (2003) and, trees, that is species that have the potential to become trees (Germishuizen & Meyer, 2003; Kremer-Köhne, 2021; Van Wyk & Gericke, 2017). The proportion of shrub and tree species was calculated as follows: the number of shrub/tree species were divided by the total number of woody species.

The occurrence and cover of these woody species were compared for the 2011 and 2020 surveys. The standard deviation of cover means was determined, and a paired t-test was performed to test whether the changes in cover between the 2011 and 2020 sampling were significantly different.

5.2.2.2 Species composition of the two plant communities

To assess the effects of open and closed community type using the 2019/2020 data on herbaceous species composition, the Euclidean distance measure was selected in a Non-parametric Permutational Multivariate Analysis of Variance (PERMANOVA; permutations = 999; type III sum of squares) by using cover data as proxy of species abundance (Anderson & Walsh, 2013; Anderson *et al.*, 2008). Log(X+1) transformation was applied to reduce the weighting of abundant species (Anderson *et al.*, 2008). However, a significant PERMANOVA result for a factor does not indicate whether the difference lies in means, dispersion or both (Mitchell & Bakker, 2016). The homogeneity of dispersion was consequently tested by using PERMDISP (Anderson *et al.*, 2006).

PERMDISP tests were performed by using deviations from the centroid with a selection of 999 restricted permutations. The mean distance of the sampling units (subplots) in relation to the group centroid was measured in terms of the PERMDISP analysis. In this analysis, the group heterogeneity usually increases with the dispersion of data points (Abadia *et al.*, 2018). In the space of the selected similarity measure, the rigorous identification of location versus dispersion effects in balanced designs can be accomplished by using PERMANOVA and PERMDISP, respectively (Anderson & Walsh, 2013). When the dispersions are homogenous, a significant PERMANOVA would indicate a location-only effect, which will be indicative of species sorting themselves into groups. If the dispersions are heterogenous, a significant PERMANOVA results would reflect a dispersion effect or both a dispersion and location effect (Long et al., 2014).

Non-Metric Multidimensional Scaling (NMDS) analysis was used to explore differences in species composition among communities by focussing on floristic data. The simultaneous use of PERMANOVA and PERMDISP in conjunction with the inspection of ordination diagrams may facilitate the interpretation of complex treatment effects on community composition, the latter which result from a combination of changes in the location of the multivariate data cloud (centroid shifts) and multivariate dispersion (Long *et al.*, 2014). The NMDS results have to be interpreted in terms of stress values: (i) a stress value < 0.05 indicates a very good representation with no chance of misinterpreting the data, (ii) a stress value < 0.1 indicates a good ordination with a low risk of misinterpreting the data, and (iii) a stress value > 0.2 indicates a poor representation and should be interpreted with caution (Clarke, 1993). PERMANOVA, PERMDISP, and NMDS analyses were performed in PRIMER 6 (2012). Both PERMANOVA and PERMDISP analyses are robust to deviations from normality and can be applied to uni- and multivariate data (Mitchell & Bakker, 2016).

Similarity Percentage Analysis (SIMPER) was applied to determine the grass, forb, and woody species that caused the greatest extent of differences between the two plant communities.

5.2.2.3 Plant species diversity patterns

All species were considered when it came to determining diversity. Calculation of diversity indices, including species richness (S), Shannon diversity index (H'), and Pielou's evenness index (J') was done in PRIMER 6 (2012) software. Species richness (S) is the total number of species observed and is calculated by means of the mean number of species per plot (m^2). Evenness is the distribution of individuals or abundance of species (Crowder *et al.*, 2012). The Shannon diversity index (H') takes both the species richness and evenness into account. The samples include all species present in that community (Kent, 2011).

Simple paired t-tests were applied in R version 4.0.2 (R Core Team, 2020) to test for significant differences between open and closed communities in the cases of the selected diversity measures. Percentage decrease in richness and abundance (cover) of species per growth/life form, threat status, endemism, and alien taxa were calculated in terms of the statistical means.

5.3 Results

Results presented in this chapter elucidate the shifts in species community composition and diversity between open and closed grassland sites. Results of the woody species were also compared for two sampling periods so as to detect change over time. Significant differences ($p \leq 0.05$) that were obtained are indicated with an asterisk (*) in all figures and tables.

5.3.1 Changes in woody species and cover in the open versus closed communities

The closed community presented a higher shrub cover and more shrub species. Seven woody species occurred only in the open community, 11 only in the closed community, while 13 woody species occurred in both communities (Table 5-1). Among those that occurred in both communities, four presented both a higher number of observations and cover in the closed community, in contrast with only two in the open community. Furthermore, eight of the woody species presented higher average cover in the closed community (Table 5-1).

Most of the recorded woody species from the WGG were associated with grassland habitats, followed by woodland, forest, and savanna (Figure 5-5). All the woody species found are documented to occur in grasslands, even if only occasionally although the type of grassland (ancient, secondary, pristine, transformed, or degraded) was not specified. In other words, Figure 5-5 only depicts the habitat type in which a species was found most often. Only slight differences occurred in proportion for the woodland, forest, and savanna groups. Closer examination was therefore necessary to determine which species were encroaching into the system. For this reason, the abundance and cover were compared for two sampling years, as reflected in Table 5-2.

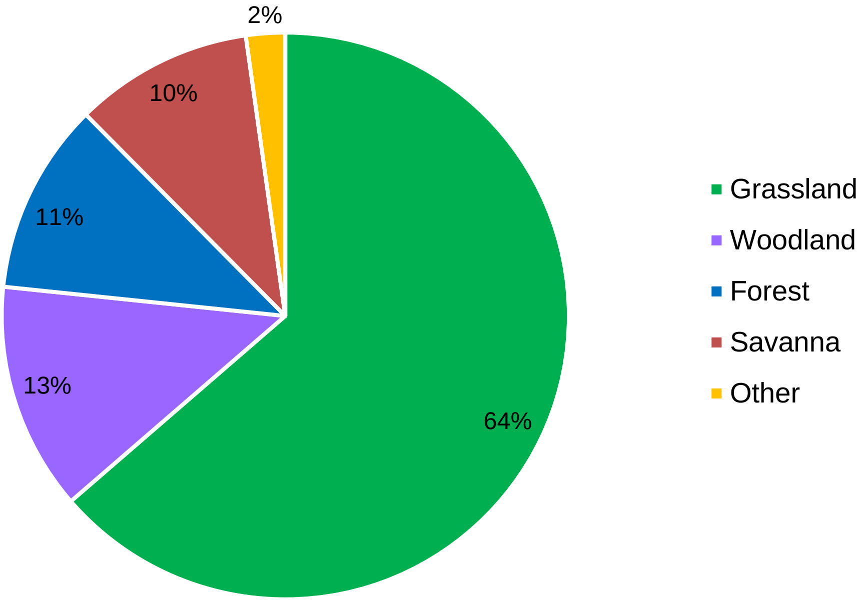


Figure 5-5: The proportion of woody plant species assigned to grassland, woodland, forest, savanna, and other habitat groups.

Table 5-1: The number of observations and the average cover of the woody species observed in open and closed communities in 2019/2020. Bold values indicate the highest observations and cover between the open and closed communities.

	Growth form	Open community		Closed community	
		Observations	**Average cover**	**Observations**	**Average cover**
Indigofera hilaris	Shrub	17	14,06		
Clutia monticola	Shrub	15	4,2		
Searsia discolor	Shrub	6	9,33		
Indigofera hedyantha	Long-lived forb	3	2,67		
Cyphia elata	Long-lived forb	2	4,5		
Argyrolobium transvaalense	Shrub	2	14,5		
Eriosema psoraleoides	Shrub	1	6		
Pteridium aquilinum	Long-lived forb			21	34,76
Indigofera homblei	Shrub			13	44,69
Otholobium wilmsii	Tree/shrub			13	41,23
Lippia javanica	Shrub			11	32,55
Fadogia homblei	Shrub			9	2,56
Helichrysum tenax	Shrub			7	17,71
Lantana rugosa	Shrub			6	16
Searsia transvaalensis	Tree/shrub			3	80
Gymnosporia senegalensis	Tree/shrub			1	45
Hypericum revolutum	Shrub			1	50
Indigofera rehmannii	Shrub			1	10
Afroaster comptonii	Long-lived forb	66	6,48	**83**	**9,29**
Tylosema fassoglense	Shrub	**40**	16,38	26	**28,15**
Triumfetta welwitschii	Long-lived forb	**24**	**7,58**	7	4,29
Pseudarthria hookeri	Shrub	19	**4,32**	**26**	3,08
Stomatanthes africanus	Long-lived forb	**13**	**10,62**	8	10,5
Rabdosiella calycina	Shrub	9	**8,67**	**14**	7,71
Lasiosiphon kraussianus	Long-lived forb	**8**	3,75	3	**6**
Trichodesma physaloides	Long-lived forb	7	3,86	**32**	**7,03**
Rhoicissus tridentata	Shrub	4	**59,25**	**9**	18,89
Athrixia phylicoides	Shrub	3	4,67	**13**	**7,46**
Artemisia afra	Shrub	2	8	2	**10,5**
Searsia pondoensis	Shrub	**2**	2	1	**4**
Cyphostemma woodii	Shrub	1	3	**4**	**18,75**

Over the span of nine years, an increase in the occurrence of species with a preference for woodland, forest, and savanna habitat types was observed. Most of these species were shrubs which included the long-lived woody forbs (Table 5-2). However, when considering cover, the increase was restricted to forest species: and both shrubs and trees (Table 5-2). The only significant difference was observed in the cover of the shrub species, which showed an increase in the 2020 sampling year (Table 5-2).

Table 5-2: A comparison between the occurrence (in 528 subplots) and cover of woody species from woodland, forest, and savanna habitat types, and the overall shrub and tree proportion in 2011 and 2020 (proportion in relation to graminoids, 17% and forbs, 59%). Bold values indicate the higher value among 2011 and 2020 data. * indicate significant differences between the two sampling years.

	Proportion (%)	Occurrence (%)		Mean cover	
		2011	2020	2011	2020
Woodland	13	37,7	**41,1**	**6,48 ± 13,74**	6,29 ± 13,99
Forest	11	54,7	**60,2**	11,53 ± 18,56	**12,99 ± 20,07**
Savanna	10	10,0	**12,3**	**1,45 ± 6,07**	1,18 ± 6,33
Shrub species	22	44,7	**54,5**	7,33 ± 14,00	**9,07 ± 16,70 ***
Tree species	2	**15,3**	12,1	4,63 ± 14,93	**5,24 ± 17,11**

5.3.2 Species composition of the two plant communities

One would expect that the dominant plant families of grasslands, savannas and forests to differ. For example, grasslands are known to have Poaceae, Asteraceae, Fabaceae and Cyperaceae as the most prominent plant families, while savannas are known for having Poaceae, Combretaceae, Myrtaceae and Fabaceae as the most prominent plant families (Kremer-Köhne, 2021; Power *et al.*, 2017). For this study, however, both communities had Asteraceae, Poaceae and Fabaceae as the top three most dominant plant families (Figure 5-6), which are more in line with what is expected for a grassland. The top eight plant families were similar for the two plant communities, except for the presence of Hyacinthaceae and Malvaceae, which were only in the top eight of the open and closed communities, respectively (Figure 5-6).

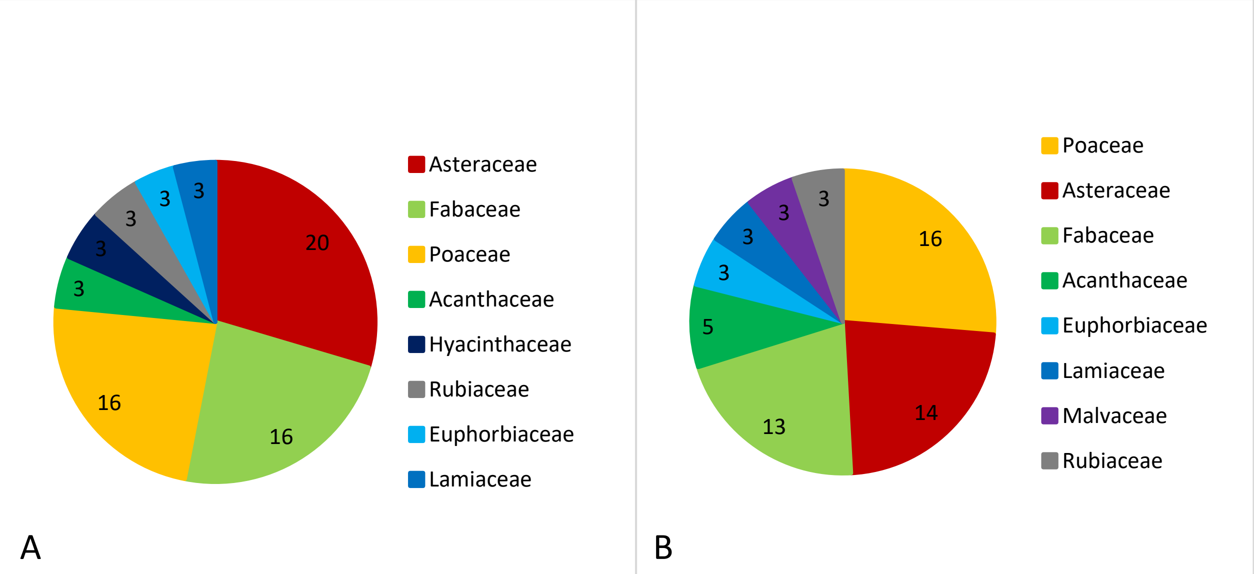


Figure 5-6: The top eight families occurring in the two plant communities in 2019/2020 based on the proportion of each family's contribution to the total species pool of each treatment expressed as a percentage. A. Open community and B. Closed community.

Seven species were endemic to South Africa and two to the WGG (Table 5-3). Of the endemic species, four were found only in the open community, while one was found only in the closed community. Of the remaining endemic species, all presented higher mean abundances in the closed community. The endangered species that occurred in both communities was *Aloe lettyae*, while *Indigofera rehmannii* was present only in the closed community (Table 5-3). The near-threatened species (*Merwilla plumbea*) occurred only in the open community. The exotic species recorded (*Duhaldea latifolia* and *Physalis peruviana*) was present only in the closed community (Table 5-3).

Table 5-3: The mean cover of endemic, endangered, near-threatened, and exotic species in each community in 2019/2020. Bold values represent the highest mean abundance per community. Superscripts: LC Least Concern, DDT Data Deficient – Taxonomically Problematic, E endangered species; NT near-threatened; F forbs; W long-lived forbs and woody species.

	Species	Open community	Closed community
Endemic to South Africa	*Agapanthus inapertus* $^{LC, F}$	0,03	**0,05**
	Alepidea peduncularis $^{DDT, F}$	**0,21**	0
	Argyrolobium transvaalense $^{LC, W}$	**0,18**	0
	Hypericum aethiopicum $^{LC, F}$	0,2	**0,38**
	Indigofera sanguinea $^{LC, W}$	**1,49**	0
	Pygmaeothamnus chamaedendrum $^{LC, F}$	**0,37**	0
	Searsia pondoensis $^{LC, W}$	0,03	0,03
Endemic to WGG	*Aloe lettyae* $^{E, F}$	0,07	**0,61**
	Indigofera rehmannii $^{E, W}$	0	**0,08**
Not endemic	*Merwilla plumbea* $^{NT, F}$	**1,46**	0
Exotic	*Duhaldea latifolia* ^{F}	0	**0,23**
	Physalis peruviana ^{F}	0	**0,02**

The grass, forb, woody, bare soil, and litter cover was calculated as a percentage of community composition (Figure 5-7). The open community was dominated by higher grass cover, while a more equal distribution of life forms was found to occur in the closed community (Figure 5-7). The litter cover was higher in the closed community than in the open one (Figure 5-7). This indicates that the cover of the long-lived forb and woody species is much higher in the closed community and that this seems to suppress the grass cover and not the forb cover.

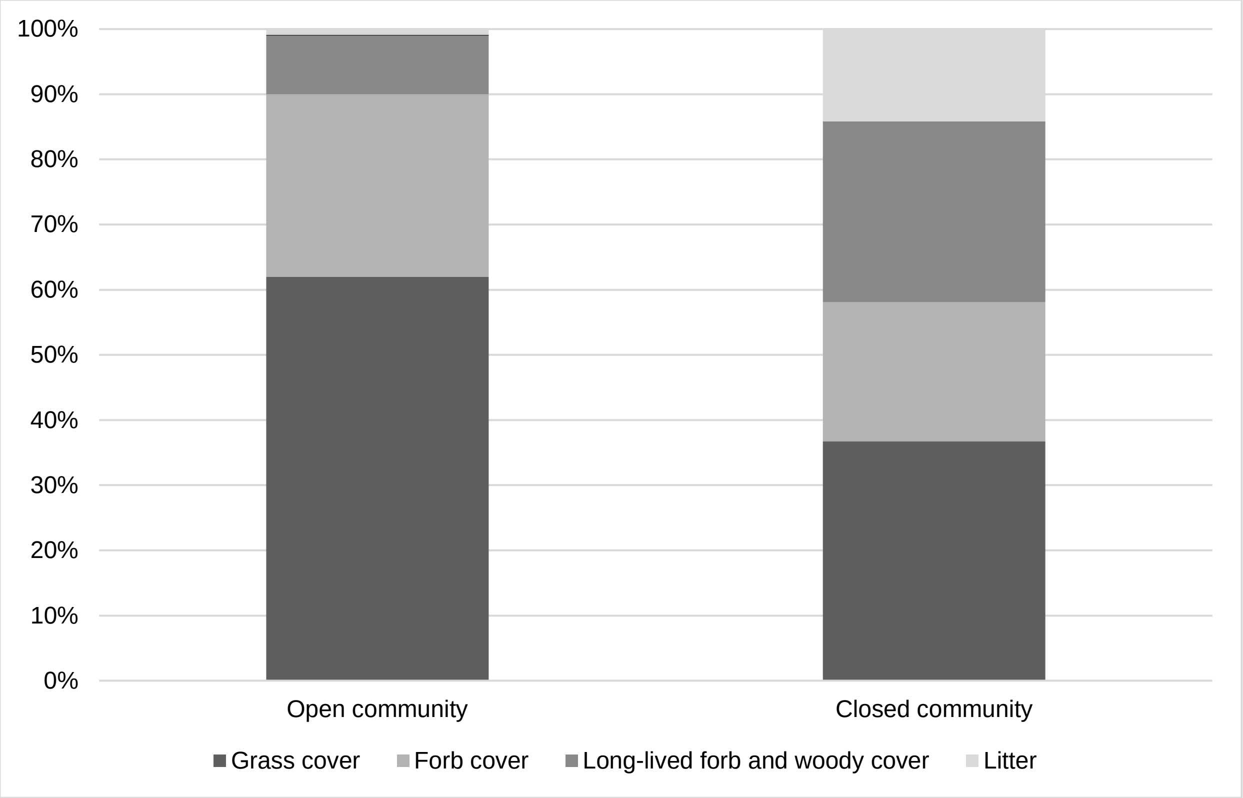


Figure 5-7: The percentage cover of grass, forb, woody species, and litter recorded in the open and closed communities of the Woodbush Granite Grassland in 2019/2020.

The changes in species richness and dominant plant families were expected to affect the composition of the open and closed communities. The PERMANOVA results (p<0.05 in all cases; Table 5-4) demonstrated that community type had a significant effect on community composition among all species groups (p<0.001 in all cases), whereas the ordinations (Figures 5-8 – 5-12) and highly significant PERMDISP results (p<0.001 in all cases; Table 5-4) indicated that this significance resulted primarily from dispersion effects rather than centroid shifts. Compositional differences were thus caused by reduced community dissimilarity rather than species sorting (Long *et al.*, 2014). Such complex effects on composition cannot be disentangled from dispersion in the factorial PERMANOVA, which likely explains the lack of an interaction.

Table 5-4: PERMDISP and permutational multivariate analysis of variance (PERMANOVA) results for comparisons between community types for 2019/2020. Asterisk denotes significant values (significance set at p ≤ 0,05).

	PERMDISP		**PERMANOVA**	
	***F*-value**	***p*-value**	***F*-value**	***p*-value**
All species	80,582	<0,001*	32,903	0,001*
Grass species	38,574	<0,001*	21,883	0,001*
Forb species	108,55	<0,001*	15,299	0,001*
Woody species	47,051	<0,001*	9,6817	0,001*

According to the NMDS interpretation guidelines (section 5.2.2.2), the NMDS results should be interpreted with caution. The NMDS results for all species (Figure 5-8) and for the grass species (Figure 5-9) showed some clustering of the two communities. The open plots were generally more scattered, while the closed plots were dispersed to a lesser degree, which could explain the NMDS outcomes.

Although there seem to be some clustering of subplots to indicate compositional shifts between the two communities on a two-dimensional scale, the stress values are too high (range 0.2-0.27; Figures 5-8 – 5-12), and the significant PERMDISP results illustrate dispersion ($p<0.05$ in all cases; Table 5-4).

Brachiaria serrata and *Diheteropogon amplectens* were only recorded in the open community (both grassland and savanna species), while all other grass species were recorded in both communities (Table 5-5). *Eulalia villosa* (grassland species), *Hyparrhenia cymbaria* (grassland and forest species), and *Melinis repens* (grassland and savanna species) were more prevalent in the closed community.

Fifteen species of forbs contributed ~18% to the dissimilarity between the open and closed communities (Table 5-5). The forb species that contributed most to the compositional changes between the two communities was *Berkheya setifera*. In turn, *B. setifera* was most abundant in the open community, which was to be expected since it is a grassland species. *Dyschoriste setigera* and *Merwilla plumbea* (both grassland species) were the only two forb species that were not found in the closed community, while all other forb species occurred in both communities. *Crabbea hirsuta* (grassland species), *Cynoglossum lanceolatum* (grassland species), *Galopina aspera* (grassland and forest species), *Helichrysum platypterum* (grassland and forest species), and *Hilliardiella oligocephala* (grassland species) were more prevalent in the closed community than in the open one.

Eleven woody species contributed ~16% to the differences observed between the open and closed communities (Table 5-5). Five woody species were present only in the closed community, while one was present only in the open community. *Indigofera hilaris* (grassland species) was the only woody species not found in the closed community. *Indigofera homblei* (grassland and forest species), *Lippia javanica*, *Otholobium wilmsii*, *Pteridium aquilinum*, and *Searsia transvaalensis* (all grassland, savanna, and forest species) did not occur in the open community.

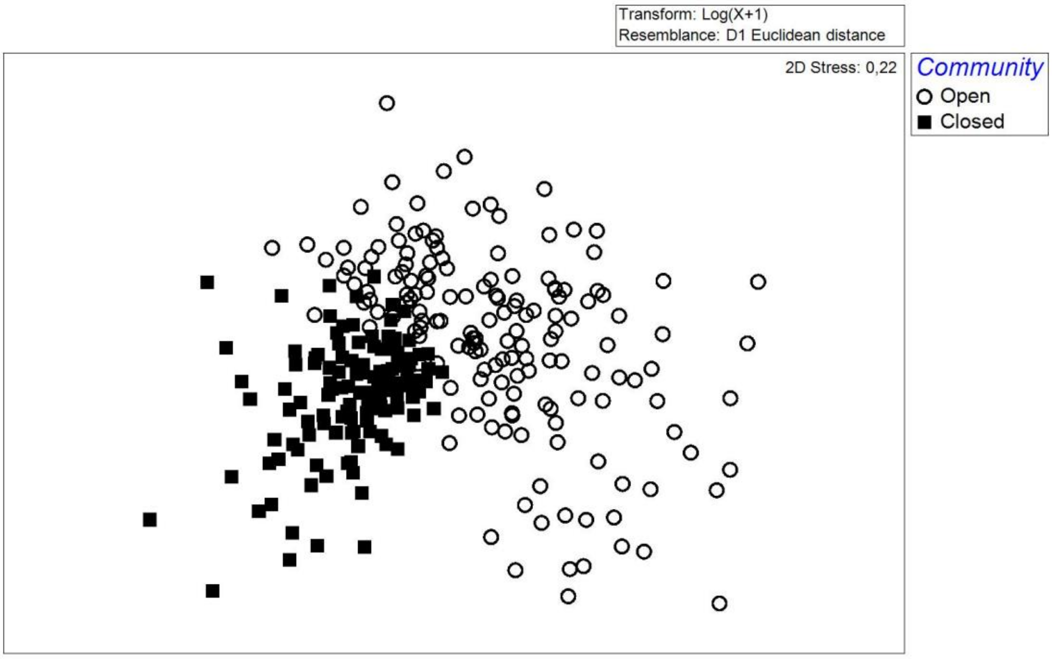


Figure 5-8: An NMDS ordination of all species recorded in subplots A-D in the open and closed communities in 2019/2020 (PERMDISP: $p < 0.05$; PERMANOVA: $F = 32.903$, $p < 0.05$).

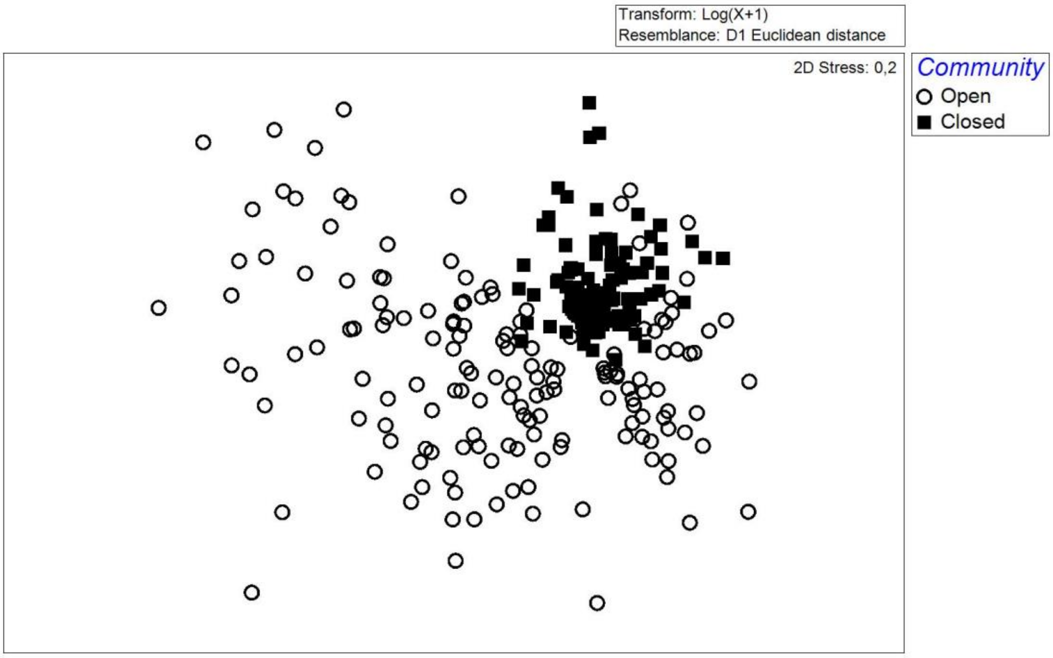


Figure 5-9: An NMDS ordination of all grass species recorded in subplots A-D in the open and closed communities in 2019/2020 (PERMDISP: $p < 0.05$; PERMANOVA: $F = 21.883$, $p < 0.05$).

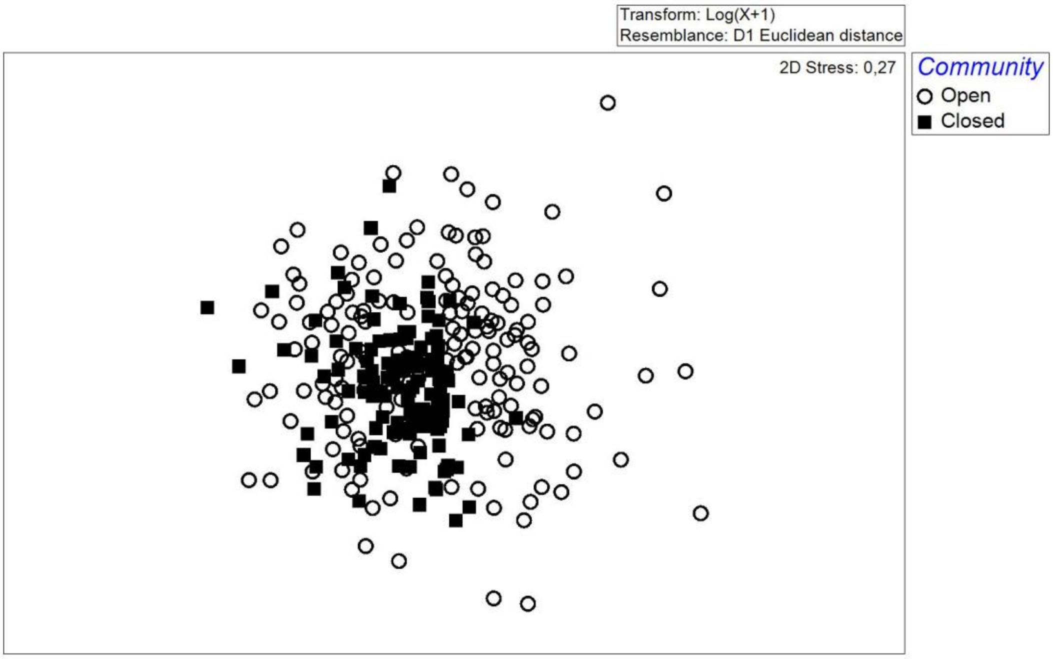


Figure 5-10: An NMDS ordination of all forb species recorded in subplots A-D in the open and closed communities in 2019/2020 (PERMDISP: p < 0.05; PERMANOVA: F = 15.299, p < 0.05).

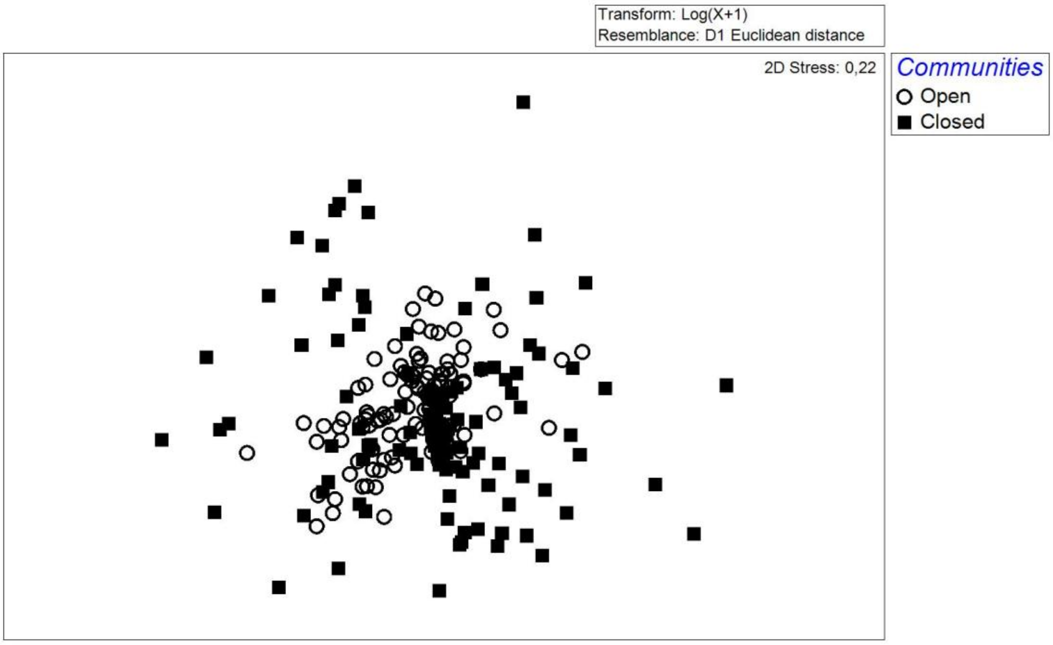


Figure 5-11: An NMDS ordination of the woody species recorded in the subplots A-D in the open and closed communities in 2019/2020 (PERMDISP: p < 0.05; PERMANOVA: F = 9.6817, p < 0.05). (Subplots that did not contain any woody species were excluded).

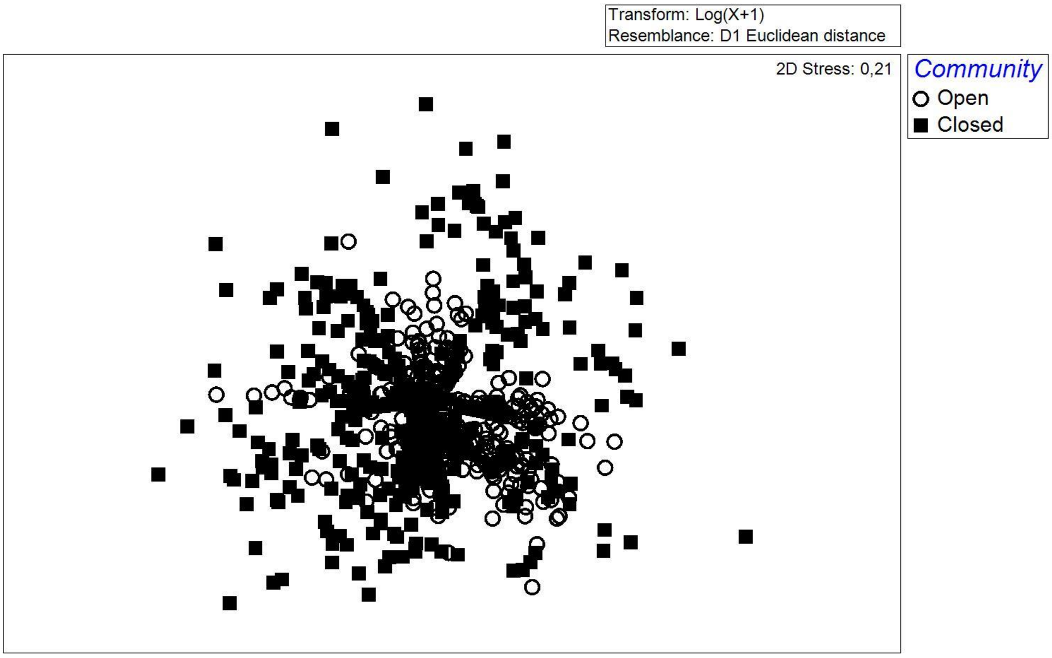


Figure 5-12: A NMDS ordination of the woody species recorded for the 4 x 4 m plots in the open and closed communities in 2019/2020 (Subplots that did not contain any woody species were excluded). (PERMDISP: $p < 0.05$; PERMANOVA: $F = 41.001$, $p < 0.05$).

Table 5-5: Similarity percentage analysis (SIMPER) of species contributing >1% to compositional differences between the open and closed communities in 2019/2020. Species are listed according to contribution % from highest to lowest. Bold values indicate the highest mean abundance (cover). Distance/ similarity measure: Euclidean distance. Av. dis.: average dissimilarity; Cont. %: contribution %; Cum. %: cumulative contribution %

Species	Life form	Plant family	Av. Dis.	Cont. %	Cum. %	Mean abundance (cover)	
						Open	Closed
Cymbopogon nardus	Grass	Poaceae	9,18	12,02	12,02	**36,8**	36
Loudetia simplex	Grass	Poaceae	6,77	8,87	20,89	**21,2**	0,39
Berkheya setifera	Forb	Asteraceae	2,96	3,88	33,08	**7,71**	7,18
Themeda triandra	Grass	Poaceae	2,63	3,45	40,00	**8,08**	0,1
Tylosema fassoglense	Woody	Fabaceae	2,62	3,44	43,44	4,09	**5,72**
Helichrysum platypterum	Forb	Asteraceae	2,07	2,71	46,15	4,08	**5,27**
Afroaster comptonii	Woody	Asteraceae	2,07	2,71	48,85	2,67	**6,02**
Trachypogon spicatus	Grass	Poaceae	1,92	2,52	51,37	**5,81**	0,08
Melinis repens subsp. *repens*	Grass	Poaceae	1,90	2,49	53,86	2,14	**4,43**
Acalypha peduncularis	Forb	Euphorbiaceae	1,90	2,48	56,34	**5,42**	1,75
Setaria sphacelata	Grass	Poaceae	1,70	2,23	58,57	**4,42**	2,02
Pteridium aquilinum	Woody	Dennstaedtiaceae	1,66	2,18	60,74	0	**5,7**
Indigofera homblei	Woody	Fabaceae	1,30	1,70	62,44	0	**4,54**
Helichrysum nudifolium var. *oxyphyllum*	Forb	Asteraceae	1,29	1,69	64,13	**2,67**	2,17
Otholobium wilmsii	Woody	Fabaceae	1,24	1,62	65,75	0	**4,19**
Diheteropogon amplectens	Grass	Poaceae	1,03	1,35	67,10	**3,14**	0
Imperata cylindrica	Grass	Poaceae	0,96	1,25	68,35	**2,58**	0,36
Cynoglossum lanceolatum	Forb	Boraginaceae	0,93	1,22	69,57	1,04	**2,52**
Hyparrhenia filipendula	Grass	Poaceae	0,89	1,17	70,74	**2,56**	0,36
Lippia javanica	Woody	Verbenaceae	0,84	1,10	71,84	0	**2,8**
Rhoicissus tridentata	Woody	Vitaceae	0,82	1,08	72,91	**1,48**	1,33
Hyparrhenia cymbaria	Grass	Poaceae	0,78	1,03	73,94	0,86	**1,8**
Eulalia villosa	Grass	Poaceae	0,74	0,97	74,91	0,73	**1,63**
Brachiaria serrata	Grass	Poaceae	0,64	0,84	75,75	**1,96**	0
Hilliardiella oligocephala	Forb	Asteraceae	0,59	0,77	76,52	1,22	**1,25**
Trichodesma physaloides	Woody	Boraginaceae	0,59	0,77	77,28	0,17	**1,76**

Crabbea hirsuta	Forb	Acanthaceae	0,56	0,73	78,01	0,58	**1,45**
Pentanisia angustifolia	Forb	Rubiaceae	0,55	0,72	78,73	**1,29**	0,79
Searsia transvaalensis	Woody	Anacardiaceae	0,53	0,69	79,42	0	**1,88**
Indigofera hilaris	Woody	Fabaceae	0,47	0,62	80,66	**1,49**	0
Merwilla plumbea	Forb	Hyacinthaceae	0,47	0,61	81,26	**1,46**	0
Thunbergia atriplicifolia	Forb	Acanthaceae	0,42	0,55	82,38	**1,29**	0,02
Triumfetta welwitschii	Woody	Malvaceae	0,42	0,55	82,93	**1,14**	0,23
Tragia rupestris	Forb	Euphorbiaceae	0,41	0,54	84,01	**1,14**	0,52
Galopina aspera	Forb	Rubiaceae	0,40	0,52	85,05	0,21	**1,14**
Justicia anagalloides	Forb	Acanthaceae	0,40	0,52	84,53	**1,06**	0,48
Acalypha depressinerva	Forb	Euphorbiaceae	0,39	0,51	85,55	**1,21**	0,01
Dyschoriste setigera	Forb	Acanthaceae	0,39	0,51	86,06	**1,19**	0

5.3.3 Plant species diversity patterns

Herbaceous species evenness (J'), diversity (H'), and richness (S) differed significantly across the community types. All three indices were higher in the open community ($p < 0.05$; Table 5-6). Species evenness was lower by 4%, diversity lower by 13%, and richness lower by 22% in the closed community. The open community also had a higher cover of endemic, perennial, grass, forb, parasitic, creeping, and suffrutex species than the closed one (Table 5-6). The closed community had 59% higher cover of threatened species and 63% higher cover of geophytes than the open one (Table 5-6).

Table 5-6: Mean values (±SD) of selected diversity measures and mean cover of growth forms, and alien, threatened, and endemic species per community type in 2019/2020. The percentage decrease is given in paranthesis. T-test results are reported for diversity measures, with significance set at p ≤ 0,05, indicated by *.

Measure	Open		Closed		Df	T	P
Pielou's evenness (J')	**0,769**±0,07		0,739±0,09	(4%)	243	3,12	<0,05*
Shannon diversity index (H')	**2,191**±0,31		1,914±0,35	(13%)	259	7,06	<0,05*
Total species (S)	**17,47**±3,58		13,67±3,61	(22%)	271	8,9	<0,05*
Alien	0		**0,125**±0,15				
Threatened	0,094±0,11	(59%)	**0,229**±0,33				
Endemic	**0,26**±0,47		0,12±0,22	(54%)			
Annual	0,425±0,85	(45%)	**0,767**±1,80				
Perennial	**0,987**±3,55		0,749±3,07	(24%)			
Grass	**3,662**±7,79		1,915±6,82*	(48%)			
Geophyte	0,147±0,37	(63%)	**0,397**±1,47				
Forb	**0,364**±0,95		0,243±0,86*	(33%)			
Parasitic	**0,499**±0,68		0				
Creeper	**0,499**±1,10		0,491±1,51	(2%)			
Shrub	0,412±0,76	(44%)	**0,731**±1,38				
Succulent	0,069	(89%)	**0,609**				
Suffrutex	**0,175**		0				
Woody	0,478±0,92*	(65%)	**1,357**±1,90				

5.4 Discussion

5.4.1 Savanna encroachment of forest expansion in the WGG

The results suggest that the closed physiognomy of the closed community could be ascribed to forest expansion rather than savanna encroachment, as there was an increase in the cover and occurrence of forest woody species over nine years, although not enough to suggest encroachment is occurring. The widespread presence of plantations in the area surrounding the study site, and immediately adjacent to the HNR, could potentially have benefitted forest species to a larger degree than grassland ones. Extant literature has established that the kind of human activity (such as forestry or agriculture) can impact the success with which natural grassland and forest species are preserved (Filloy *et al.*, 2010; Pinto *et al.*, 2018).

Results however provide weak evidence of community compositional shifts towards a specific biome, but rather of a particular growth form that caused the grassland to become shrubby by an increase in woody species which can be associated with grasslands (Figure 5-5, Figure 5-6 & Table 5-2). It could thus be argued that the closed community reflects a grassland that is being degraded into a shrubland state. Shrublands are defined as ecosystems with shrub or sub-shrub densities of at least 30% cover, and tree densities of up to 10 trees per hectare (Stavi, 2019) Increasing in size and abundance, woody plants cause soil resources to become more spatially heterogeneous, concentrated under shrub canopies, and depleted in shrub interspaces, thus establishing positive abiotic feedbacks that encourage the transition to a shrubland state (Schlesinger *et al.*, 1996; Schlesinger *et al.*, 1990; Ward *et al.*, 2018). Shrublands are still considered to be open ecosystems (Bond, 2019)

The comparison of the cover of litter, grass, forb, and woody species supports that encroachment/expansion theory, since the grass and forb cover in the closed community decreased, whereas the woody and litter cover increased. The present project confirms that the closed community supported not only a higher cover but also a greater diversity of woody species, and not dominated by only one or two species (Table 5-1). In addition, the occurrence of all the woody species associated with woodland, forest and savanna had increased from 2011 to 2020, even though these species are documented to occur in grasslands, even if only occasionally (Table 5-2).

It is proposed that the closed community is at the beginning of encroachment, and more specifically woody thickening or brush encroachment (Box 5-2), and not savanna encroachment or forest expansion as such. The increase in density and cover of native shrubs is a global phenomenon that occurs in various ecosystems (Maestre *et al.*, 2016). The present study resonates with this finding, as all the observed woody species were indigenous grassland species, meaning that they had been reported in grasslands before as well as in the other habitat types.

In grasslands adjacent to woody communities, proximity usually serves as a catalyst for encroachment (Barbosa da Silva *et al.*, 2016). Seeds reach the neighbouring open grassland from these woody communities to create shrub islands (Barbosa da Silva *et al.*, 2016). In the present case, these communities were forest and savanna. There is however little evidence to conclusively state that it was forest or savanna woody species that caused the encroachment. Most of the increase in woodiness (~2/3) was due to an increase in woody shrubs commonly associated with grasslands, while ~1/3 of woodiness could be primarily associated with savanna and forest biomes, although also associated with grasslands. Two processes were therefore at work: 1) densification of grassland woody species already present in the system, and 2) the encroachment/expansion of woody species from savanna and forest biomes. These two processes could be taking place simultaneously, process 2 may be occurring as a result of process 1 through alteration of the microhabitat beneath the shrub layer. The increase in woody species will create suitable environments for an increase in germination and establishment of other shade tolerant woody species, hence creating a feedback loop (D'Odorico *et al.*, 2010; He *et al.*, 2010; Russell *et al.*, 2014).

Furthermore, trees and shrub species typically have a more extensive rooting system, allowing them to make use of water sources deeper in the soil layer, especially during drier times (Archer *et al.*, 2017; Bond, 2008; Schenk & Jackson, 2002). This would give them a competitive advantage over herbaceous species that generally access only the top 50 cm of soil (Yu *et al.*, 2017).

All grasslands are, however, not equal since there are old growth/primary grasslands and degraded/secondary grasslands (Bond, 2019; Cadman *et al.*, 2013; Veldman *et al.*, 2015b). The latter consists of vegetation that forms over short timescales usually as a result of human land use and was formerly an old growth grassland (Veldman *et al.*, 2015b). These secondary grasslands differ from primary grasslands in terms of species composition, vegetation structure, ecological functioning, and the ecosystem services they provide (Cadman *et al.*, 2013). The presence of more woody species (structure) could therefore be an indication of a degraded/secondary grassland and not necessarily of another biome (Buisson *et al.*, 2019). As previously mentioned, succession in the southern hemisphere is not linear and grasslands can exist as multiple alternate stable vegetation states (Dantas *et al.*, 2016; Pausas & Bond, 2020; Sankaran *et al.*, 2005; Staver *et al.*, 2011). This is especially true since all the woody species that increased are associated with grasslands. Grasslands are described as having sparse to no tree cover, but scattered shrubs may occur (Bond, 2019; Bond & Parr, 2010; Pillay & Ward, 2022). Alternatively, the term ‘hybrid’ can be applied which is the state between old growth and degraded/secondary (novel) grasslands (Buisson *et al.*, 2019). Therefore, it might be a case of an old growth versus a degraded grassland rather than a grassland versus savanna/forest, or in fact an untransformed versus a transformed grassland (Chapter 4) or a grassland versus a shrubland.

5.4.2 Effects of encroachment

The results indicated a mix of effects of encroachment on grassland diversity and structure; positive, negative, and neutral outcomes were found as centred on the increase in shrub cover in the WGG. It has been reported that shrub encroachment has multiple outcomes for the biota, functions and services provided by the encroached ecosystem (Maestre *et al.*, 2016). There is increasing evidence that suggests that the positive effects of encroachment might outweigh the negative In the present case, a positive outcome of encroachment was the presence of endemic and endangered plant species in the closed community (Table 5-3). This is a positive outcome at the moment, unfortunately it is unknown for how long they would remain in the closed community if the canopy cover continued to increase. It would be expected that these species would disappear from the closed community if the encroachment persisted. A negative outcome was the presence of two exotic species in the closed community, which could have been engendered by historic disturbances that made the colonisation by these species easier (Pysek *et al.*, 1995). A neutral outcome was that encroachment did not affect the top four plant families occurring in the two plant communities (Figure 5-4).

Hyacinthaceae did not make it to the top eight plant families in the closed community (Figure 5-6). This family generally consists of perennial bulbous forbs with basal leaves (Koekemoer *et al.*, 2014). The positioning of the leaves could have hampered these species when it came to successfully competing with taller growing species in the closed community, since most of the species in this family prefer open grassland (Kremer-Köhne, 2021), which suggests that they are shade-intolerant. This is supported by the central plant database of SANBI, that species of the Hyacinthaceae family are 79.8% associated with grasslands. This would mean that encroachment could have negatively affected the species of the Hyacinthaceae. Similarly, the Malvaceae did not make it to the top eight in the open community (Figure 5-6). Even so, there were three species that occurred in both communities. Therefore, encroachment had no significant effect on the Malvaceae, and the species of this family did not contribute to the shrub encroachment.

Encroachment negatively affected the grass species *Brachiaria serrata* and *Diheteropogon amplectens* (Table 5-5). Both prefer short open grasslands and are ‘decreaser’ species (Van Oudtshoorn, 2012). Species in the present study site that are usually associated with forests but were absent from the closed community were *Alloteropsis semialata* and *Oplismenus* *hirtellus*. *A. semialata* is an 'Increaser I' perennial climax grass that is abundant in underutilised fields (Van Oudtshoorn, 2012). Biodiversity is threatened at both extremes of a disturbance gradient, namely too much disturbance and lack of disturbance (Stankeva Terziyska *et al.*, 2020). The absence of *A. semialata* could indicate that there was too much disturbance for the species to survive, contributing to the degraded/secondary grassland theory. In turn, *O. hirtellus* was only recorded in one subplot where there was high cover of *Rhoicissus tridentata* (55%), which could have provided sufficient shade for *O. hirtellus* to establish and survive since it is a forest grass species adapted to shade. Species that were positively affected by encroachment included *Hyparrhenia cymbaria*, an 'Increaser I' species that often grows in shaded areas, and *Melinis repens* an 'Increaser II' that grows in disrupted soil (Table 5-5) (Van Oudtshoorn, 2012). The grass *Themeda triandra* was negatively affected by encroachment (Table 5-5), which is to be expected since this grass was a keystone species and indicator of undisturbed grassland (Koekemoer *et al.*, 2014; Snyman *et al.*, 2013).

Forbs that were negatively affected by encroachment included *Dyschoriste setigera, Hilliardiella hirsuta,* and *Rhynchosia resinosa* (Table 5-5). *D. setigera* is a low-growing groundcover (Maphanga, 2014) which is generally associated with grasslands (Kremer-Köhne, 2021). *H. hirsuta* occurs in forest margins, but it requires full sun to light shade (Johnson, 2004). *R. resinosa* is a scrambling plant that is mainly found in forest and savanna systems (Foden & Potter, 2011) but it might prefer these systems to be more open as opposed to densely vegetated. Many forb species are shade intolerant (Weigl & Knowles, 2014) and are therefore resource-limited by taller vegetation (Bråthen *et al.*, 2021; Salazar *et al.*, 2012). This could explain the higher mean abundance (cover) of forb species in the open community in contrast with a loss of these in the closed community (Table 5-5 & Table 5-6). Extant literature established a positive relationship between increased light availability and species richness in grassy biomes (Borer *et al.*, 2014), which could explain the result of the present study of a higher species richness in the open than in the closed community (Table 5-6). Furthermore, the soil surface temperature could have become cooler due to a closed canopy and the forming of a thick litter layer on the soil surface (Figure 5-7), which might have inhibited the germination of seeds that require fluctuations in temperature to germinate (Dairel & Fidelis, 2020; Kolb *et al.*, 2016). Contrastingly, the more closed conditions might have created suitable environments for an increase in germination and establishment of shade tolerant woody species, hence providing a feedback loop for these (D'Odorico *et al.*, 2010; He *et al.*, 2010; Russell *et al.*, 2014). The idea of a feedback loop is supported by the fact that there was an increase in the occurrence of woodland, forest and savanna species from 2011 to 2020.

The grassland diversity (Shannon diversity), species richness and species evenness were negatively affected by encroachment (Table 5-6). Encroachment generally leads to a decrease in herbaceous species, since woody species compete for space previously occupied by herbaceous ones (Eldridge *et al.*, 2011; Laliberte *et al.*, 2004; Ratajczak *et al.*, 2012; Velázquez & Gómez-Sal, 2009). Such a decline in grassland species richness could then lead to altered community composition (Pykälä *et al.*, 2005; Ratajczak *et al.*, 2012; Siebert, 2011; Wieczorkowski & Lehmann, 2022). This suggests that some grassland species are not well adapted to woody plant dominance, which could be attributed to a shading effect caused by the woody canopy (Mogashoa *et al.*, 2021; Pykälä *et al.*, 2005; Ratajczak *et al.*, 2012). The establishment of woody species in a grassland has also been found to alter soil nutrients, nitrogen fixation, and decrease pH (Kinnebrew *et al.*, 2020; Zehnder *et al.*, 2020). Changes in soil nutrients have been found to reduce species diversity, as faster-growing species outcompete those adapted to lower nutrient levels (Bobbink *et al.*, 2010; Bustamante *et al.*, 2012). Changes in light availability and soil attributes promoted by woody encroachment will thus favour a different species pool with different life strategies (Barbosa da Silva *et al.*, 2016). This is reflected by the presence of exotic species in the closed community that do not occur in the open community (Table 5-3).

A loss of grassland diversity, as was the case in the closed community (Table 5-6), could result in altered ecosystem functioning ultimately affecting ecosystem services. This is because ecosystem functioning is promoted by higher biodiversity, which also drives ecosystem services (Balvanera *et al.*, 2006; Duncan *et al.*, 2015; Quijas *et al.*, 2010). To ensure the supply of ecosystem goods and services, more, rather than less, species are needed in a system (Hooper *et al.*, 2005), as is the case with the open community (Table 5-6).

Encroachment negatively affected species of specific growth forms such as forbs, parasitic plants, and suffrutices, of which many species have belowground storage organs (Table 5-6). It has been found that grasses and forbs shift the allocation of biomass production from mainly belowground in open grasslands to mainly aboveground in areas dominated by woody vegetation (Lett & Knapp, 2005). In this way, plants better compete for light aboveground (Tilman, 1988).

5.4.3 Two grassland communities: possible alternative stable states

All grasslands are not equal, there are old growth/primary and degraded/secondary grasslands (Nerlekar & Veldman, 2020; Veldman *et al.*, 2015b). An encroached grassland cannot be viewed as an old growth pristine grassland since it has been degraded (Buisson *et al.*, 2019) and should be seen as a secondary (Veldman *et al.*, 2015b) or transformed grassland (Muller *et al.*, 2021). There is thus an argument to be made that the two communities represent current alternative states, or that the closed community is an intermediate towards a different end point, which would be a different stable state. The closed community could also be said to be at a tipping point between two alternate stable states (Beisner *et al.*, 2003; Folke *et al.*, 2004; Gillson, 2015; Holling, 1973; Scheffer & Carpenter, 2003).

It has been found that, in fire-maintained grassland-forest-savanna ecotones, spatial heterogeneity in plant resource availability likely promotes community heterogeneity of grassland and forest/savanna plant species (Peterson & Reich, 2008). The fact that frequent fires still occur in the two plant communities examined might entail that the closed community was restricted from completely transitioning to a forest or savanna system, thus promoting heterogeneity.

There has been an increase in the occurrence of forest/savanna species over time (Table 5-2). This change is gradual and might be because of the regular fire that has occurred in the system (Figure 5-3). The community would likely continue the current trajectory of becoming shrubbier and support (even if gradually) more species associated with non-grassland biomes. The progression to this new state may be sped up due to feedback loops. Once a grassland has been converted to savanna or forest, it is unlikely that the grassland would return to its previous state, since the feedback loop between fire and vegetation maintain mutually exclusive assemblages of fire and shade tolerant communities (Gillson, 2015; Parr *et al.*, 2012; Staver *et al.*, 2011; Warman & Moles, 2009). For example, forests have a closed canopy and relatively little cover at ground level, which has insufficient flammable biomass to carry fire (Gillson, 2015). Furthermore, under the current increase of CO_2 levels in the atmosphere, more woody species can escape the ‘fire trap’, because tree growth is faster, even if fire intervals remain the same. This means fewer young woody species will be top-killed and more will reach larger size classes thus contributing to the feedback loop (Bond, 2008; Bond & Midgley, 2000; Higgins & Scheiter, 2012; Parr *et al.*, 2014).

Disturbances (such as fire) reduce the abundance and competitive ability of dominant species, thus slowing rates of encroachment and competitive exclusion (Peterson & Reich, 2008). Intermediate fire frequencies have also been found to result in the greatest community heterogeneity (Peterson & Reich, 2008). However, in the present study the NMDS results did not support significant compositional differences between the two plant communities. What was observed in the closed community of the WGG could therefore be the beginning of the formation of an alternative stable state. It would then be expected that, should the driver of encroachment persist, the closed community could become an alternative stable state to the open community.

The woody species composition in grasslands will be influenced by phenomena that affect the timing and frequency of exposure to damage (Botha *et al.*, 2020). For example, the timing of fire events could determine the damage to woody species: woody species that burn in autumn may still have fresh, humid foliage, making them less flammable than species with dry foliage in late winter (Hoffman *et al.*, 2019). The timing of fire events should therefore be well considered to effectively control the increase of woody species in grasslands.

At this stage it is unlikely that the two communities are two different states. The closed community is probably facing the beginning of the encroachment process. A shift in states would become more apparent when there is a shift from flammable, shade-intolerant plants (mostly grasses) to non-flammable, shade-intolerant plants (mostly trees).

5.5 Summary

In this study, open and closed communities of a vulnerable escarpment grassland habitat were compared in terms of composition and diversity to determine if savanna encroachment or forest expansion was occurring. The results indicate that the closed community of the grassland is experiencing an increase in shrubby plant species over the recent decade, but that the increased cover is not primarily through species with forest or savanna affiliation. It is therefore proposed that shrub encroachment of locally occurring grassland species is occurring in the closed community.

It was found that major plant families in the closed community remain floristically dominant after encroachment, while other families, such as the geophytic Hyacinthaceae, disappeared. The compositional changes observed were likely due to the higher forb species diversity in the open community and higher woody species diversity in the closed one. The diversity in the closed community was less than that of the open community for all diversity measures, possibly due to shading, litter accumulation, and competition for resources. The two plant communities did however both contain endemic and threatened species.

This study set out to assess whether the closed grassland community in the WGG occurring within the HNR was in the process of transitioning to a forest or savanna system. It cannot yet be conclusively stated that a grassland-forest/savanna transition was occurring but, if woody species continue to increase and change the composition of the grassland, the system may transition to a forest or savanna. Since an increase in woody species will ultimately reduce the presence of shade-intolerant species such as grassland forbs and grass thus altering the amount of biomass available for fire. The closed community could also be viewed as a degraded/secondary grassland or a shrubland. Further studies are needed to determine if the open community will also become transformed in the future due to the increase in woody species.

The results therefore do not conclusively indicate that the closed community had changed to an alternative stable state, but they do indicate that change was occurring, and the two communities were becoming distinct from one another in terms of diversity.

5.6 Supporting information

Supplement S1: Woodbush Granite Grassland Composition

Table 5-7: Top ten families occurring in the two plant communities based on the proportion of each family's contribution to the total species pools of each community. Values in second column under each heading indicate the ranking of each family for the specific community.

Family	The proportion of all species (%)			
	Open community		Closed community	
Acanthaceae	2,5	6	3,7	4
Asteraceae	19,2	1	15,9	2
Apocynaceae	2,5	7	1,8	11
Cucurbitaceae	1,5	14	2,5	7
Euphorbiaceae	2,0	9	2,5	8
Fabaceae	14,7	3	13,5	3
Geraniaceae	1,5	15	1,8	13
Lamiaceae	3,0	5	3,1	6
Poaceae	17,7	2	17,2	1
Rubiaceae	3,5	4	3,7	5

CHAPTER 6 EFFECTS OF DROUGHT ON GRASSLAND COMMUNITIES OF THE WOODBUSH GRANITE GRASSLAND

6.1 Introduction

Grasslands cover 31-43% of the global land surface (Gibson, 2009; White *et al.*, 2000) and are known for hosting an exceptionally high diversity of animal and plant species, habitats, and communities (Bond & Parr, 2010; Carbutt *et al.*, 2017). Globally grasslands provide many ecosystem services required to support the livelihoods of approximately one billion people (Egoh *et al.*, 2011; Gao *et al.*, 2016). Most importantly, grasslands provide forage to wild and domestic herbivores (Guuroh *et al.*, 2018; Maestre *et al.*, 2022). Water supply is another valuable service provided by grasslands (Egoh *et al.*, 2011). Grasslands further play a role in the mitigation of greenhouse gas emissions, as carbon is stored and sequestered, primarily belowground, within grasslands (O'Mara, 2012).

Determining the mechanisms underlying, and quantifying ecosystem responses to global environmental change is an important concern in ecological research (Ruppert *et al.*, 2015). Despite the extensive terrestrial cover of grasslands globally (Gibson, 2009; White *et al.*, 2000), our understanding of how the composition and diversity of these ecosystems will respond to changing climate is still poor (Adler & Levine, 2007; Cleland *et al.*, 2013; Evans *et al.*, 2011; Jonas *et al.*, 2015). One of the reasons for this research gap is that ecological research on the effects of climate change has for a long time been dominated by a focus on chronic environmental changes rather than discrete climate extremes (Jentsch *et al.*, 2007; Smith, 2011). However, climate change will not only lead to increasing temperature worldwide; in many ecosystems, the variation of precipitation will also increase, which means that drought events will increase in duration and severity (Bodner & Robles, 2017; Hoffman *et al.*, 2019; IPCC, 2012). Consequently, there is an urgent need to better understand how grassland ecosystems will respond to drought.

Drought is a naturally occurring phenomenon characterized by negative rainfall anomalies, with subsequent negative effects on soil moisture content, groundwater recharge, and streamflow (Botai *et al.*, 2019). How a system is likely to respond ecologically to climate extremes such as drought is still poorly understood, since these extremes are rare (Smith, 2011). This research gap has also not been satisfactorily closed yet by the increasing body of literature on the response of various terrestrial ecosystems to drought (Cherwin & Knapp, 2012; Hoover *et al.*, 2014; Knapp *et al.*, 2008; Stuart-Haëntjens *et al.*, 2018). Studies on the effect of drought on the diversity and composition of natural grassland ecosystems remain particularly limited. What we do know is that droughts can have positive effects on grassland ecosystems in terms of the reduction of herbivore numbers due to shortages of food and water, consequently reducing the impact of grazing; reducing tree densities, and thus reducing bush encroachment; lastly, they facilitate the persistence of drought-tolerant flora and fauna (Swemmer *et al.*, 2018).

The ability of an ecosystem to maintain a healthy and productive state, regardless of changes in climate or land-use, is of great interest for all stakeholders (landowners, decision makers, and scientists) involved in the evaluation and mitigation of global change (Ruppert *et al.*, 2015). This ability is referred to as ecosystem stability (Donohue *et al.*, 2013; Pimm, 1984). There are multiple ways in which ecosystem stability can be defined and estimated, including resistance, recovery, and resilience (Donohue *et al.*, 2013). Resistance refers to a system's ability to withstand disturbance such as drought (Pimm, 1984). Resilience refers to the amount of disturbance a system can absorb without changing the state (Gunderson, 2000), also refers to the capacity for recovery of function (Pimm, 1984). Another aspect to consider when studying droughts is drought legacy effects or recovery. Drought legacies can be defined as the change in resources or ecosystem properties that continue to affect an ecosystem after a drought event (Griffin-Nolan *et al.*, 2018; Yahdjian & Sala, 2006). It has been found that drought impact and legacies can either be negative of positive (Sala *et al.*, 2012).

There is limited knowledge on how drought affects ecosystem stability in natural grasslands (Stampfli *et al.*, 2018). One important reason for this research gap is that the analysis of all aspects of ecosystem stability in the face of drought requires good pre-drought data, which is required for assessing drought impact and drought legacy (Ruppert *et al.*, 2015). Most empirical drought studies only start during a drought (Van Coller *et al.*, 2018), as result we have most knowledge on drought resistance. Only long-term ecological observations are a suitable approach to capture all stability dimensions in the face of ecological perturbations such as drought (Ruppert *et al.*, 2015).

In summary, a better understanding of drought impacts on grassland ecosystems is urgently needed, as it is prerequisite to identify sustainable management measures of current and future grasslands in the face of drought (Bodner & Robles, 2017). This requires and improved understanding of the floristic diversity with a view to greater clarity about the community shifts related to climate change. According to Knapp *et al.* (2002), grasslands are ideal for the study of the effect of rainfall variability, as they are among the ecosystems that are most responsive to inter-annual variability in precipitation.

Different land use and management-induced changes in plant communities have been found to either amplify or buffer drought responses and patterns of recovery (Vogel *et al.*, 2012).

different grassland types have also been found to respond differently to drought conditions (Wang *et al.*, 2019). One of the reasons for this was ascribed to the different grassland types having different resistance and resilience to drought. Differences in grassland net primary productivity and evaporation are other reasons for the differences observed (Wang *et al.*, 2019).

Climate change also is projected to cause significant change and loss of habitat in the Grassland Biome in South Africa (DEA, 2013). Grasslands of South Africa could retreat to high-altitude areas in the worst-case scenario (Driver *et al.*, 2012). This will likely be accompanied by an increase in woody plant cover and an expansion of savanna and forest biomes (Higgins & Scheiter, 2012; Moncrieff *et al.*, 2015). This phenomenon is already visible in some areas of South African grasslands, leading to a mosaic of more open and more closed, that is encroached patches (Moncrieff *et al.*, 2015; Parr *et al.*, 2014; Pausas & Bond, 2020). If the climate becomes warmer and drier or the frequency, magnitude, and/or duration of drought intensifies, present-day grasslands could become desert shrublands in some areas (Archer *et al.*, 2017). Furthermore, if rainfall variability increases, as is predicted, more variable soil moisture content in grasslands are likely to increase plant water stress in the short term, and alter key carbon cycling processes such as net photosynthesis, aboveground productivity, and soil CO_2 flux, which hold long term consequences for carbon storage and biotic-atmospheric feedbacks (Knapp *et al.*, 2002; Munjonji *et al.*, 2020). It is also expected that there will be shifts in species composition towards more diverse communities characteristic of more xeric environments (Knapp *et al.*, 2002). Even so, the long-term impacts of drought in grasslands remain unknown, especially when it comes to the impact of drought on vegetation (Swemmer *et al.*, 2018), species composition, and ecosystem productivity (Yu *et al.*, 2017).

The aim of this study was therefore to assess how the encroachment state of a native South African grassland modulates its drought stability (i.e. its drought resistance and resilience, as well as the legacy effect of a drought event). Particularly, the following questions were addressed:

1. Do grassland communities with different encroachment state (open versus closed) differ in their drought resistance and resilience, and how do these grasslands recover, after a drought?
2. What are the implications of a severe drought for encroachment processes?

The following hypothesis were formulated from the above:

The composition and diversity of closed (encroached) grasslands reflects lower drought resistance and resilience and stronger legacy effects than in open grasslands. Higher variability in species abundances in time or space equates to reduced stability (Cottingham *et al.*, 2001; Ives & Carpenter, 2007). Community composition over time, including species invasions, extinctions, resistance and variability determines stability: less stable communities experience higher rates of compositional change (Donohue *et al.*, 2013; Pimm, 1984). Communities with higher resistance show less structural change (Donohue *et al.*, 2013).

Long-lived woody species complete their life cycles over decades to centuries and should therefore be more resistant and less resilient to drought. This is because these species have a high energy and time cost associated with the rebuilding of biomass before reproduction (MacGillivray *et al.*, 1995). On the other hand, grassland species may be less resistant but more resilient. These species are more immediately vulnerable to severe drought, but they can rapidly re-establish, grow, and reproduce (Hoover *et al.*, 2014).

6.2 Materials and methods

Two adjacent plant communities within the same vegetation type, the Woodbush Granite Grassland (WGG) will be compared. The one community is more in a natural state (open), while the other is experiencing shrub encroachment (closed) (Box 6-1).

Box 6-1: Definitions

Woody species	Includes shrubs and long-lived mound forming forbs that have the potential to dominate the herbaceous layer. The term ‘woody species’ will be used in this chapter.
Long-lived forbs	‘Mound-forming’ species that have the potential to dominate the herbaceous layer and many of them have been described as both herb (forb) and suffruticose in the literature.
Open community	A grassland with none or very few woody plants, typically with less than 10% tree and shrub cover (Mucina & Rutherford, 2006; Veldman *et al.*, 2015b; White *et al.*, 2000).
Closed community	A grassland with a discontinuous open grass layer caused by an increase in woody cover.
Encroachment	The increase in the cover and abundance of indigenous woody shrub species in grassland. Encroachment can be defined as an increase in the density, cover, and biomass of indigenous woody species (Van Auken, 2009). The term ‘encroachment’ is synonymous with woody thickening, densification, regrowth, thicketisation, woody weed invasion, xerification, shrub invasion, bush encroachment, and Brush encroachment (Belayneh & Tessema, 2017; Eldridge *et al.*, 2011).

Pre-drought	Refers to the sampling years before the drought: pre-drought sampling was undertaken in 2009 in the open community, and in 2011 in the closed community.
Drought year	Refers to the 2015 sample year.
Post-drought	Refers to the sampling years after the drought: post-drought sampling was undertaken in 2019 in the open community and in 2020 in the closed community.
Resistance	See Box 6.3
Resilience	See Box 6.3
Legacy effect or Recovery	See Box 6.3
Abundance	Refers to the species cover (Andrade *et al.*, 2019; Legendre, 2019).
Occurrence	Refers to the presence or absence of plant species (Legendre, 2019).
Species richness	The number of species in a system (Pimm, 1984).
Evenness	Refers to the distribution of individuals across different species in a community (Crowder *et al.*, 2012).
Diversity	A combination of richness and evenness (Jonas *et al.*, 2015).
Alpha diversity	Diversity at habitat level expressed as species richness and evenness indices (Thukral, 2017).
Beta diversity	Diversity that accumulates as a result of differences between sites (Socolar *et al.*, 2016)

From 2013 to 2016, South Africa experienced a mild to extreme drought event associated with an El Niño event (Malherbe *et al.*, 2020; Swemmer *et al.*, 2018; Yuan *et al.*, 2018). Severe drought was recorded in the summer rainfall regions of South Africa from 2013 to 2015 (Figure 6-1; Box 6-2). The eastern parts of South Africa were affected the most (Wilcox *et al.*, 2020). The drought was intensified by a soil moisture deficit carried over as a legacy from previous years (Yuan *et al.*, 2018). Moreover, the negative rainfall anomalies in the years 2013 to 2016 coincided with higher temperatures, and a considerable number of areas experiencing record-breaking monthly mean maximum temperatures (Swemmer *et al.*, 2018) as well as episodes of excessive heat and heatwaves in 2015 and 2016 (Malherbe *et al.*, 2020).

Box 6-2: Standardised Precipitation Index for Haenertsburg, South Africa

According to the data provided by the South African Weather Service (SAWS) and data obtained from the South African Environmental Observation Network (SAEON) weather station in Haenertsburg, precipitation in the 2015 hydrological year was well below the long-term site mean and was classified as an extremely dry year according to the Standardised Precipitation Index (SPI; Figure 6-1). This event was one of ten “extremely dry” events since 1903 up until 2021.

Drought intensity was quantified using SPI*,* *a* well-supported precipitation index (Vicente-Serrano *et al.*, 2012). SPI values were calculated based on annual precipitation sums representing Haenertsburg's hydrological years from 1904 to 2022. SPI values were assigned to drought intensity classes based on the classification of the US National Drought Mitigation Center (http://droughtmonitor.unl.edu), and adding the class of 'normal precipitation' (SPI of -1 to +1) (Ruppert et al., 2015). The SPI is a preferred method because of its solid theoretical development, robustness, and versatility in drought analyses (Redmond, 2002).

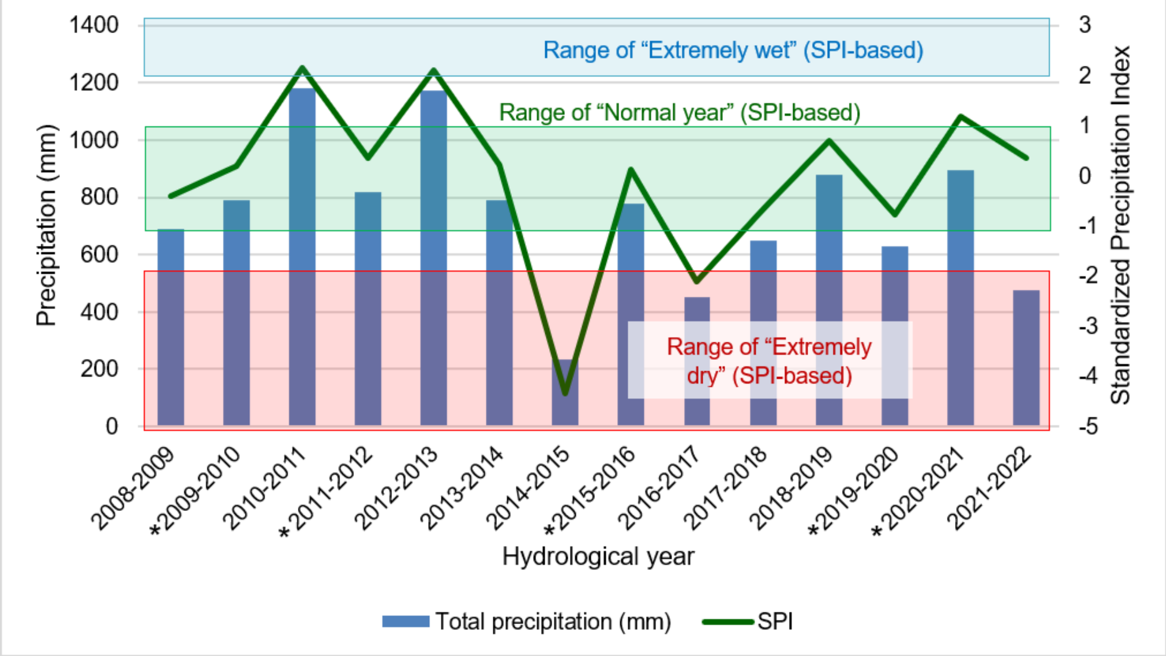


Figure 6-1: The total precipitation (mm) and Standardised Precipitation Index (SPI) for Haenertsburg for hydrological years since 2009, when sampling in the WGG was initiated. Sampled years are indicated by an asterisk (based on a draft by A Linstädter (unpublished)).

6.2.1 Study site, experimental design, and sampling

The study was conducted in the Haenertsburg Nature Reserve (HNR) in the WGG located in the Limpopo Province, South Africa. To assess the effects of encroachment state on the stability of the grassland ecosystem towards a severe drought, pre-drought, within-drought and post-drought data on vegetation composition and diversity were compared between an untransformed, open grassland community to a community in which woody plant cover was observed to be considerably higher. In both communities, permanent plots (4 x 4 m) were established for long-term field observations, with 40 plots situated in the open and 33 in the closed community. Sampling was done during the vegetation periods 2009/2011 (pre-drought), 2015 (drought) and 2019/2020 (post-drought), and was always performed in February, that is at the time of peak standing crop.

During each visit, we conducted floristic surveys in the permanent plots. To this end, we divided plots into 16 subplots of 1 x 1 m (Figure 6-2) and performed visual cover estimations for all vascular plant species in the four corner subplots (A-D) to determine species abundance (Andrade *et al.*, 2019).

The remaining twelve subplots were individually searched for woody plants (long-lived forbs, shrubs, and trees). We recorded ground cover, stem number and stem height per species and subplot, with stem height measured as the height from ground-level to the upper border of the canopy, disregarding any inflorescence (Cornelissen *et al.*, 2003; Pérez-Harguindeguy *et al.*, 2016).

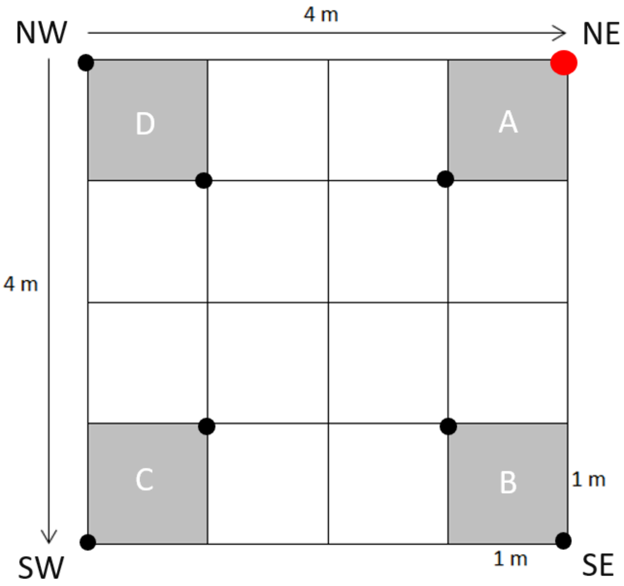


Figure 6-2: An illustration of the 4 x 4 m sampling plots and the 16 subplots. The red dot marks the location of the tallest metal rod located at the outside corner of subplot A. The black dots mark the location of the shorter metal rods to facilitate plot and subplot orientation. Subplots A-D were sampled for herbaceous vegetation, while the entire plot area was sampled for woody species.

6.2.2 Data preparation and analyses

To assess drought resistance and resilience as well as drought recovery (Box 6-3) across the two grassland communities, we focused on five variables capturing different aspects of floristic diversity. Three of them were related to plant communities’ alpha diversity (Box 6-1), namely species richness, species evenness and Shannon diversity on the square meter level. The remaining two were related to temporal beta diversity, that is to the similarity of plant communities in space and time. We quantified spatial beta diversity as the species turnover between the open and closed communities. While the temporal beta-diversity was quantified from pre-drought to drought to post-drought conditions.

A species-plot matrix was compiled and consolidated in Excel 2016. The data matrix consisted of quantitative floristic (that is, species cover) data for the herbaceous and woody layer for each permanent plot over the rainfall years. Data were formatted so as to be easily imported into PRIMER 6 and R software.

6.2.2.1 The temporal effect of drought on an encroached grassland to determine grassland resistance, resilience and recovery after drought

The collected data were used in a temporal beta-diversity index (TBI) approach to determine the losses and gains of plant species and their respective abundances in response to rainfall conditions (Legendre, 2019). TBI measures the change in community composition between time 1 (T1) and time 2 (T2) (Legendre, 2019). Since change in time is directional, species presence or abundance may have been gained and/or lost between T1 and T2 (Legendre, 2019). According to Legendre (2019), in the TBI test, aerial cover data can be treated as if these were species abundances. The occurrence refers to the presence/absence of species. TBI calculations were implemented in the *TBI.R* function available in the R package *adespatial* by using R software (version 4.0.2). The percentage difference (%diff), also known as the Bray-Curtis dissimilarity index, was used to this end, as it contains in its equations the quantities *B* and *C*, that represent the species loss and gain components of the dissimilarities (Legendre 2019). B-C plots were created with the *B*/den and *C*/den statistics as coordinates of points, representing sites (Legendre 2019). These graphs visually display the relative importance of the loss and gain processes across the study sites (Legendre 2019). The function tpaired.krandtest.R was used to identify the species whose abundances/occurrences had changed in a significant way between T1 and T2 (Legendre 2019).

In the B-C plots (see Figure 6-7) a diagonal green line (slope = 1) goes through the origin and represents the theoretical positions of sites where the gain would be equal to the loss (Legendre 2019). The red line, parallel to the green line, passes through the centroid of all points (Legendre 2019). When the red line is below the green line, it indicates that species loss dominated over gain across the sites, and the opposite holds if the red line is above the green one (Legendre 2019). Furthermore, points found higher in the plot (towards the upper-right corner) represented higher temporal beta diversity than points found lower down (towards the lower-left corner) (Legendre 2019).

Box 6-3: How drought effects were operationalised

Comparison 1: Pre-drought to drought = resistance. Resistance refers to a system's ability to withstand disturbance (Pimm, 1984). The time before the drought (pre-drought to drought) was used to determine community resistance to drought. Usually, it is assumed that there is a negative effect of a disturbance on measures of ecosystem function, such as above-ground net primary productivity (ANPP). Hence, one measures the negative impact as resistance, as reflected in the definition of drought resistance by Sadiqi *et al.* (2022): resistance is "*the ability to withstand a water shortage while remaining within the upper limit of performance".* This "remaining within the upper limit" would correspond to a loss of biodiversity. Drought resistance has also been defined as growth insensitivity to stress (van Mantgem *et al.*, 2020) and the capacity of an ecosystem to withstand change during severe drought (Stuart-Haëntjens *et al.*, 2018). The grassland plant community will be considered 'resistant to drought' when there are no significant losses of species cover, abundance, diversity, or composition shifts before the onset of the drought.

Comparison 2: Drought to post-drought = recovery. Recovery has been defined as a system's potential to (or rate of) return to a previous state after a disturbance (Pimm, 1984), the growth rate following a drought relative to the growth during the drought (van Mantgem *et al.*, 2020) and the capacity of an ecosystem to return to an undisturbed state after a disturbance has occurred (Capdevila *et al.*, 2020). The time from the drought year to after the drought (drought to post-drought) was used to determine community recovery after the drought. The grassland plant community will be considered 'recovered from drought' when the pre- and post-drought species cover, abundance, diversity, and composition are similar. The term recovery will be used in this chapter.

Comparison 3: Pre-drought to post-drought = resilience. Resilience has been defined as the amount of disturbance a system can absorb without changing the state (Gunderson, 2000), the capacity for recovery of function (Pimm, 1984), and "t*he ability to recover from a stress or threshold response after favourable hydrologic conditions have returned*" (Sadiqi et al., 2022). Furthermore, drought resilience is measured as the magnitude of return to pre-drought growth (van Mantgem *et al.*, 2020) and resilience is the degree to which an ecosystem recovers to its original composition (Stuart-Haëntjens *et al.*, 2018). The overall change from before the drought to after the drought (pre-drought to post-drought) was used to determine community resilience to drought. The grassland plant community will be considered 'resilient to drought' when there are no significant losses of species cover, abundance, diversity, or composition shifts.

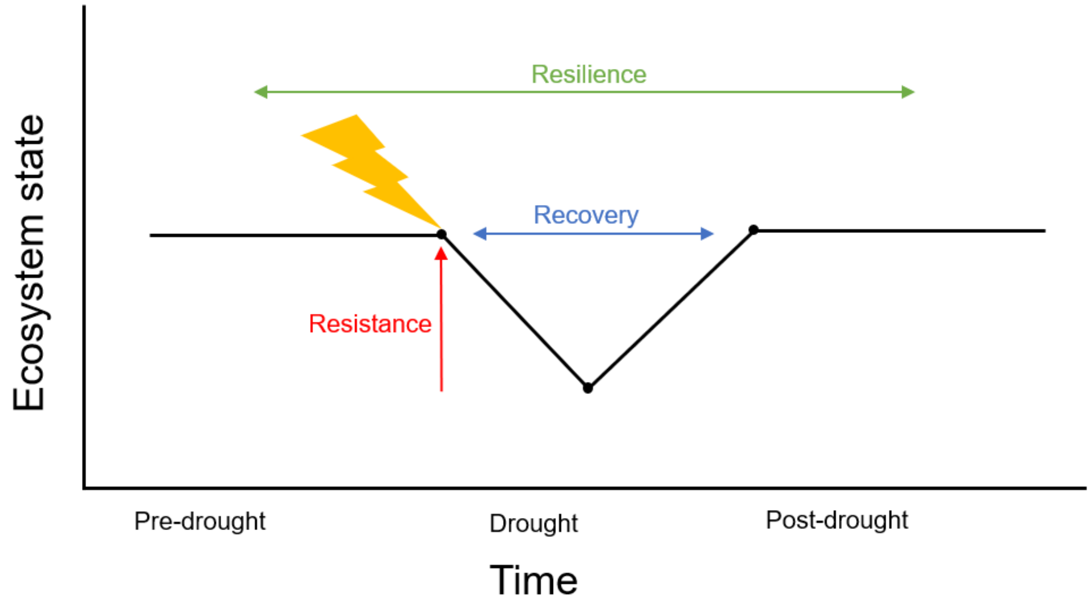


Figure 6-3: Conceptual framework for plant community responses to extreme drought.

6.2.2.2 Drought effects on species turnover between the open and closed grassland

To assess the effects of drought on herbaceous species composition across the two community types (open and closed), the Euclidean distance measure was selected in a Non-parametric Permutational Multivariate Analysis of Variance (PERMANOVA; permutations = 999; type III sum of squares) by using cover data as a proxy of species abundance (Anderson & Walsh, 2013; Anderson *et al.*, 2008). Log(X+1) transformation was applied to reduce the weighting of abundant species (Anderson *et al.*, 2008). A significant PERMANOVA result for a factor does not indicate whether the difference lies in means, dispersion or both (Mitchell & Bakker, 2016). Therefore, the homogeneity of dispersion was tested by using PERMDISP (Anderson *et al.*, 2006). PERMDISP tests were performed by using deviations from the centroid with 999 restricted permutations selected. The mean distance of the sampling units (subplots) in relation to the group centroid is measured with the PERMDISP analysis. In this analysis, the group heterogeneity increases with the dispersion of the data points. (Abadia *et al.*, 2018). In the space of the selected similarity measure, the rigorous identification of location vs. dispersion effects in balanced designs can be accomplished using PERMANOVA and PERMDISP, respectively (Anderson & Walsh, 2013). When the dispersions are homogenous, a significant PERMANOVA would indicate a location only effect, indicative of species sorting themselves into the groups. If the dispersions are heterogenous, a significant PERMANOVA results would reflect a dispersion effect or both a dispersion and location effect (Long et al., 2014).

Non-Metric Multidimensional Scaling (NMDS) analysis was used to explore differences in species composition between communities and rainfall years by focusing on floristic data. It is possible to interpret complex treatment effects on community composition that result from a combination of changes in the location of the multivariate data cloud (centroid shifts) and changes in the multivariate dispersion when PERMANOVA and PERMDISP are used simultaneously and in conjunction with the inspection of ordination diagrams (Long *et al.*, 2014). The NMDS analysis results in a stress value that can be interpreted as follows: (i) a stress value < 0.05 indicates a very good representation with no chance of misinterpreting the data, (ii) a stress value < 0.1 indicates a good ordination with a low risk of misinterpreting the data, (iii) a stress value < 0.2 indicates a poor representation and should be interpreted with caution (Clarke, 1993). PERMANOVA, PERMDISP, and NMDS analyses were performed in PRIMER 6 2012 where rainfall year was used as the fixed effect.

All plant species were considered for diversity analysis. Diversity indices (species richness (S), Shannon diversity index (H’), and Pielou’s evenness index (J’)) was done in PRIMER 6 software. Species richness (S) refers to the total number of species observed and is calculated as the mean number of species per plot (m^2). Evenness refers to the distribution of individuals (abundance) of species (Crowder *et al.*, 2012). The Shannon diversity index (H’) takes bot the species richness and evenness into consideration. The samples include all species present in that community (Kent, 2011).

Two-way ANOVA type Generalized Linear Model (GLM) was applied to the richness, abundance, and diversity index data. This was done to determine the presence of significant differences among the diversity measures across community types (open and closed) over the three rainfall years (pre-drought, drought, and post-drought). The GLM analyses was done by using R version 4.0.2 of the R Core Team, 2020, where rainfall year (pre-drought, drought, post-drought) was included as a fixed effect, and plot was nested within community type (open and closed) as random effects so as to account for repeated measures sampling. A pairwise test was then conducted to determine whether changes observed among rainfall years and between community types were statistically significant.

Significance was determined at the standard level of $p \leq 0.05$ (Manderscheid, 1965). Normality assumptions were tested by using the Shapiro-Wilk’s test for each diversity measure. If the data were normally distributed, the p-value would be greater than 0.05. Homogeneity of variance assumption was checked by using Levene’s test ($p > 0.05$). The homogeneity of covariances assumption was evaluated by using the Box M-test. If this test presents $p < 0.001$, no equal covariances exist.

6.3 Results

This chapter's results centre on drought response effects and community type in terms of shifts in species community composition and diversity. Significant differences (p ≤ 0,05) are indicated with an asterisk (*) in all figures and tables.

6.3.1 Grassland resistance, resilience, and recovery from drought

To get an overview of the changes over time in the two plant communities of the species richness of grass, forb and woody species simple bar graphs were created (Figure 6-4). Grass species in both communities and woody species and the forbs in the closed community, increased from the pre-drought to drought year and then decreased after the drought. Only the number of forb and woody species in the open community increased going into and continued to increase after the drought.

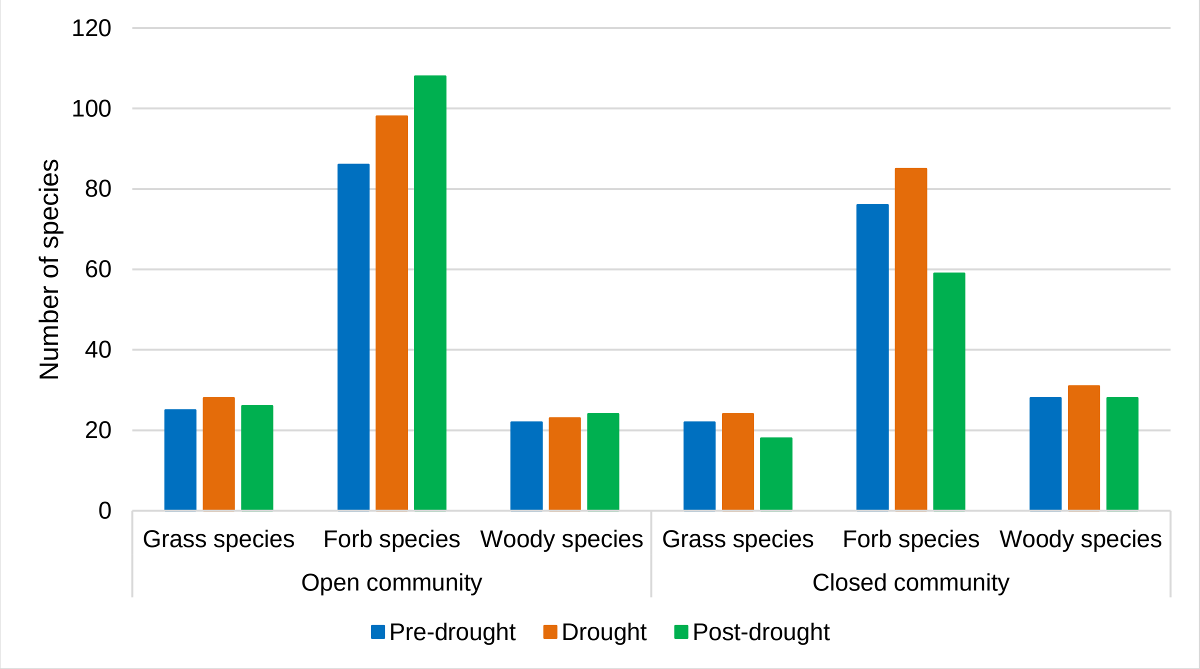
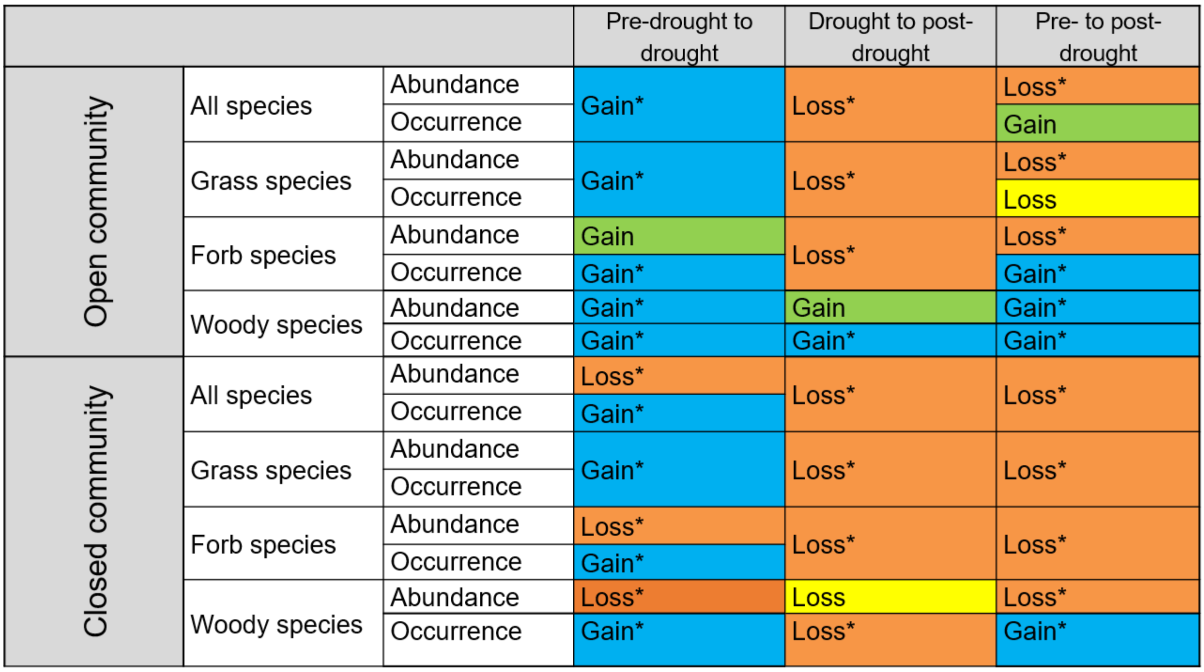


Figure 6-4: The number of grass, forb, and woody species observed in the three different rainfall years for the open and closed communities.

To further explore the changes in plant species composition in the two plant communities over time, TBI analyses were applied. Figure 6-5 provides the gain/loss statistics for each species group across rainfall years as a compilation of plot-level data. A gain means there was an overall gain in species abundance or occurrence for a specific plot (Figure 6-7 and Figure 6-8: red line above the green line). A loss refers to an overall loss of species abundance or occurrence for the sampled plots (Figure 6-7 and Figure 6-8: red line below the green line).

There was a gain in species occurrence for all species groups from the pre-drought to drought year, indicating possible resistance to drought (Figure 6-5). In the closed community there was however some loss of species abundance. The recovery from the drought was negative since there was only significant loss of species abundance and occurrence, meaning the communities were not able to recover after the drought. Only the forb species of the open community showed resilience to drought (Figure 6-4 and Figure 6-5). The B-C plots in Figure 6-7 and Figure 6-8 show these losses and gains in detail for all the species (See Supplement S1 for the B-C plots of the grass, forb and woody species).

The woody species showed resistance to the drought, except for the abundance of woody species in the closed community which experienced a significant loss (Figure 6-4 and Figure 6-5). The recovery from the drought was positive for the open community and negative for the closed community (Figure 6-5). Generally, the woody species showed resilience to drought, with only the abundance of woody species in the closed community not exhibiting resilience.



			Pre-drought to drought	Drought to post-drought	Pre- to post-drought
Open community	All species	Abundance	Gain*	Loss*	Loss*
		Occurrence			Gain
	Grass species	Abundance	Gain*	Loss*	Loss*
		Occurrence			Loss
	Forb species	Abundance	Gain	Loss*	Loss*
		Occurrence	Gain*		Gain*
	Woody species	Abundance	Gain*	Gain	Gain*
		Occurrence	Gain*	Gain*	Gain*
Closed community	All species	Abundance	Loss*	Loss*	Loss*
		Occurrence	Gain*		
	Grass species	Abundance	Gain*	Loss*	Loss*
		Occurrence			
	Forb species	Abundance	Loss*	Loss*	Loss*
		Occurrence	Gain*		
	Woody species	Abundance	Loss*	Loss	Loss*
		Occurrence	Gain*	Loss*	Gain*

Figure 6-5: A summary of the species abundance and occurrence gain/loss results obtained from the TBI analyses for the open and closed communities for all species observed, grass only, forbs only and woodies only. Asterisk * indicates whether the gain or loss was significant. Significant losses are shaded in orange, loss in yellow, gain in green, and significant gain in blue.

The B-C plots for all the species observed in the open and closed communities are presented in Figure 6-6 for the abundance and in Figure 6-7 for occurrence. For the abundance data (Figure 6-6), the open community showed more resistance to drought, while both communities were negatively affected by the legacy effect of the drought and neither community showed resilience. For the occurrence data (Figure 6-7), both communities showed resistance to drought, both communities were negatively affected by the legacy effect of drought, while the open community was slightly resilient to drought and the closed community not.

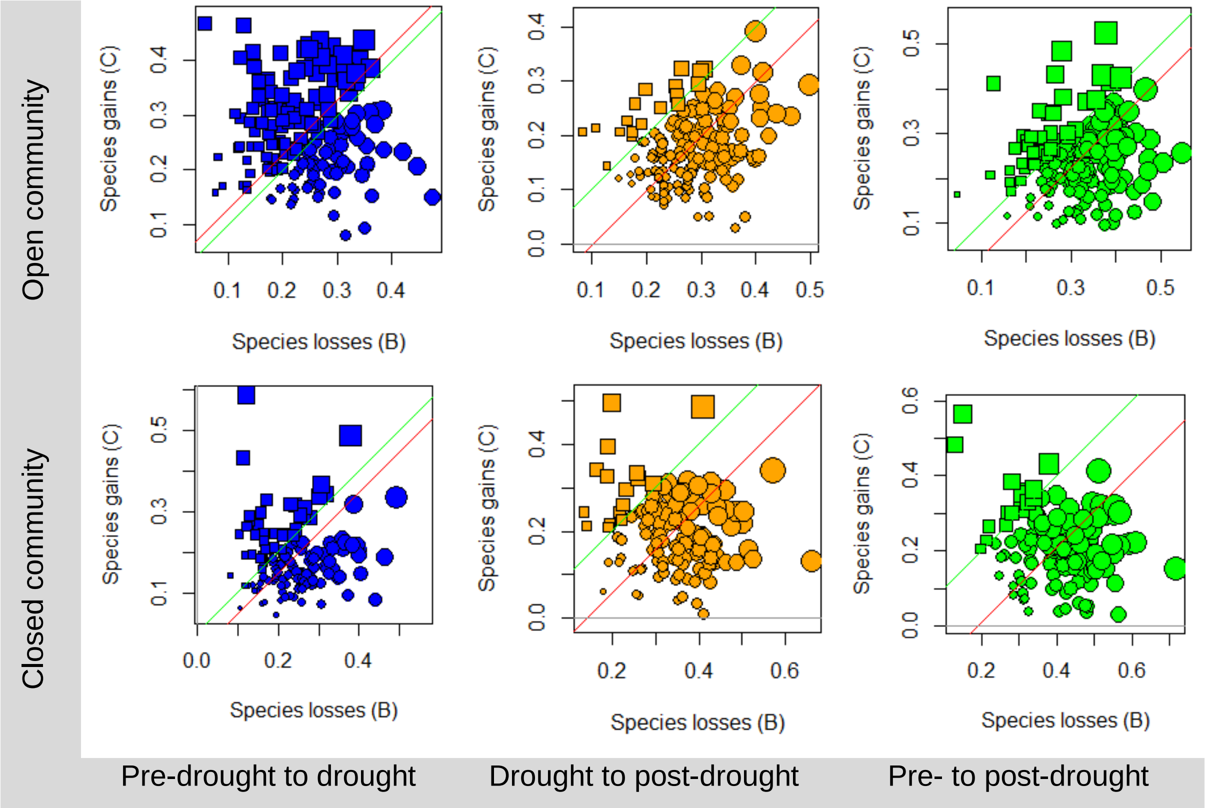


Figure 6-6: B-C plots of the plant species abundance in the two plant communities, namely open and closed across the three rainfall years, namely pre-drought to drought; drought to post-drought and pre- to post-drought. Symbol diameters are proportional to the TBI statistics. A gain means there was an overall gain in species abundance for a specific plot indicated with the red line above the green line. A loss refers to an overall loss of species abundance for the sampled plots indicated by a red line below the green line.

To shed light on which species are being gained or lost, further analyses were done on species of conservation value, i.e., endemic, threatened, and exotic species (Table 6-1). The open community displayed increases in South African endemic and threatened species while the closed community experienced decreases for both South African and WGG endemics as well as threatened species. The endemic and threatened species in the open community therefore displayed more resistance, while the exotic species in the closed community displayed resistance and resilience to drought. The WGG endemics of the open community, the WGG endemics, threatened species and exotic species of the closed community were positively affected by the drought legacy effect. The WGG endemics showed resilience in both communities, while the threatened species showed resilience in the open community and the exotic species in the closed community.

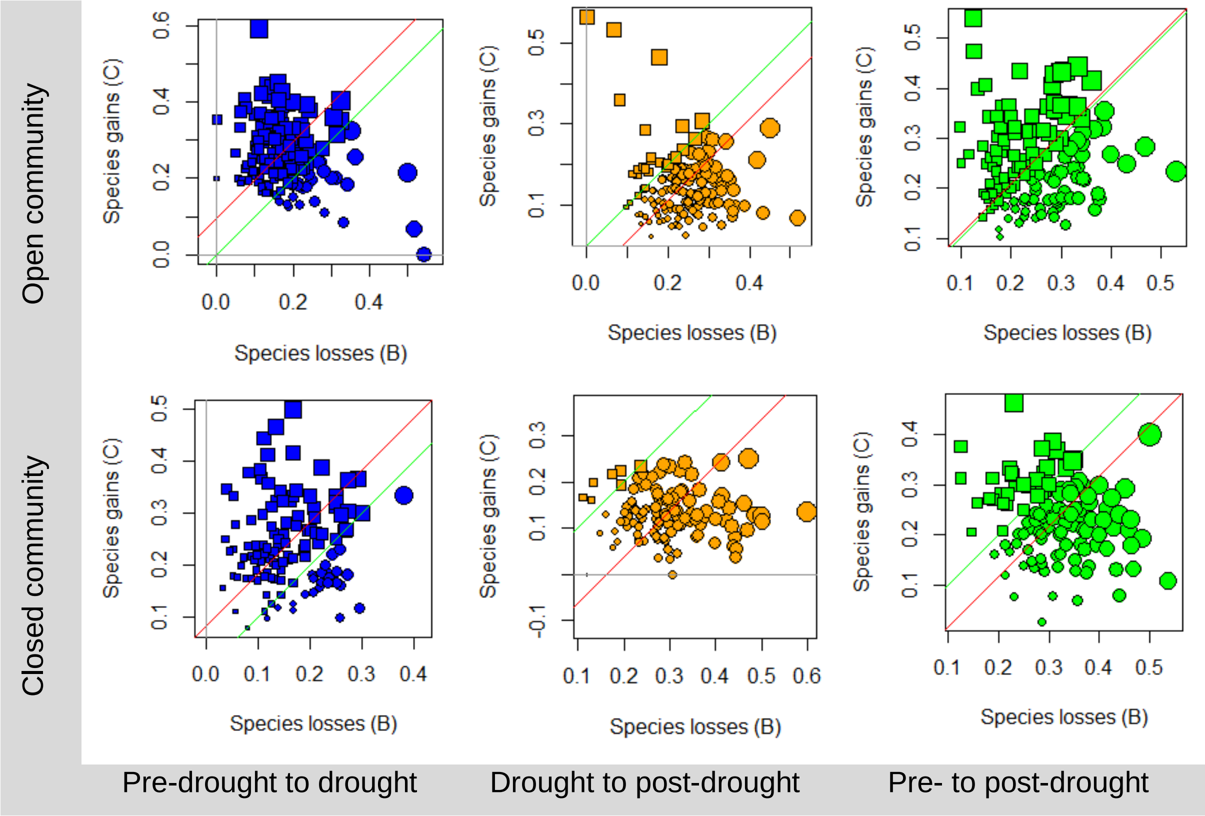


Figure 6-7: B-C plots for the plant species occurrence in the two plant communities, namely open and closed across the three rainfall years. Symbol diameters are proportional to the TBI statistics. A gain means there was an overall gain in species abundance for a specific plot indicated with the red line above the green line. A loss refers to an overall loss of species abundance for the sampled plots indicated by a red line below the green line.

Table 6-1: Percentage increase (+) or decrease (-) of endemic, threatened, and exotic species of the WGG across the two communities.

	Open community			**Closed community**		
	Pre-drought to drought	**Drought to post-drought**	**Pre- to post-drought**	**Pre-drought to drought**	**Drought to post-drought**	**Pre- to post-drought**
SA endemics	+18.0%	-20.0%	-1.9%	-2.1%	-32.9%	-34.7%
WGG endemics	0%	+16.7%	+16.7%	-2.5%	+7.8%	+5.3%
Threatened	+19.4%	-15.6%	+3.9%	-13.9%	+5.3%	-8.6%
Exotic species	-33.3%	-100%	-100%	+4.8%	+37.1%	+41.2%

To understand the effect of drought on the entire open and closed community, the compositional changes over the years were determined (Figure 6-8). The open community displayed some clustering for the pre-drought and post-drought communities, which would indicate lower resilience, but the resistance and recovery capacity seem high. The closed community displays high resistance, while the drought and post-drought communities seem to differ lowering the recovery capacity and pre- and post-drought communities seem to differ lowering resilience.

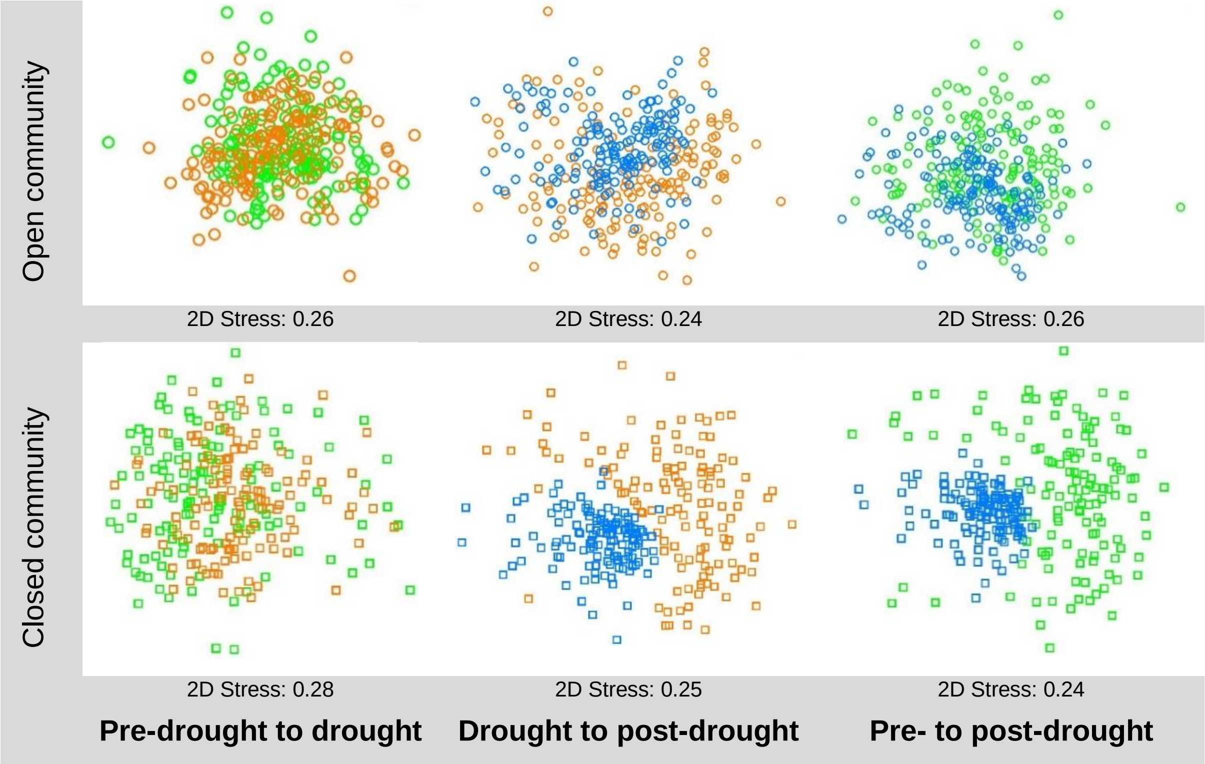


Figure 6-8: Non-Metric Multidimensional Scaling (NMDS) ordinations for the two communities, open and closed. Legend: ○ open community and □ closed community. Green: pre-drought, orange: drought, and blue: post-drought.

Species richness and diversity increased significantly in both community types in the drought year relative to pre-drought richness (Figure 6-9). Both communities showed resistance to change in species richness, evenness, and diversity since there were only significant increases. The open comm8nity was able to recover after the drought for all three diversity measures, while the closed community was unable to recover. The open community showed resilience to drought since the pre- and post-drought diversity measures were similar, while the closed community did not exhibit resilience to drought.

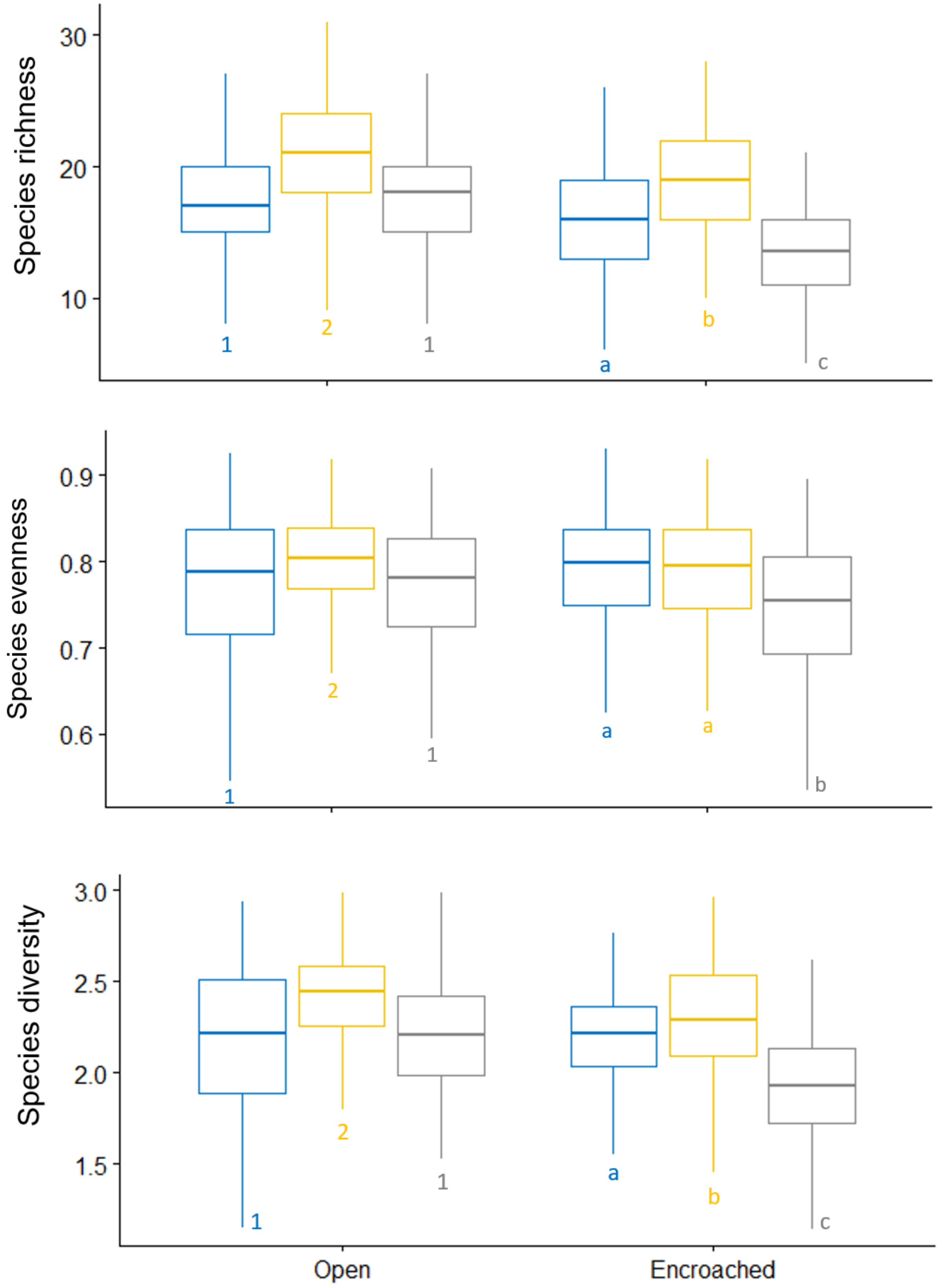


Figure 6-9: Mean (±SE) of plant species richness, evenness, and diversity over time (pre-drought, drought and post-drought) for the two community types (open and encroached) with outliers removed. The numbers and letters below the boxplot indicate the change within each community between rainfall years. Legend: Blue: pre-drought, yellow: drought, and grey: post-drought.

6.3.2 The spatial effect of drought on the floristic composition and diversity of an encroached site

To explore if there were compositional differences between the open and closed communities, the composition was compared for the pre-drought, drought, and post-drought years (Figure 6-10). In the pre-drought years, the two communities seemed to be quite distinct in terms of all the species, shown by two distinguishable clusters. The forb species also show relatively distinct clusters although there is more overlap. The woody species show the least amount of clustering, but the open community cluster is much tighter than the closed community. During the drought, there was only a relatively clear distinction when considering all the plant species and not for forbs and woody species alone. In the post-drought years, the two communities again seemed to be quite distinct in terms of all the species, shown by two distinguishable clusters. And the forb and woody species clustering was less distinct, and it appears that the two communities are more similar. These results should however be interpreted with caution since the stress values are high.

Species evenness remained similar for all rainfall years in the open and closed communities (Figure 6-11). Even so, the species diversity between the open and closed communities are different meaning a major contributing factor was the species richness. The species richness before the drought in both communities were similar (indicated with an ‘a’ Figure 6-11) and became significantly different at the onset of the drought and onwards. The species evenness when comparing the open and closed communities were only similar during the drought (indicated with a ‘c’; Figure 6-11). Regarding the Shannon species diversity, only during the drought was the diversity similar for both the open and closed communities (indicated with a ‘c’; Figure 6-11).

Before the drought, the open and closed communities had similar species richness, but the evenness and diversity differed significantly. During the drought the evenness and diversity between the open and closed communities were similar while the species richness was different. After the drought all the diversity measures were different between the two communities.

To understand if the encroached closed community is becoming more or less like the open community, the stem count and stem heights of the woody species were compared between the two communities (Figure 6-12 and Figure 6-13). The closed community had more stems than the open community in the pre-drought years but were similar for the drought and post-drought years (Figure 6-12). The stem height in the closed community was higher than in the open community for all rainfall years and they were significantly different between the two communities (Figure 6-13).

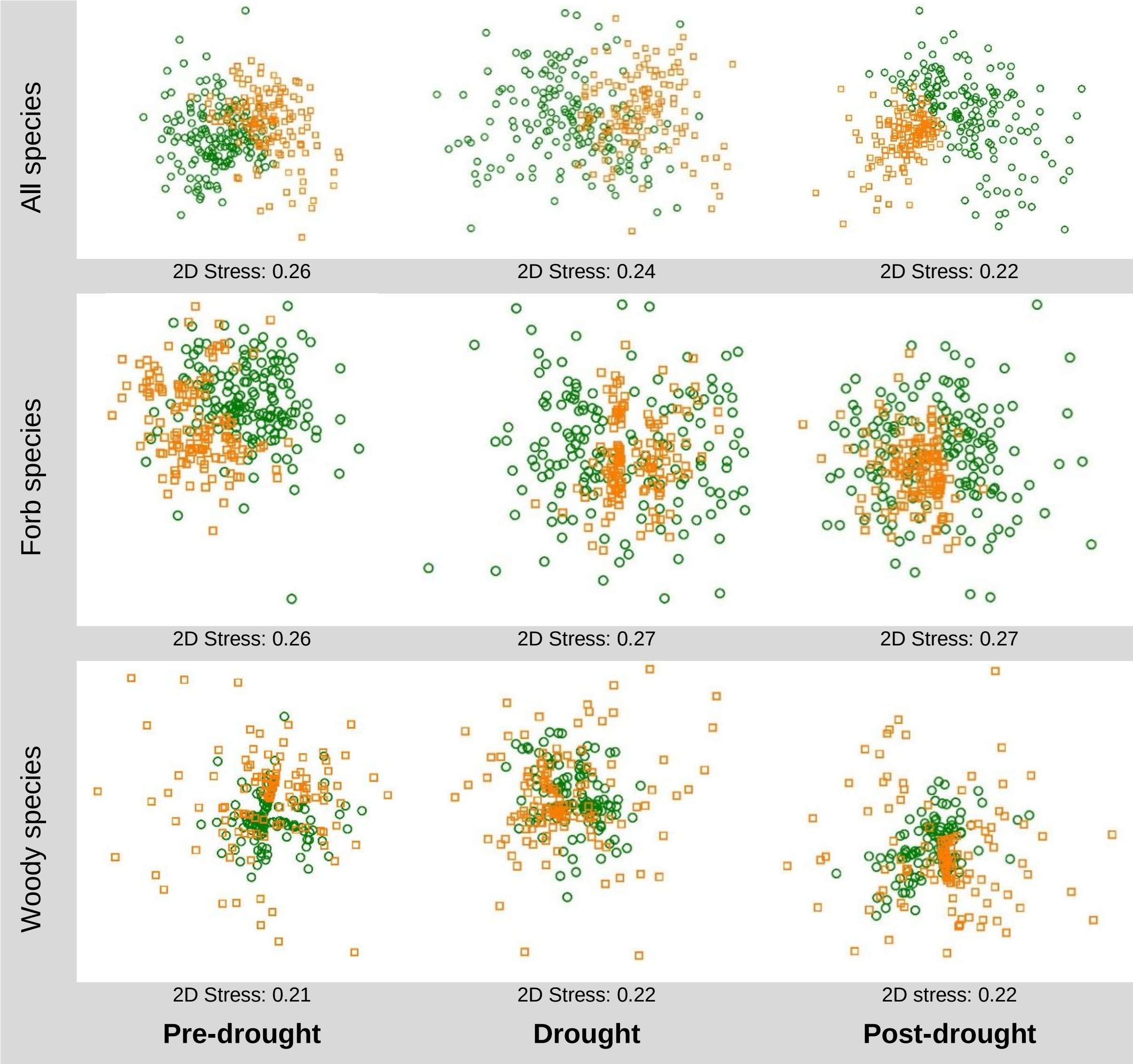


Figure 6-10: Non-Metric Multidimensional Scaling (NMDS) ordinations for the three rainfall years. Legend: ○ open community and □ closed community.

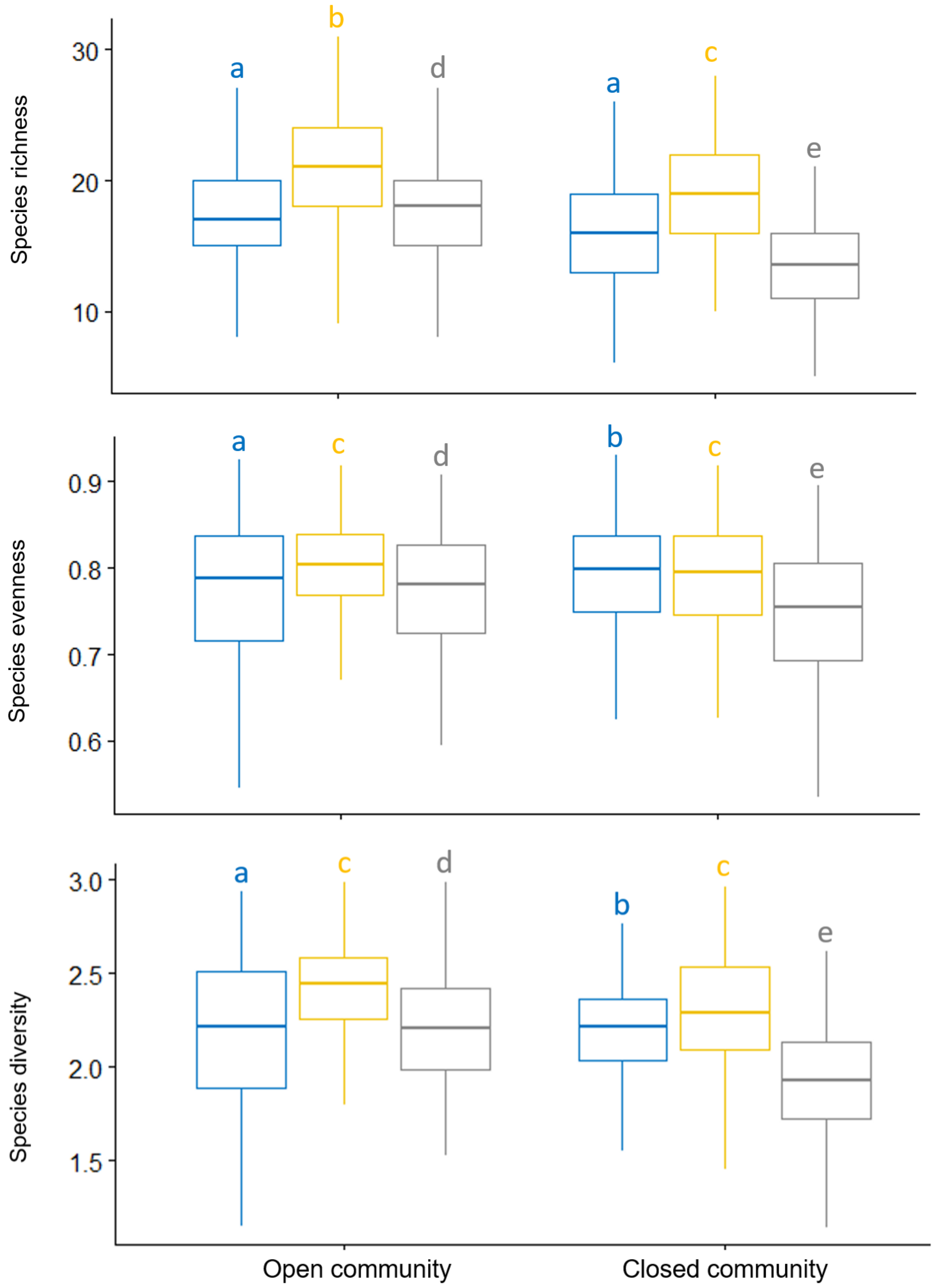


Figure 6-11: Mean (±SE) of plant species richness, evenness, and diversity across community types with outliers removed. The letters above the boxplots indicate the differences between the communities for the specific years – pre-drought letters must be compared to each other, drought to each other and the same goes for post-drought years. Legend: Blue: pre-drought, yellow: drought, and grey: post-drought.

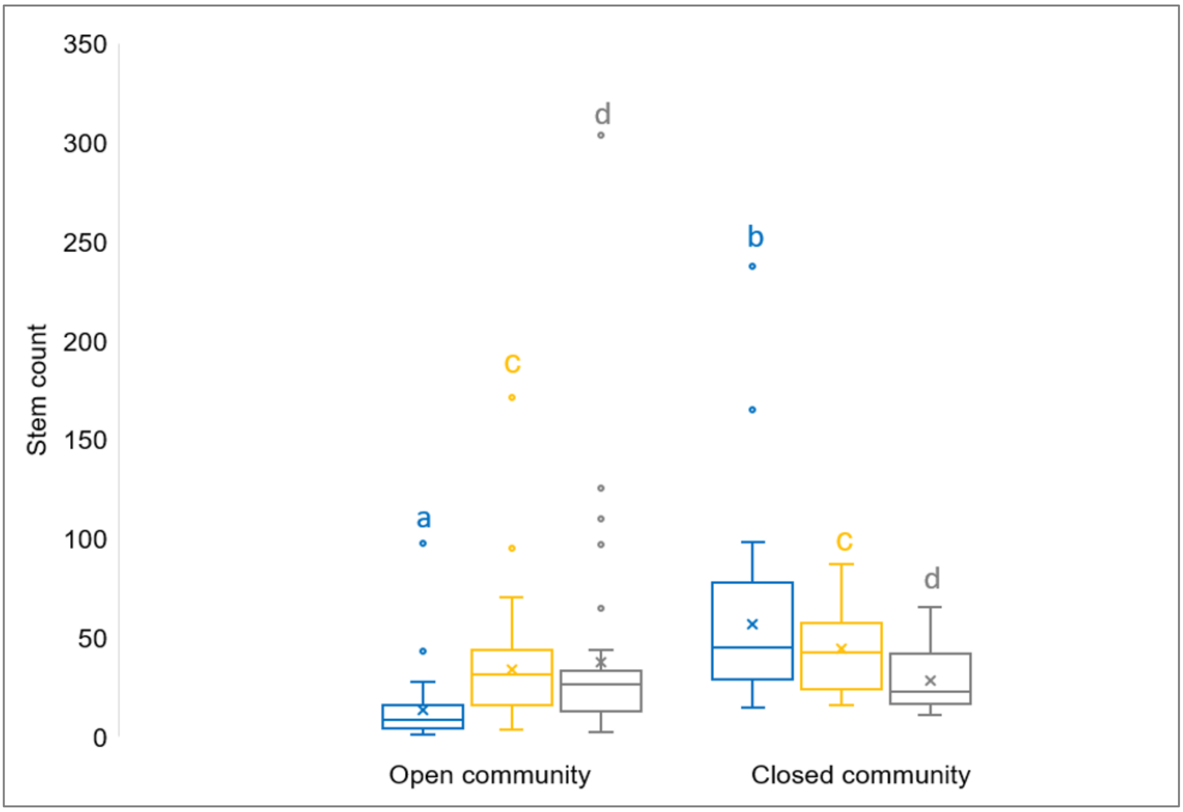


Figure 6-12: The stem count of the woody species of the open and closed communities. The letters above the boxplots indicate the differences between the communities for the specific years – pre-drought letters must be compared to one another, and the same goes for drought and post-drought years. Legend: Blue: pre-drought, yellow: drought, and grey: post-drought.

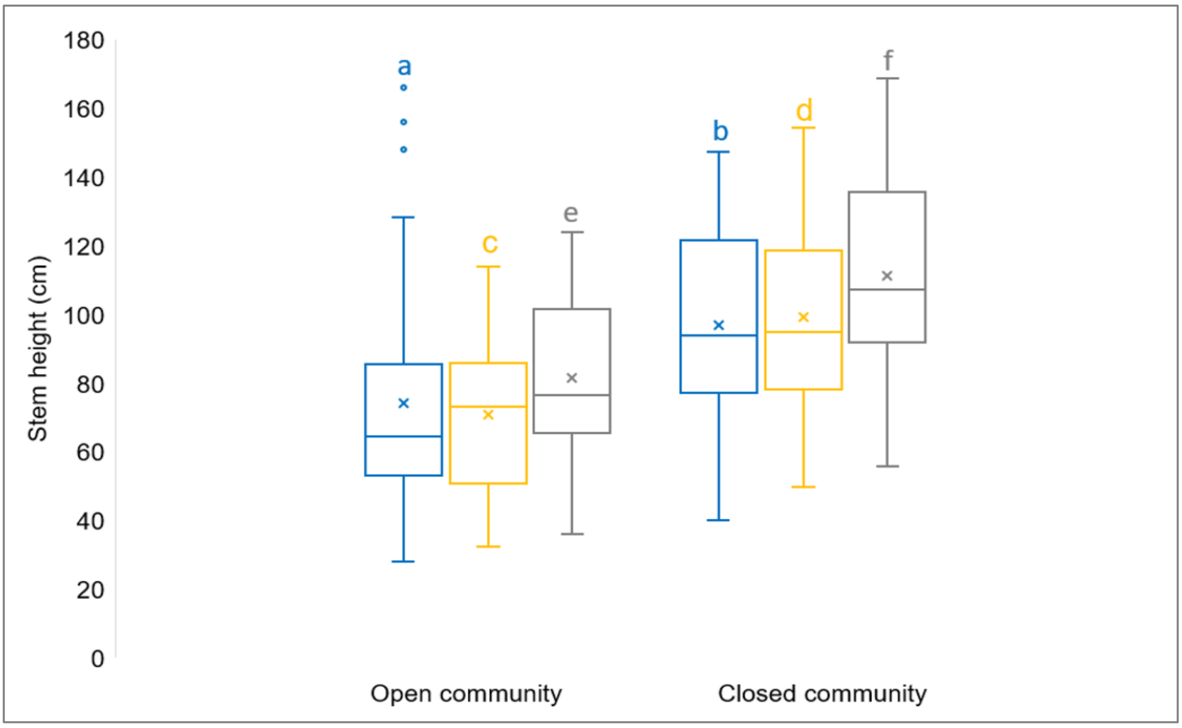


Figure 6-13: The stem height (cm) of the woody species of the open and closed communities. The letters indicate significance and non-significance. The letters above the boxplots indicate the differences between the communities for the specific years – pre-drought letters must be compared one another and the same goes for the drought and post-drought years. Legend: Blue: pre-drought, yellow: drought, and grey: post-drought.

6.4 Discussion

6.4.1 Grassland resistance, resilience, and recovery from drought

Both communities, but especially the open community, were resistant to drought. Grassland systems worldwide have been found to be resistant to severe water limitation, and up to four years in a drought experiment (Evans *et al.*, 2011; Mirzaei *et al.*, 2008; Morecroft *et al.*, 2004). These findings suggest that drought duration is as, or even more, important than drought severity in determining the response of vegetation to drought (Evans *et al.*, 2011). Therefore, it would be expected that if the drought was prolonged it would have had a bigger effect on the resistance of the system.

Different species exhibit different resistance to drought and mortality after drought (Stampfli *et al.*, 2018). The insurance hypothesis predicts that resistance and resilience should both increase with diversity (Yachi & Loreau, 1999). From this one would expect a more diverse community to have a bigger chance of housing species that will increase its performance and compensate for the loss of other species during the disturbance (Van Ruijven & Berendse, 2010). Generally, this was true about the open community, especially concerning its resistance to drought.

A possible explanation for the resistance of both communities to the drought could be that; there was enough water available in the soil to withstand the initial drought effects. It has been found that in mesic ecosystems, the soil water content is usually moderately high and any ambient rainfall (intermediate and small rain input) will keep the soil water levels above drought stress levels much of the time (Knapp *et al.*, 2008).

Both communities had similar species richness which explains the resistance. Additionally species richness affects resistance due to increasing productivity with species number, and not due to the number of species *per se* (Van Ruijven & Berendse, 2010). Long- versus short-lived life histories may explain resistance to change (Pimm, 1984). Under some circumstances, resistant systems, because they change less under a given disturbance, will appear more persistent and less variable (Pimm, 1984). One typically assumes that woody species have longer life histories, but many forb species have been found to persist for years with the aid of underground storage organs. These underground organs can survive for many years belowground, without the allocation of resources to new shoot formations (Fidelis *et al.*, 2014; Shefferson, 2009). Some forbs have been found that can produce large storage organs up to 40 cm deep (Zaloumis, 2013).

Higher drought resistance could result in quicker recovery post-drought, meaning the system has higher resilience (Hoover *et al.*, 2021). The forb species in the open community seemed to display resilience to drought (expressed as a gain in species occurrence; Figure 6-5), consistent with trends observed by Ratajczak *et al.* (2019). It has also been found that frequent disturbance in a grassland, both spatially and temporally, benefit forb species (Bråthen *et al.*, 2021). Forbs that contributed to this gain include *Acalypha peduncularis*, *Berkheya setifera*, *Helichrysum nudifolium* var. *oxyphyllum, H. platypterum* and *H. nudifolium* var. *nudifolium* and *Merwilla plumbea* that had the highest cover in the drought year. These forb species typically have belowground organs (Kremer-Köhne, 2021) which allows them to persist during disturbances.

Many forb species are shade-intolerant (Weigl & Knowles, 2014) and are therefore resource limited by taller vegetation (Bråthen *et al.*, 2021). This could also explain the resilience of forb species in the open community where there is less competition for resources in comparison to a loss in the closed community. There is also a positive relationship between increased light availability and species richness (Borer *et al.*, 2014). This could explain the higher species richness in the open community for the drought and post-drought years when compared to the closed community.

The open community's species richness, evenness and diversity was comparable pre- and post-drought, with all three measures increasing in the drought year. It has been found that ecosystems may resist change during a perturbation, or show resilience by returning to the state before the perturbation occurred (Van Ruijven & Berendse, 2010). The open community could therefore be described as resilient to drought as it displayed a gain in species abundance and occurrence and there was no loss of species richness, evenness or diversity. This suggests that the grassland is pre-adapted to rainfall variability and that the grassland experienced similar variation during its evolutionary history (Bartha *et al.*, 2022).

The WGG communities showed more resistance to drought and less resilience. Resistance may either correlate positively or negatively with resilience, depending on the nature of population growth and their sensitivity to the environment (Donohue *et al.*, 2013). Variability in ecological resistance and resilience may be due to differences in ecosystem attributes, the magnitude, duration, and timing of the climate extreme (Hoover *et al.*, 2014). Ecosystems might either resist change and/or show resilience by returning to the original state (Van Ruijven & Berendse, 2010) while a loss in resilience is often linked to a change in a system state (Gunderson, 2000). This further supports the hypothesis that the closed community could be transferring to an alternative state since it showed lower resilience.

When concerning the species of conservation value, the endemic and threatened species in the open community showed resistance to change, while they were negatively affected in the closed community. On the other hand, the exotic species in the closed community showed resistance to drought. The WGG endemics showed resilience in both communities. Grasslands, and thus the endemic species they house, have evolved in more variable and often drier climates, and biomass reducing disturbance regimes such as grazing and burning, therefore they likely possess traits that support rapid re-establishment and regrowth following a perturbation such as drought (Ingrisch *et al.*, 2018; Stampfli *et al.*, 2018).

The recovery after the drought was overall negative, except for a positive effect on woody species in the open community, since there was a loss of species abundance, occurrence, and richness. Other studies have reported a delayed drought recovery linked to the mortality of dominant species or drought-sensitive species (Sala *et al.*, 2012; Smith *et al.*, 2009). The legacy effect of drought slows the recovery of species richness (Tilman & El Haddi, 1992). In the six years preceding the drought, the recorded rainfall for the area was above the long-term average, with some years even being considered as ‘extremely wet’ according to the SPI (Box 6-2). This high rainfall before the drought could have created favourable conditions for the gain in species abundance and occurrence going into the drought (Adler & Levine, 2007). After the drought there was a significant loss in species abundance and occurrence across both communities. After the drought, the annual rainfall increased to ‘normal’ conditions according to the SPI. There were however some plant species such as *Setaria sphacelata*, *Berkheya setifera*, and *Hyparrhenia filipendula*, that were not able to recover to pre-drought levels. As water becomes limited, the intensity of competition between grasses and woody species increases, while dead grass facilitates seedling recruitment by increasing soil moisture content (De Dios *et al.*, 2014). It was observed that the woody species were more resilient to drought and was positively affected by the drought’s legacy effect in comparison with the grass species. Drought could have a positive effect on woody plant recruitment, as competing grasses are killed before rains return (Scholes & Archer, 1997). This might have been the case in the open community where there was a gain in woody species occurrence and abundance across all rainfall years.

A legacy effect of drought could also be observed in changes in soil moisture, especially in the shallow soil layers (Hoover *et al.*, 2021; Schwinning & Sala, 2004). These changes in the soil will ultimately affect the phenology and above-ground biomass of the vegetation (Hoover *et al.*, 2021). As a result fast recovery after a drought is likely if the soil moisture has been recharged after drought, since belowground plant organs are highly resistant to drought (VanderWeide *et al.*, 2014). Legacy effects of drought could even affect species differently, for example Hoover *et al.* (2021) found that two grass species had differential responses to drought legacies. This is reflected by the woody species that increased in abundance and occurrence in the open community but decreased in the closed community (Figure 6-5).

An important consideration when analysing drought legacy is that when a system recovers slowly from a past drought, it may reduce the resistance to future droughts, as reduced growth or mortality of species impact the starting baseline of the new drought event (Hoover *et al.*, 2021).

6.4.2 The spatial effect of drought on the floristic composition and diversity of an encroached site

The loss of abundance of species and not of occurrence in the closed community (pre-drought to drought) could be because the plants reduced their above-ground cover (abundance), and invested more energy and resources below-ground in their organs to be able to survive the harsh above-ground conditions (Klimešová *et al.*, 2017). It is therefore expected that the cover will recover over time to be what was experienced before the drought event. Up to 99% of graminoid and forb regrowth in grasslands stems from belowground buds (Benson & Hartnett, 2006; Russell *et al.*, 2019). Belowground bud banks enable plants to persist even if the aboveground parts have been removed (Reintal *et al.*, 2010). This enables belowground plant diversity to buffer biodiversity loss by slowing the process of species extinctions (Hiiesalu *et al.*, 2021).

It has been reported that plant community diversity and turnover of rare and uncommon species increase when there is an increase in the variability in precipitation (Knapp *et al.*, 2002). These species seemingly benefitted from the rainfall variability seen between 2009-2020, allowing them to persist in both communities. If drought conditions continue to persist, there may however be a shift to more drought resistant species that are able to tolerate drier and warmer climates at the expense of grassland specialists (Gottfried *et al.*, 2012). Even though there were many losses in species abundance and occurrence after the drought event, there was not a huge influx of exotic species that tend to colonise areas quickly after a disturbance (Pysek *et al.*, 1995).

The sampling sites in the HNR are heterogenous across space and time (Figure 6-10). Ecological models have found that an ecosystem that is diverse and heterogenous, is more ecologically resilient to environmental stress, as was the case for the open community. This is possible because it has higher potential to absorb disturbance without major change to properties and processes of the ecosystem (Virah-Sawmy *et al.*, 2009).

A microclimate warming effect together with the global climate warming, could explain the co-occurrence of heterogenetic plant communities (D'Odorico *et al.*, 2012). Community composition has been found to respond to increasing variation in rainfall (Knapp *et al.*, 2002). In mesic grasslands, species richness may be buffered to interannual rainfall variability due to having long-lived, bud-banks reducing variation in composition over years (Cleland *et al.*, 2013). Belowground bud-banks are generally reflective of the present community, and play a role in stabilising community composition in response to rainfall variation, in addition to their role as a reservoir of potential vegetative regrowth, allowing communities to recover quickly following disturbances (Cleland *et al.*, 2013; Klimešová & Klimes, 2007). This may explain why the NMDS results were not significant.

Plant species richness was significantly affected by rainfall year in all plant communities. The species richness was this highest in the drought year for both communities. There was an increase in the forb and woody species in the drought year which continued after the drought. This result resonates with reports that forbs are persistent during dry conditions in savannas (Kallah *et al.*, 2000) and in tallgrass prairie, since they avoid drought with deep rooting profiles (Nippert & Knapp, 2007). However, in a grassland drought experiment, forbs were found to be the most stress sensitive species and the community shifted to a more grass-dominated state (Reynaert *et al.*, 2021). This was attributed to a reduction in water availability, which invoked more physiological stress and ultimately mortality. Furthermore, broadleaved forb species were found to suffer more in a drought than grass species since the anatomy of the broadleaved species could have caused excess heat stress: they capture more midday radiation and dissipate the heat more slowly than graminoids that have narrow and more vertical leaves (De Boeck *et al.*, 2016). There is also the possibility that the abundance of dominant perennial grass species reduced during the drought, releasing more resources to the forb species in the following wet year, assuming forbs have superior dormancy (Levine & Rees, 2004). In this study, the community did not shift to a grass-dominated state, as the number of grass species remained relatively heterogenous, and slightly decreased after the drought.

There was an increase in species richness from the pre-drought to drought years in both communities, followed by a decrease in the post-drought year. This could be due to the fact that more species-rich communities generally have higher water demands, while lower water availability prevails in drought conditions, resulting in higher senescence (Elst *et al.*, 2017; Van Peer *et al.*, 2004). Species richness has furthermore been found to recover extremely slowly following an extreme drought (Tilman & El Haddi, 1992). Alternatively it has been suggested that with higher species richness there is less chance of system stability (Pimm, 1984), which could explain the decrease of species richness from the drought to the post-drought years in both communities. It has also been suggested that dominant species maintain stability at the cost of species richness (Evans *et al.*, 2011; Sasaki & Lauenroth, 2011)

Species evenness remained largely unaffected in both communities. This could be attributed to the fact that forbs have below-ground storage organs that allow them to resprout after above-ground disturbances (Bond & Parr, 2010; Uys, 2006). These traits likely aided the increase in floristic richness during the drought season, also improving the resilience of grassland forbs to adapt, tolerate, persist and continue recruitment during environmental changes caused by rainfall variability (Uys, 2006).

A possible explanation for the lower species richness, evenness, and diversity in post-drought conditions (especially in the closed community) and loss of species abundance and occurrence could be that the species have not been lost, but they are in the soil in a dormant state. Plants will remain in a dormant state until the return of more ‘normal’ climatic conditions (Tilman & El Haddi, 1992). Another possibility is that species extinctions occurred caused by the drought thus causing species loss (Tilman & El Haddi, 1992). The rainfall in the post-drought conditions were still below-average meaning plant diversity could not yet return to pre-drought diversity. It has also been found that lagged effects from a drought year were equally or more important than precipitation in the time following a drought (Jonas *et al.*, 2015).

In the case of most of the diversity measures, the greatest response was observed in drought years, including increase in species richness, evenness, and Shannon diversity. Knapp *et al.* (2002) similarly found that responses to rainfall variability were greatest in dry years, which suggests that greater impacts on vegetation will occur when drought occurs or in more arid systems. This could be attributed to the formation of gaps during drought, while the consequent empty niches would give other species the opportunity to establish (Evans *et al.*, 2011; Liao *et al.*, 2015; Reynaert *et al.*, 2021; Winkler *et al.*, 2019). It has also been found that plants can shift the allocation of assimilated carbohydrates to roots to maximise water capture during a drought (Hasibeder *et al.*, 2015). Plants are able in this way to invest in root length without necessarily having to change root biomass, thereby improving the overall efficiency of the root system (Frank, 2007; Ma *et al.*, 2020). These findings confirm the importance of recognising that most grasslands have multiple drivers of diversity and not only rainfall variability (Chesson, 2000). Because grasslands cover 31-43% of terrestrial land cover, their responses to changing rainfall patterns may have important consequences for global patterns of productivity and diversity under future climate scenarios (Fay *et al.*, 2003).

The overall increase observed of the occurrence of woody species in the open and closed communities could be attributed to the fact that woody species might be able to adapt to drier conditions. It has been found by Yang *et al.* (2020) that shrub species have the potential to adapt to drying environments as the soil water content increased in the deep soil layer where generally only woody species can access it. The soil clay content changed which allowed water to penetrate deeper into the soil layer (Yang *et al.*, 2020). The drought is however not seen as the driver of the increase in woody species, since this increase has been observed long before the drought occurred. A possible driver of increased woody species dominance and/or higher biomass is the leaf-level physiological response to CO_2 concentration and temperature increase that favours species with a C_3 photosynthetic pathway over C_4 grass species (Higgins & Scheiter, 2012). C_3 species have been reported to mitigate the adverse effects of drought on plant growth by making use of conditions such as early season high soil water availability and low air temperature, including winter precipitation (Knapp *et al.*, 2020; Smoliak, 1986). It is therefore predicted that as droughts increase in future, there would be a decrease in C_4 grassland cover and increases in C_3 abundance (Knapp *et al.*, 2020).

The number of stems of the woody species differed significantly between the open and closed community in the pre-drought years, with the open community having less. The number of woody stems in the open community increased over time as to be like that of the closed community in the drought year. After the drought both communities' woody stem counts reduced but were still similar. It could therefore be said that the closed community did become more like the open community in terms of number of woody stems.

In terms of stem height, there were only significant differences between the communities. As would be expected from tall vegetation, the stem height increases over time. The drought did not seem to make an impact on the height of the woody species, it might only have slowed it down somewhat. A possible reason for the closed community having taller woody species, could be the age of the individuals. The closed community has been encroached for several years, giving it a time advantage in terms of height.

In the closed community there was a decrease in the number of stems from pre- to post-drought conditions and in the open community from drought to post-drought conditions, while there was an increase in stem height. It might be necessary for the woody species to invest in height rather than stems to be better able to compete for light. It has been reported that mean species height increases with heightened competition for light (Liancourt *et al.*, 2009; Weiher *et al.*, 1999). As woody species reach their upper size limit for an area with a specific soil texture, depth, topographic setting, etc., their ability to effectively transport xylem water may be put at risk and lead to an increased possibility of branch or canopy mortality (Archer *et al.*, 2017; Browning *et al.*, 2014). This could explain why there was a reduction in the number of stems as the stem heights increased. Furthermore, the loss of branches or canopy would increase the probability of new species being recruited and establishing (Archer *et al.*, 2017; Browning *et al.*, 2014). This could then explain why there was a gain in the occurrence of species (pre- to post-drought) but a loss in species abundance for the closed community. For there to be an increase in the number of species, the abundance of the species should decrease.

6.4.3 Implications for grassland ecosystems in the light of climate change

Global change will not affect all ecosystems the same, temperature and precipitation changes will vary across regions, and responses will depend on the ecosystem’s sensitivity to climate change (Evans *et al.*, 2011). Reduced vegetation cover and compositional changes are common responses to global change, however, the time period over which they occur and the reaction to different drought severities vary among sites (Evans *et al.*, 2011).

In mesic ecosystems, the soil water content is usually moderately full and any ambient rainfall (intermediate and small rain input) will keep the soil water levels above drought stress levels much of the time, thus maintaining most ecosystem processes in an unstressed state (Knapp *et al.*, 2008). Larger drought events are expected to cause more fluctuations in soil water content, leading to prolonged dry periods and an increase in the duration and occurrence of drought stress (Knapp *et al.*, 2008; Porporato *et al.*, 2006). In these conditions, mesic ecosystems would likely experience more frequent and longer periods of plant and soil water stress (Knapp *et al.*, 2008). Frequent drying of the upper soil layers would have a negative effect on shallow-rooted herbaceous species that are adapted to smaller and more regular rainfall events (Knapp *et al.*, 2008; Schwinning & Ehleringer, 2001).

It is also important to consider temperature changes in addition to changes in precipitation (Jonas *et al.*, 2015). Species-level productivity and demographic processes can be significantly impacted by temperature (Alward *et al.*, 1999; Epstein *et al.*, 1998; Williams *et al.*, 2007). Plant assemblage metrics may also be more sensitive to temperature rather than precipitation (Jonas *et al.*, 2015).

When systems surpass extreme response thresholds, which are reached when the tolerance of one or more species in a community is surpassed, extreme ecological responses are projected to occur (Kardol *et al.*, 2012; Smith, 2011). Due to decreased growth, reproduction, and/or mortality of individuals, this would cause a considerable decrease in a species' abundance. As a result, the composition of the plant community would change because of species reordering. (Hoover *et al.*, 2014).

6.5 Summary

This chapter aimed to determine the effect of drought on ecosystem stability, composition, and diversity of an encroached grassland. Even though the composition changed across rainfall years and across community types, the results indicate that most of these compositional changes are due to the system being heterogenous, which would likely benefit the WGG in being resilient to disturbances. Compositional changes observed between communities could be ascribed to higher forb species diversity in the open community and higher woody species diversity in the closed community.

Encroached grasslands are somewhat less resistant and resilient to drought than an un-encroached grassland. Encroached grasslands could therefore be seen to be more vulnerable to drought, but the results are not definitive. Extreme drought does not necessarily mean that there will be extremity in the ecological response. There might be other factors affecting the resistance and resilience of the communities to drought. While the open community was somewhat resilient to the short-term drought, drought of extended duration would likely reduce the resilience significantly (Hoover *et al.*, 2014).

The woody species were overall positively affected by drought as they showed resistance and resilience and were positively affected by the drought's legacy effect. The number of stems did however decrease but stem height increased.

The open and closed communities remained significantly different in terms of species richness, evenness, and diversity from each other after the drought. During the drought the two communities were more similar. Furthermore, species richness, evenness, and diversity did not decrease during the drought year. For most of the diversity measures the drought year had the highest diversity. Only in the open community did the diversity measures return to pre-drought diversity after the drought. The diversity in the closed community was less than that of the open community for all diversity measures, for different rainfall years.

The open community experienced less species loss in terms of both abundance and occurrence measurements. The closed community gained woody species from pre-drought to the drought, and overall, from pre- to post-drought, which was to be expected at a site with a comparatively higher component of woody species. Before the onset of the drought, the species richness of the open and closed sites was similar. This changed with the onset of the drought and onwards, and reduced diversity was measured in the closed community after the drought.

The gain in woody species abundance and occurrence in the open community, irrespective of the drought, is worrisome. Therefore, it is important to continue to monitor the spread and increase in cover of long-lived forb and woody species by implementing burn programmes in such a way as not to cause shrub encroachment. Understanding how grasslands respond to climate variability is critical, since it is expected that droughts will be a more common occurrence in the future.

It was anticipated that the drought event would trigger a substantial diversity loss, which was not supported by the results obtained in the studied WGG system. It is important to note that the effects of rainfall variability on long-term diversity patterns of grasslands vary with location and management (Jonas *et al.*, 2015).

6.6 Supporting information

Supplement S1: Temporal beta-diversity

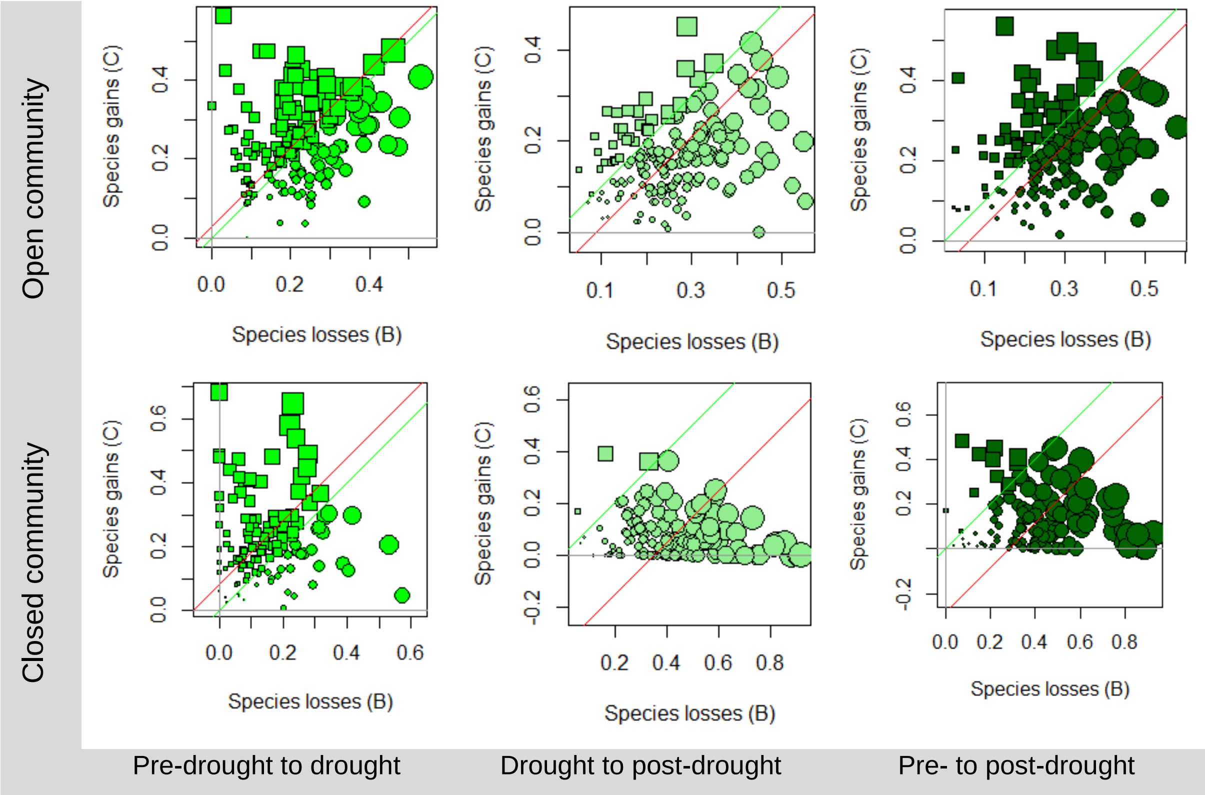


Figure 6-14: B-C plots of grass abundance in the open and closed communities across the three rainfall years.

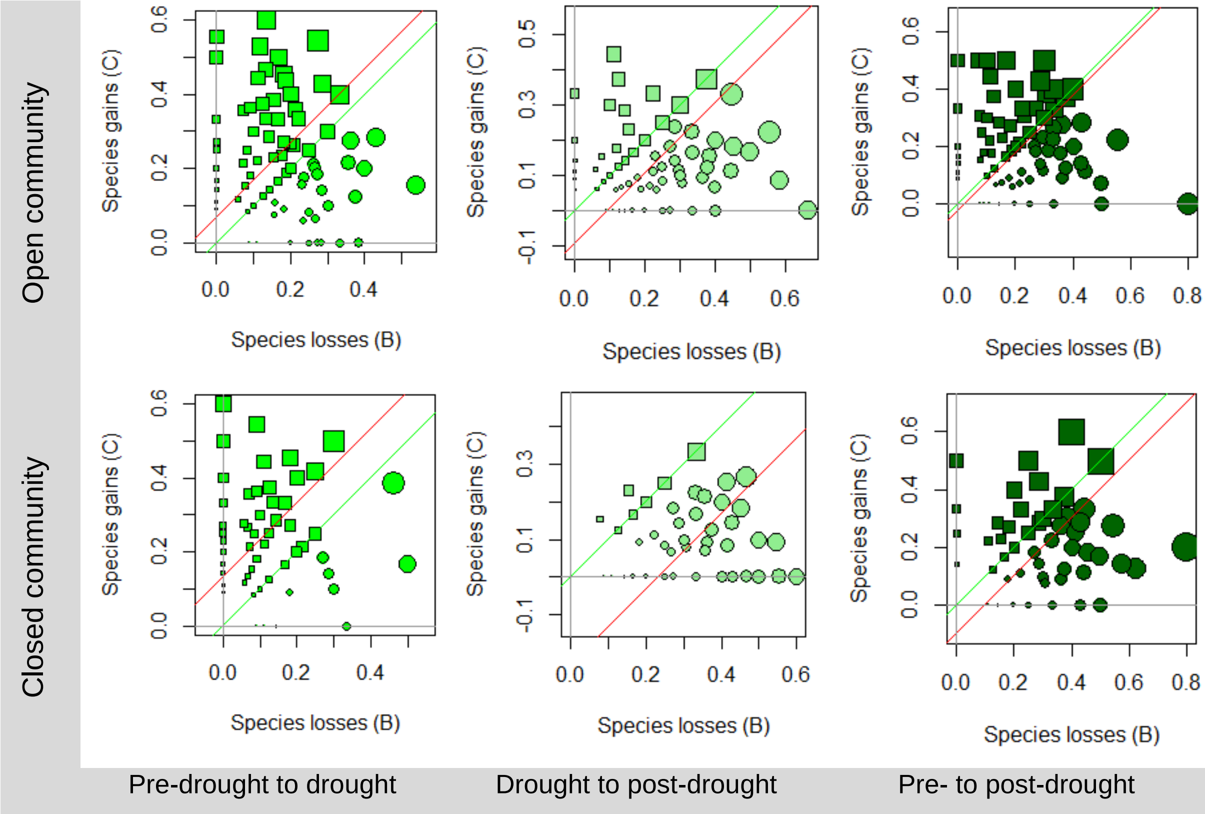


Figure 6-15: B-C plots of grass occurrence in the open and closed communities across the three rainfall years.

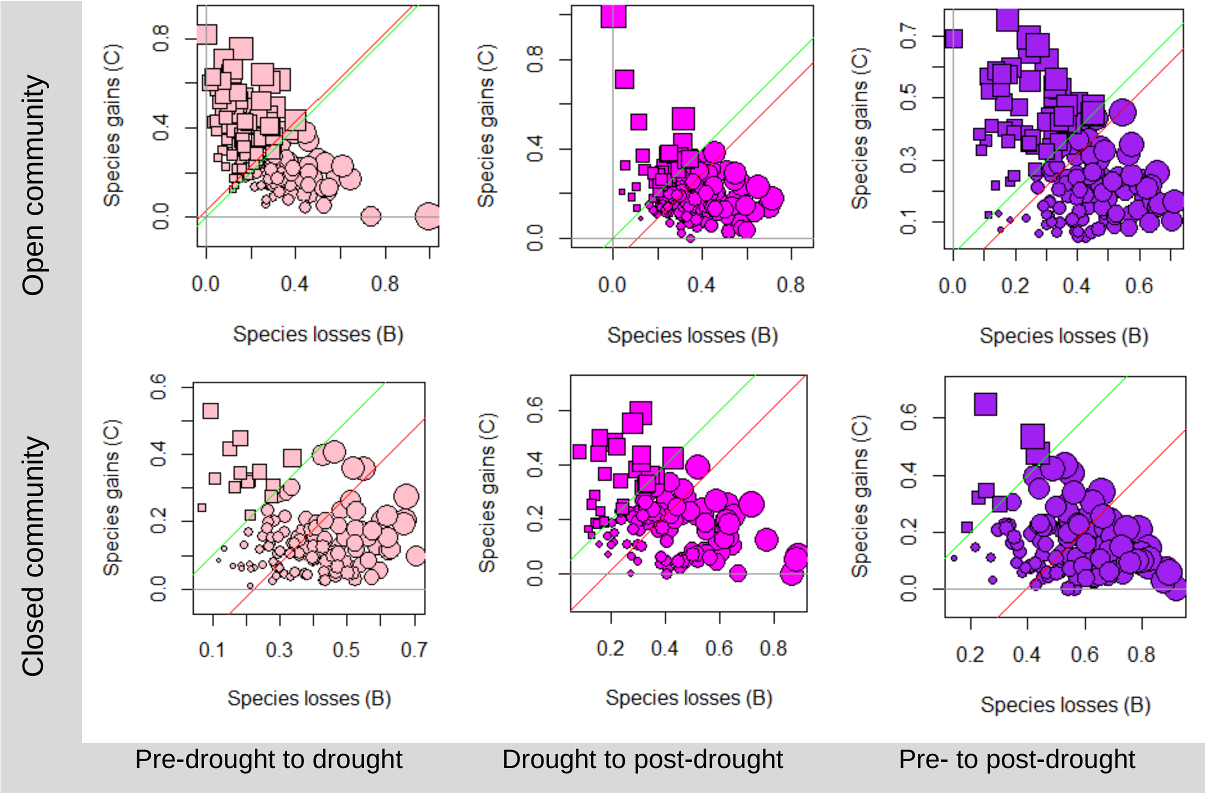


Figure 6-16: B-C plots of forb abundance in the two plant communities, namely the open and closed ones across the three rainfall years.

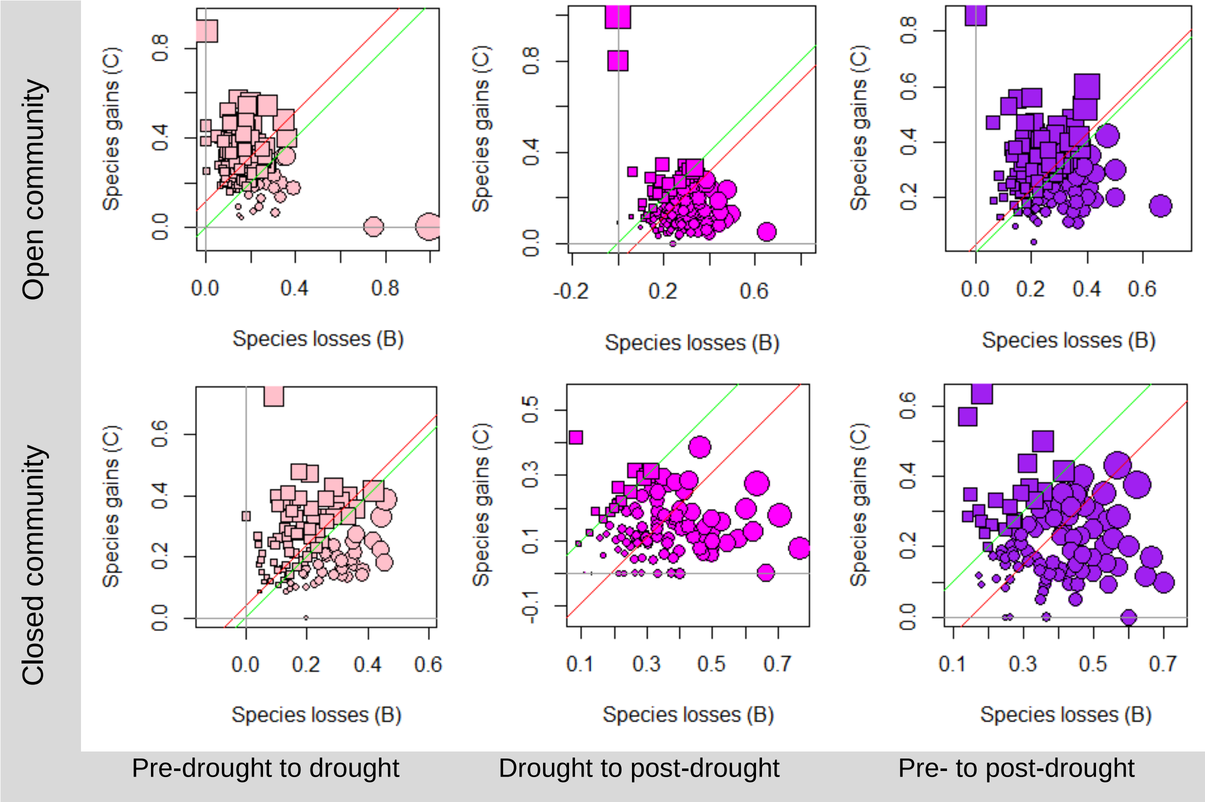


Figure 6-17: B-C plots of forb occurrence in the two plant communities, namely the open and closed ones, across the three rainfall years.

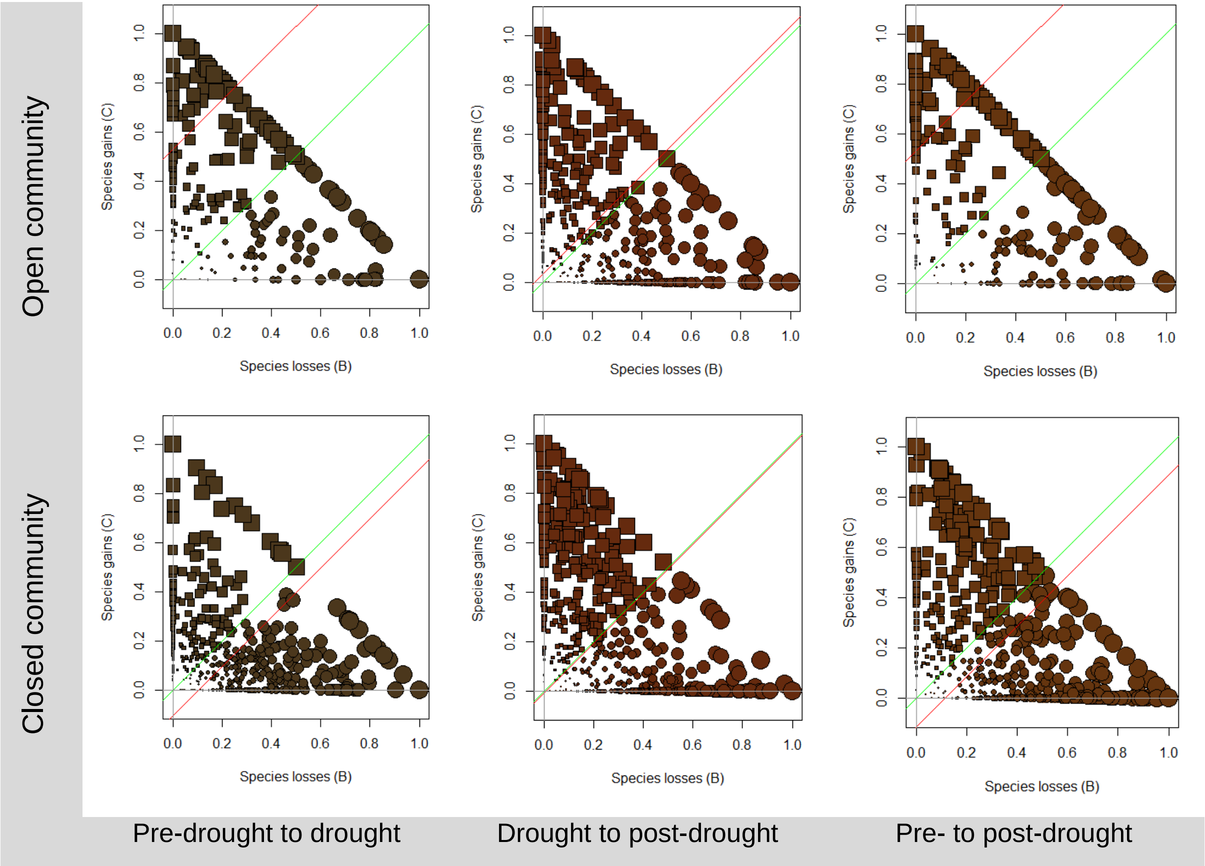


Figure 6-18: B-C plots of the long-lived forb and woody species abundance in the two plant communities for the entire plot (16 1x1 m subplots per plot) across the rainfall years.

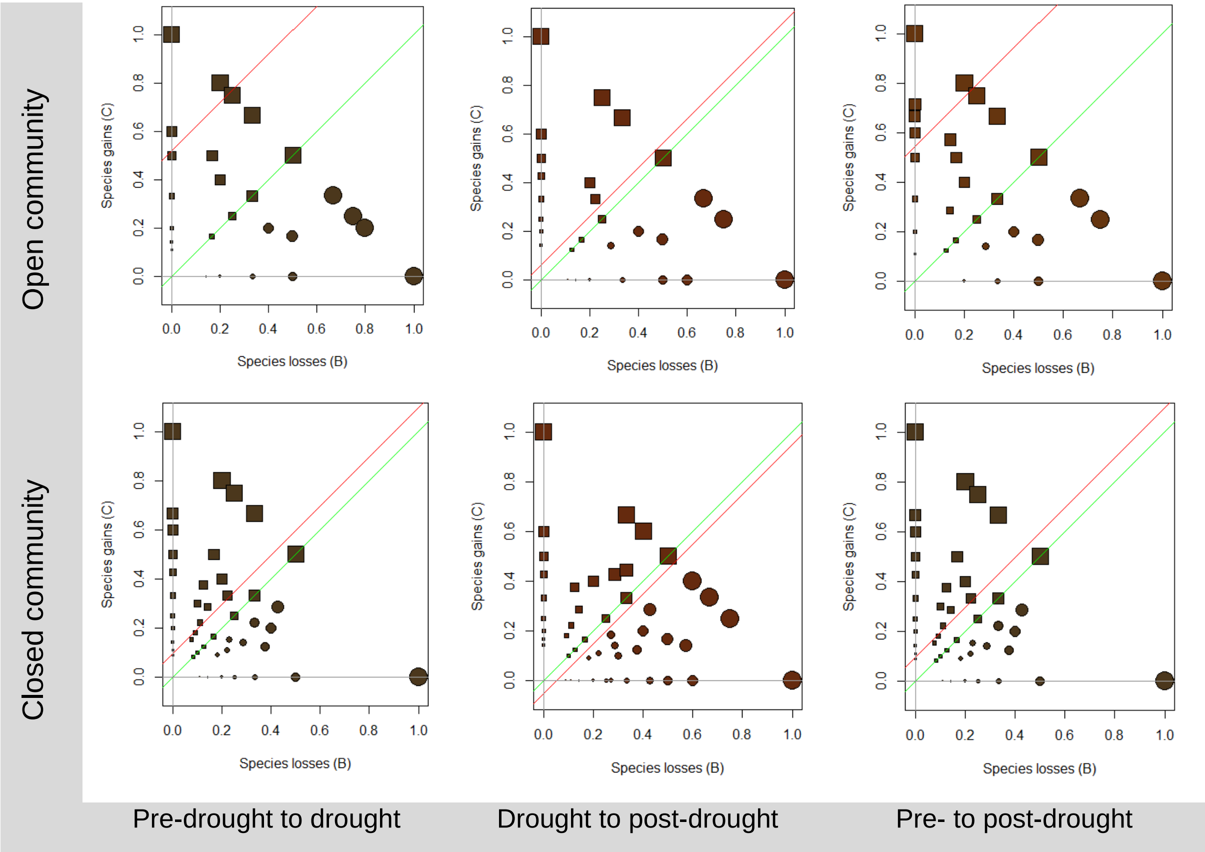


Figure 6-19: B-C plots of the long-lived forb and woody species occurrence in the two plant communities for the entire plot (16 1x1 m subplots per plot) across the rainfall years.

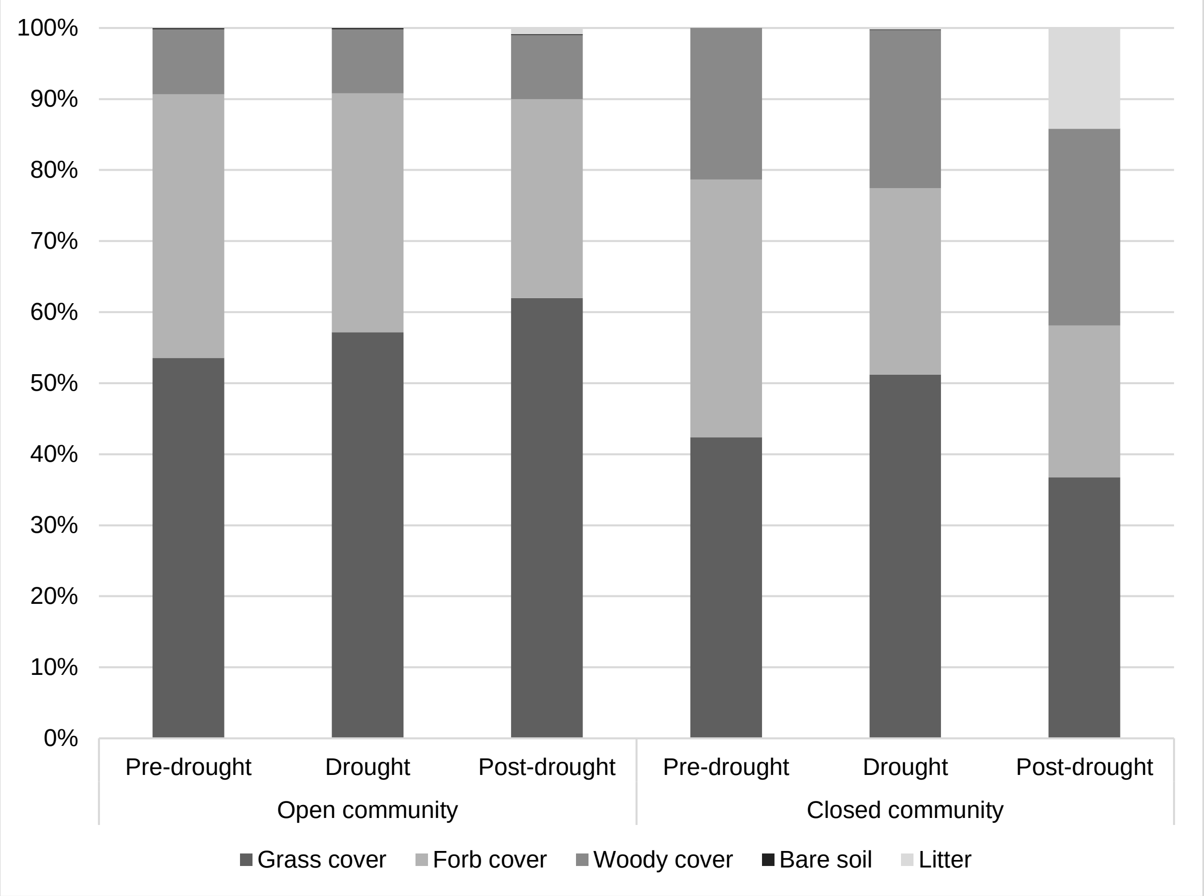


Figure 6-20: The percentage cover of grass, forb, woody species, bare soil, and litter for the open and closed communities of the HNR.

PERMANOVA results revealed significant effects of community type and rainfall year on the species composition (Table 6-2). There were groups for which the PERMDISP test, after transformation, still had a significant p-value. The PERMANOVA results for these groups should therefore be interpreted with caution.

Table 6-2: PERMDISP and Permutational multivariate analysis of variance (PERMANOVA) results for comparisons between rainfall years. Asterisk denotes significant values (significance set at p ≤ 0.05).

	PERMDISP	PERMANOVA	
	***p*-value**	***F*-value**	***p*-value**
Open community	0,001*	10,489	0,001*
Grasses	<0,05*	12,788	0,001*
Forbs	0,001*	11,267	0,001*
Woody species	<0,05*	1,914	<0,05*
Closed community	0,001*	21,672	0,001*
Grasses	0,001*	22,758	0,001*
Forbs	0,001*	20,96	0,001*
Woody species	>0,05	2,258	0,004*

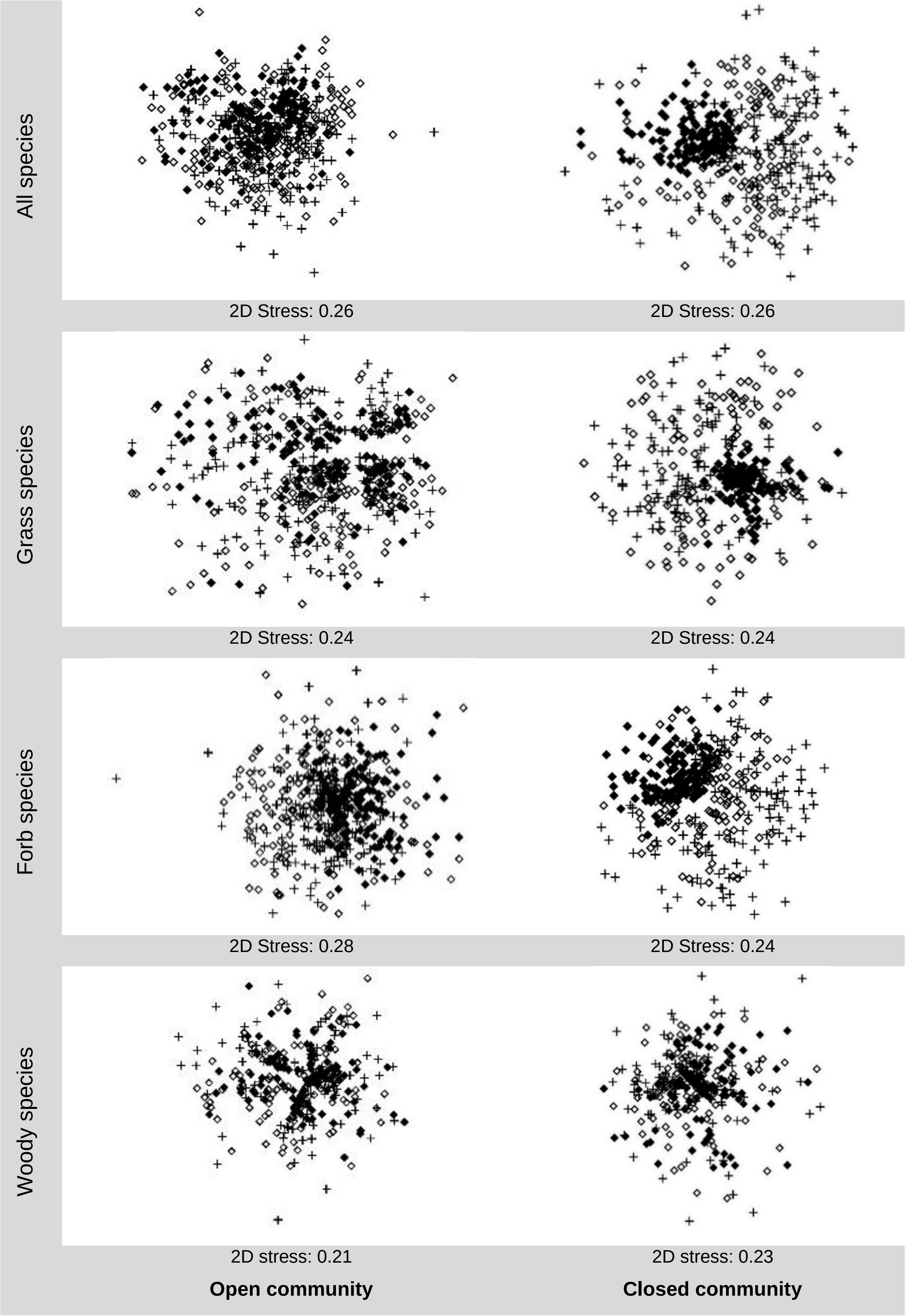


Figure 6-21: Non-Metric Multidimensional Scaling (NMDS) ordinations for the two communities, open and closed. Legend: + pre-drought; ◇ drought; and ◆ post-drought.

Table 6-3: PERMDISP and Permutational multivariate analysis of variance (PERMANOVA) results for comparisons between community types. Asterisk denotes significant values (significance set at p ≤ 0.05).

	PERMDISP	**PERMANOVA**	
	***p*-value**	***F*-value**	***p*-value**
Pre-drought	>0,05	21,036	0,001*
Grasses	<0,01*	21,883	0,001*
Forbs	>0,05	25,168	0,001*
Woody species	0,001*	10,703	0,001*
Drought	<0,05*	20,429	0,001*
Grasses	<0,05*	27,99	0,001*
Forbs	0,001*	16,097	0,001*
Woody species	0,001*	11,092	0,001*
Post-drought	0,001*	32,903	0,001*
Grasses	0,001*	21,883	0,001*
Forbs	0,001*	15,299	0,001*
Woody species	0,001*	9,682	0,001*

Table 6-4: Similarity percentage analysis (SIMPER) of species contributing >1% to compositional differences between pre-drought, drought, and post-drought years in the open community. Species are listed according to the contribution % from highest to lowest. Bold values indicate the highest mean abundance. Distance/similarity measure: Bray-Curtis.

Species	Life form	Plant family	Av. Dis.	Cont. %	Cum. %	Mean cover		
						Pre-drought	Drought	Post-drought
Cymbopogon nardus	Grass	Poaceae	7,8	10,9	10,87	30,8	30,3	**36,8**
Loudetia simplex	Grass	Poaceae	6,8	9,5	20,39	22,3	**22,8**	21,2
Themeda triandra	Grass	Poaceae	5,1	7,1	27,47	13,2	**20,9**	8,08
Setaria sphacelata	Grass	Poaceae	4,2	5,9	33,37	**16,5**	12,9	4,42
Berkheya setifera	Forb	Asteraceae	2,7	3,8	37,19	**9,59**	9,01	7,71
Acalypha peduncularis	Forb	Euphorbiaceae	2,3	3,2	43,66	**9,32**	5,27	5,42
Tylosema fassoglense	Woody	Fabaceae	2,1	2,9	46,55	4,7	**5,48**	4,09
Diheteropogon amplectens	Grass	Poaceae	1,95	2,7	49,27	2,87	**7,62**	3,14
Hyparrhenia filipendula	Grass	Poaceae	1,8	2,6	57,09	**7,49**	3	2,56
Trachypogon spicatus	Grass	Poaceae	1,9	2,6	54,54	4,27	0,91	**5,81**
Helichrysum nudifolium var. *nudifolium*	Forb	Asteraceae	1,9	2,6	51,9	5,57	**6,74**	0,02
Afroaster comptonii	Woody	Asteraceae	1,4	1,99	59,09	3,4	**4,47**	2,67
Helichrysum nudifolium var. *oxyphyllum*	Forb	Asteraceae	1,3	1,8	60,85	**3,16**	2,81	2,67
Hyparrhenia cymbaria	Grass	Poaceae	0,9	1,3	65,97	**2,52**	2,25	0,86
Imperata cylindrica	Grass	Poaceae	0,9	1,3	63,45	0	**2,63**	2,58
Monocymbium ceresiiforme	Grass	Poaceae	0,96	1,3	62,18	**3,47**	1,39	0,64
Helichrysum platypterum	Forb	Asteraceae	0,9	1,3	64,71	0,79	0,73	**4,08**
Indigofera hilaris	Woody	Woody	0,8	1,2	67,14	1,16	**2,39**	1,49
Thunbergia atriplicifolia	Forb	Acanthaceae	0,8	1,09	68,23	**2,91**	1,89	1,29
Merwilla plumbea	Forb	Hyacinthaceae	0,7	1,02	69,25	1,36	**2,08**	1,46

Av, dis,: average dissimilarity; Cont. %: contribution %; Cum. %: cumulative contribution %

Table 6-5: Similarity percentage analysis (SIMPER) of species contributing >1% to compositional differences between pre-drought, drought and post-drought years in the closed community. Species are listed according to the contribution % from highest to lowest. Bold values indicate the highest mean abundance. Distance/similarity measure: Bray-Curtis.

Species	Life form	Plant family	Av. Dis.	Cont. %	Cum. %	Mean cover		
						Pre-drought	**Drought**	**Post-drought**
Cymbopogon nardus	Grass	Poaceae	7,69	10,35	10,35	38,8	42,1	36
Berkheya setifera	Forb	Asteraceae	4,65	6,26	16,61	**19,7**	11,7	7,18
Hyparrhenia filipendula	Grass	Poaceae	3,59	4,83	26,71	**13,3**	10,4	0,36
Afroaster comptonii	Woody	Asteraceae	3,39	4,56	31,27	**13,2**	9,58	6,02
Themeda triandra	Grass	Poaceae	3,38	4,54	35,81	6,94	**13,6**	0,1
Helichrysum nudifolium var. *nudifolium*	Forb	Asteraceae	3,09	4,16	39,97	**14,7**	5,38	0
Tylosema fassoglense	Woody	Fabaceae	3,01	4,06	44,03	**9,1**	5,17	5,72
Pteridium aquilinum	Woody	Dennstaedtiaceae	2,44	3,29	50,82	**5,91**	3,84	5,7
Setaria sphacelata	Grass	Poaceae	2,07	2,79	53,61	**8,48**	5,7	2,02
Helichrysum nudifolium var. *oxyphyllum*	Forb	Asteraceae	1,9	2,55	56,16	**7,45**	3,59	2,17
Otholobium wilmsii	Woody	Fabaceae	1,55	2,08	58,24	3,26	1,93	**4,19**
Indigofera homblei	Woody	Fabaceae	1,5	2,02	60,26	1,76	2,84	**4,54**
Hyparrhenia cymbaria	Grass	Poaceae	1,34	1,8	62,06	1,65	**4,62**	1,8
Lippia javanica	Woody	Verbenaceae	1,31	1,76	63,83	2,29	2,77	**2,8**
Melinis repens	Grass	Poaceae	1,25	1,68	65,5	0,03	2,85	**4,43**
Eulalia villosa	Grass	Poaceae	1,21	1,62	67,13	**3,16**	2,5	1,63
Loudetia simplex	Grass	Poaceae	1,18	1,59	68,72	**4,52**	2,26	0,39
Trichodesma physaloides	Woody	Boraginaceae	1,18	1,59	70,31	**3,35**	2,9	1,76
Helichrysum platypterum	Forb	Asteraceae	1,04	1,4	71,71	0,04	0,01	**5,27**
Bewsia biflora	Grass	Poaceae	0,99	1,34	73,05	**3,23**	3,04	0,03
Helichrysum tenax	Woody	Asteraceae	0,88	1,18	74,23	2,09	**2,11**	0,97

Eragrostis chloromelas	Grass	Poaceae	0,87	1,17	75,4	**3,59**	1,35	0,22
Acalypha peduncularis	Forb	Euphorbiaceae	0,85	1,14	76,54	**2,56**	0,93	1,75
Rabdosiella calycina	Woody	Lamiaceae	0,78	1,05	77,59	**2,15**	1,62	0,84
Cynoglossum lanceolatum	Forb	Boraginaceae	0,76	1,02	78,61	1,55	1,25	**2,52**

Av. dis.: average dissimilarity; Cont. %: contribution %; Cum. %: cumulative contribution %

Table 6-6: Similarity percentage analysis (SIMPER) of species contributing >1% to compositional differences between open and closed communities during the pre-drought years. Species are listed according to the contribution % from highest to lowest. Bold values indicate the highest mean abundance. Distance/similarity measure: Bray-Curtis.

Species	Life form	Plant family	Av. Dis.	Cont. %	Cum. %	Mean cover	
						Open	Closed
Cymbopogon nardus	Grass	Poaceae	8,71	10,39	10,39	30,8	39,8
Setaria sphacelata	Grass	Poaceae	5,56	6,63	17,02	**16,5**	8,22
Loudetia simplex	Grass	Poaceae	5,28	6,29	23,31	**22,3**	4,42
Trachypogon spicatus	Grass	Poaceae	4,39	5,24	28,55	**4,27**	0
Berkheya setifera	Forb	Asteraceae	4,38	5,22	33,78	9,59	**20,4**
Themeda triandra	Grass	Poaceae	3,9	4,65	38,42	**13,2**	6,73
Helichrysum nudifolium var. *nudifolium*	Forb	Asteraceae	3,16	3,77	42,19	5,57	**14,8**
Hyparrhenia filipendula	Grass	Poaceae	3,1	3,7	45,9	7,49	**12,8**
Afroaster comptonii	Woody	Asteraceae	2,56	3,05	48,95	3,4	**12,8**
Eulalia villosa	Grass	Poaceae	2,22	2,65	51,6	0,3	**3,07**
Tylosema fassoglense	Woody	Fabaceae	2,21	2,64	54,24	4,7	**8,83**
Acalypha peduncularis	Forb	Euphorbiaceae	2,05	2,45	59,32	**9,32**	2,48
Helichrysum nudifolium var. *oxyphyllum*	Forb	Asteraceae	1,71	2,03	61,36	3,16	**7,23**
Hyparrhenia hirta	Grass	Poaceae	1,01	1,2	64,54	**3,25**	0,47
Pteridium aquilinum	Woody	Dennstaedtiaceae	0,96	1,14	65,68	0	**5,73**

Av. dis.: average dissimilarity; Cont. %: contribution %; Cum. %: cumulative contribution %

Table 6-7: Similarity percentage analysis (SIMPER) of species contributing >1% to compositional differences between open and closed communities in the drought year. Species are listed according to the contribution % from highest to lowest. Bold values indicate the highest mean abundance. Distance/similarity measure: Bray-Curtis.

Species	Life form	Plant family	Av. Dis.	Cont. %	Cum. %	Mean cover	
						Open	Closed
Cymbopogon nardus	Grass	Poaceae	8,05	10,06	10,06	30,3	42,1
Themeda triandra	Grass	Poaceae	5,45	6,81	16,87	**20,9**	13,6
Loudetia simplex	Grass	Poaceae	5,3	6,62	23,49	**22,8**	2,26
Setaria sphacelata	Grass	Poaceae	3,7	4,62	28,11	**12,9**	5,7
Berkheya setifera	Forb	Asteraceae	3,09	3,87	31,98	9,01	**11,7**
Diheteropogon amplectens	Grass	Poaceae	2,57	3,21	35,19	**7,62**	2,16
Hyparrhenia filipendula	Grass	Poaceae	2,17	2,71	40,84	3	**10,4**
Afroaster comptonii	Woody	Asteraceae	2,09	2,61	43,45	4,47	**9,58**
Helichrysum nudifolium var. *nudifolium*	Forb	Asteraceae	1,99	2,5	48,52	**6,74**	5,38
Tylosema fassoglense	Woody	Fabaceae	1,76	2,2	53,11	**5,48**	5,17
Acalypha peduncularis	Forb	Euphorbiaceae	1,38	1,72	54,83	**5,27**	0,93
Hyparrhenia cymbaria	Grass	Poaceae	1,21	1,51	56,34	2,25	**4,62**
Helichrysum nudifolium var, Oxyphyllum	Forb	Asteraceae	1,19	1,48	57,82	2,81	**3,59**
Eulalia villosa	Grass	Poaceae	1,16	1,45	59,28	0,19	**2,5**

Av. dis.: average dissimilarity; Cont. %: contribution %; Cum. %: cumulative contribution %

Table 6-8: Similarity percentage analysis (SIMPER) of species contributing >1% to compositional differences between open and closed communities in the post-drought years. Species are listed according to the contribution % from highest to lowest. Bold values indicate the highest mean abundance. Distance/similarity measure: Bray-Curtis.

Species	Life form	Plant family	Av. Dis.	Cont. %	Cum. %	Mean cover	
						Open	Closed
Cymbopogon nardus	Grass	Poaceae	10,13	12,36	12,36	36,8	36
Loudetia simplex	Grass	Poaceae	6,52	7,96	20,32	**21,2**	0,39
Trachypogon spicatus	Grass	Poaceae	4,68	5,71	31,89	**5,81**	0,08
Berkheya setifera	Forb	Asteraceae	2,77	3,38	39	**7,71**	7,18
Themeda triandra	Grass	Poaceae	2,5	3,06	42,06	**8,08**	0,1
Tylosema fassoglense	Woody	Fabaceae	2,04	2,49	44,54	4,09	**5,72**
Eulalia villosa	Grass	Poaceae	1,89	2,31	49,25	0,73	**1,63**
Helichrysum platypterum	Forb	Asteraceae	1,84	2,25	51,5	4,08	**5,27**
Afroaster comptonii	Woody	Asteraceae	1,73	2,11	53,61	2,67	**6,02**
Acalypha peduncularis	Forb	Euphorbiaceae	1,56	1,91	57,43	**5,42**	1,75
Melinis repens	Grass	Poaceae	1,47	1,8	59,23	2,14	**4,43**
Setaria sphacelata	Grass	Poaceae	1,44	1,76	60,99	**4,42**	2,02
Pteridium aquilinum	Woody	Dennstaedtiaceae	1,22	1,48	62,47	0	**5,7**
Helichrysum nudifolium var. *oxyphyllum*	Forb	Asteraceae	1,15	1,4	63,87	**2,67**	2,17
Indigofera homblei	Woody	Fabaceae	0,95	1,16	66,24	0	**4,54**
Otholobium wilmsii	Woody	Fabaceae	0,91	1,11	67,34	0	**4,19**
Acalypha depressinerva	Forb	Euphorbiaceae	0,89	1,09	68,43	**1,21**	0,01

Av. dis.: average dissimilarity; Cont. %: contribution %; Cum. %: cumulative contribution %

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Table 6-9: Summary of the two-way ANOVA type generalized linear mixed model results for variation in plant species richness, evenness and diversity per community type and rainfall year, as well as their interaction.

	Fixed effects		
	Community type	Rainfall year	Community type*Rainfall year
Species richness (S)			
F	11,43	81,98	9,05
Mean Sq	11,43	81,98	9,05
p	**< 0,001***	**<0,001***	**<0,001***
Species evenness (J’)			
F	2,06	17,13	6,25
Mean Sq	0,009	0,08	0,03
p	0,41	**<0,001***	**<0,01***
Species diversity (H’)			
F	11,67	78,14	15,12
Mean Sq	0,82	5,52	1,07
p	0,05	**<0,001***	**< 0,001***

Rainfall year refers to pre-drought (2009 or 2011), drought (2015) and post-drought (2019 or 2020); Community type refers to open and closed communities, Significant effects (at p < 0,05) indicated in bold with *.

Table 6-10: The mean abundance of exotic species in each community type across the three rainfall years (pre-drought, drought, and post-drought). ^{G} Superscript refers to grass species and ^{F} refers to forb species.

Species	**Open**			**Closed**		
	Pre-drought	**Drought**	**Post-drought**	**Pre-drought**	**Drought**	**Post-drought**
Duhaldea latifolia ^{F}	0	0	0	0	0	0,23
Nassella trichotoma ^{G}	0	0,2	0	0	0,09	0
Paspalum urvillei ^{G}	0	0	0	0,08	0	0
Physalis peruviana ^{F}	0,04	0	0	0	0,02	0,01
Tagetes minuta ^{F}	0	0	0	0,02	0	0

Table 6-11: The mean abundance of endemic, endangered, and near-threatened species in each community across the three rainfall years (pre-drought, drought, and post-drought). All species, except *Merwilla plumbea*, are endemic to South Africa. Bold values represent the highest mean abundance for each species in each rainfall year. Superscripts: LC Least Concern, DDT Data Deficient – Taxonomically Problematic, E endangered species; NT near-threatened; F forbs; W woody species.

		Open			**Closed**		
Endemism	**Species**	**Pre-drought**	**Drought**	**Post-drought**	**Pre-drought**	**Drought**	**Post-drought**
South Africa endemic	*Agapanthus inapertus* $^{LC, F}$	0	**0,1**	0,03	0	0	**0,06**
	Alepidea peduncularis $^{DDT, F}$	**0,8**	0,5	0,2			
	Argyrolobium transvaalense $^{LC, W}$	**0,34**	0,03	0,18	**0,13**	0,04	0
	Cyphia heterophylla $^{LC, F}$	**0,01**	0	0	0	**0,04**	0
	Cyphia tysonii $^{LC, F}$	0	**0,02**	0	**0,05**	0,02	0
	Hypericum aethiopicum $^{LC, F}$	0,26	**0,34**	0,2	0,71	**0,72**	0,38
	Indigofera sanguinea $^{LC, W}$	1,16	**2,39**	1,49			
	Lichtensteinia interrupta $^{LC, F}$				0	**0,06**	0
	Pygmaeothamnus chamaedendrum $^{LC, F}$	0,23	0,6	**0,7**	**0,02**	0,01	0
	Searsia pondoensis $^{LC, W}$	**0,13**	0,06	0,02	**0,06**	0,02	0,03
	Senecio pentactinus $^{LC, F}$	0	**0,19**	0	0	**0,02**	0
WGG endemic	*Aloe lettyae* $^{E, F}$	0,05	0,05	**0,07**	**0,62**	0,53	0,61
	Indigofera rehmannii $^{E, W}$				0	0,06	**0,08**
Not endemic	*Merwilla plumbea* $^{NT, F}$	1,4	**2,1**	1,5	**0,2**	0,03	0

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CHAPTER 7 SYNTHESIS AND CONCLUSION

7.1 Summary of research

Systems that are not forested cannot simply be viewed as degraded. Instead, they are open ecosystems with unique species assemblages and functions. These open ecosystems have been neglected in research and management practices, consequently their responses to environmental and anthropogenic change are poorly understood, especially in the case of grasslands. For this reason, this thesis investigated the effects of climate variability, land-use change, and transformation on grassland plant diversity and ecosystem function of the grasslands in South Africa. The analysis focused on patterns in species diversity, species change over time, and floristic composition across the Grassland Biome. This was done across different land-use types in the Grassland Biome and across open and closed sites of a specific grassland across rainfall years. In summary, the relevant objectives and hypotheses (Table 7-1) for each results chapter will briefly be stated, followed by an itemization and brief further discussion of the most important findings.

Table 7-1: The research questions, objectives, and hypotheses for this study.

Research questions	Objectives	Hypotheses
Q1: How do land-use change and -intensification affect floristic diversity in the Grassland Biome of South Africa? (Chapter 4)	O1a: To illustrate how plant community composition changes when old-growth grasslands are being transformed into alternative land-uses, such as afforestation, agriculture, mining, and urbanisation. O1b: To document plant diversity changes, as well as floristic gains and -losses after grassland transformation, and to summarise the observed changes in taxa (families, species (endemic, native, and introduced)), life forms, and growth forms.	H1: Considerable shifts in plant communities are expected due to the expected increase of exotic species and the decrease in forb species richness. The floristic diversity of transformed grasslands in South Africa is expected to be significantly lower compared to untransformed, old-growth grasslands due to the higher abundance of alien species and dominance of disturbance-tolerant life forms and growth forms in transformed grassland sites.
Q2: Are the observed changes in woody cover in parts of the threatened Woodbush Granite Grassland related to forest expansion or savanna encroachment, and what are the potential consequences of increased woody cover on floristic composition and diversity? (Chapter 5)	O2a: To compare woody cover and woody species abundance differences between the community with observed higher woody cover (that is 'closed' grassland community) and the 'open' community in the WGG. O2b: To determine whether the woody species present in the WGG represent savanna encroacher or forest precursor species. O2c: To evaluate the potential effects of increased woody cover on species composition, plant diversity and abundance of endemic, threatened, and exotic species.	H2: The closed plant community reflects a previously open system experiencing savanna encroachment. This increase in woody abundance is expected to have negative impacts on herbaceous richness and diversity, since the encroached grassland no longer maintains the old-growth grassland diversity.
Q3: Will a severe drought affect the floristic composition and diversity of an open and encroached grassland similarly? (Chapter 6)	O3a: To understand how an open and encroached grassland responds to a severe drought in terms of their floristic composition and diversity and relating this to ecosystem resistance, recovery, and resilience. O3b: To assess whether an encroached grassland will become more or less encroached due to a severe drought. O3c: To Investigate the effect of a severe drought on the woody species of an encroached grassland.	H3: Encroached grasslands are less resistant and resilient to drought than open grasslands, since they no longer support the old-growth grassland diversity with more resistance. The encroached community will have experienced a loss in species diversity, thus changing the community composition over time, meaning recovery the initial state will be more difficult.

7.1.1 Chapter 4

Land-use change is a considerable threat to grassland ecosystems, mainly because transformation affects soil integrity and removes plant biomass. It is therefore necessary to assess the effects of transformation especially on forb dynamics. The objectives of this chapter were to (1) compare plant community composition of untransformed and transformed grasslands and (2) provide a summary of floristic gains and -losses in transformed grasslands. It was hypothesised that the floristic diversity of grasslands would decrease when subjected to land-use changes, that is transformation. Lower indigenous floristic diversity was expected in transformed grasslands as well as shifts in plant communities (Hypothesis 1).

The main findings presented in this chapter are listed below:

- Major plant families remained floristically dominant after transformation (Asteraceae, Poaceae, Fabaceae, and Cyperaceae).
- The Poaceae and Cyperaceae were promoted by the disturbance.
- Non-typical grassland families, which harbour disturbance-tolerant traits, showed an increase in phylogenetic diversity, mainly as a consequence of colonisation by alien weedy species.
- Species composition shifted due to disturbance as evidenced by the fact that better-adapted species entered the system or that existing pre-adapted ones became more dominant.
- A proportional increase of grass species occurred, but also a large reduction in forb species with belowground storage organs.
- No evidence was found for extensive woody encroachment in transformed areas.
- Untransformed and transformed grasslands were characterised by unique herbaceous plant communities with specific indicator species as driven by anthropogenic change. Therefore, Hypothesis 1 is accepted.

7.1.2 Chapter 5

From extant literature and this project, it has been found that soil and climate are not the main determinants of vegetation distribution (Bond, 2019; Cheng *et al.*, 2021; Pausas & Bond, 2020). The same climate and soil can support grassland, savanna, and forest ecosystems. The presence of aboveground consumers (such as fire and herbivory) usually determines vegetation patterns. If changes are made to these aboveground consumers, vegetation pattern shifts from open to closed systems may be facilitated. Objectives of this chapter included (1) comparing woody cover and woody species abundance differences between the community with observed higher woody cover (that is ‘closed’ grassland community) and the ‘open’ community in the Woodbush Granite

Grassland (WGG), (2) determining whether the woody species present in the WGG represent savanna encroacher or forest precursor species, and (3) evaluating the potential effects of increased woody cover on species composition, plant diversity and abundance of endemic, threatened, and exotic species. It was hypothesised that the closed plant community reflects a previously open system experiencing savanna encroachment. This increase in woody abundance is expected to have negative impacts on herbaceous richness and diversity, since the encroached grassland no longer maintains the old-growth grassland diversity (Hypothesis 2).

The main findings as presented in this chapter include the following:

- An increase in woody species cover and occurrence was observed in the closed community.
- The woody species that contributed to the increase in cover and occurrence were indigenous and are associated with grasslands, savannas, forests, and woodlands. Therefore, whether savanna encroachment or forest expansion occurred cannot be conclusively determined. Instead, a general shrub encroachment can be confirmed.
- The closed community can be alternatively described as a transformed grassland or shrubland.
- Major plant families remained floristically dominant after encroachment (Asteraceae, Fabaceae, and Poaceae).
- Families such as the geophytic Hyacinthaceae family disappeared when the grassland became encroached, while the fern family, Dennstaedtiaceae, was only present in the closed community.
- The open and closed communities differed somewhat in terms of their composition, most likely due to the higher forb species diversity in the open community and higher long-lived forb and woody species diversity in the closed one.
- The diversity in the closed community was less than that of the open community in the case of all diversity measurements.
- Grassland species richness and diversity were impacted by the shrub encroachment but not pertinently by savanna encroachment therefore, Hypothesis 2 can be partially accepted.

7.1.3 Chapter 6

Grasslands are adapted to and reliant on rainfall variability (Hoffman *et al.*, 2019; Masubelele *et al.*, 2014). Drastic changes to rainfall patterns may however hold dire consequences for the survival of grassland species (Masih *et al.*, 2014; Rao & Chaitanya, 2016). There is also the potential that climatic extremes may drive biome switches from grasslands to savannas or forests.

Chapter 6 firstly determined the way in which open and encroached grasslands respond to a severe drought in terms of their floristic composition and diversity and relating this to ecosystem resistance, recovery, and resilience. Secondly, it was assessed whether and encroached grassland will become more or less encroached due to a severe drought. Lastly, the effect of a severe drought on the woody species of an encroached grassland was investigated.

It was hypothesised that encroached grasslands are less resistant and resilient to drought than open grasslands, since they no longer support the old-growth grassland diversity with more resistance. The encroached community will have experienced a loss in species diversity, thus changing the community composition over time, meaning recovery the initial state will be more difficult (Hypothesis 3).

The main findings from this chapter include the following:

- The community composition changed across rainfall periods and across community types, but the sampling sites showed heterogeneity, which would likely benefit the resilience to disturbances of the WGG.
- Compositional changes observed between communities were likely caused by higher forb species diversity in the open community and higher long-lived forb and woody species diversity in the closed one.
- Encroached grasslands are somewhat less resistant and resilient to drought than an un-encroached grassland. Encroached grasslands could therefore be seen to be more vulnerable to drought, but the results are not definitive.
- The open community experienced fewer species losses both in terms of abundance and occurrence.
- The closed community gained woody species from pre-drought to the drought periods, and, overall, from pre- to post-drought, which was to be expected at a site with a comparatively higher component of woody species.
- The open and closed communities remained significantly different in terms of species richness, evenness, and diversity from each other after the drought. During the drought the two communities were more similar. Furthermore, species richness, evenness, and diversity did not decrease during the drought year. For most of the diversity measures the drought year had the highest diversity. Only in the open community did the diversity measures return to pre-drought diversity after the drought. The diversity in the closed community was less than that of the open community for all diversity measures, for different rainfall years.
- The open community experienced less species loss in terms of both abundance and occurrence measurements. The closed community gained woody species from pre-

drought to the drought, and overall, from pre- to post-drought, which was to be expected at a site with a comparatively higher component of woody species. Before the onset of the drought, the species richness of the open and closed sites was similar. This changed with the onset of the drought and onwards, and reduced diversity was measured in the closed community after the drought.

- The woody species were overall positively affected by drought as they showed resistance and resilience and were positively affected by the drought's legacy effect. The number of stems did however decrease but stem height increased.
- Hypothesis 4 can only be partially accepted since species richness, evenness, and diversity did not decrease during the drought year. For most of the diversity measurements, the drought year had the highest diversity. Only in the open community did the diversity measurements return to pre-drought diversity after the drought. The diversity in the closed community was indeed less than that of the open community for all diversity measurements in the case of all the different rainfall periods, as was expected.

7.2 Synthesis

The old-growth grasslands of South Africa are diverse (Chapters 4 and 5), consisting of a wide variety of grass, forb, and woody species. Grassland diversity generally resides in its forb diversity, whereas the biomass is dominated by grass species. This correlates with what has been found for other southern African grasslands (Bredenkamp *et al.*, 2002). In the event of alterations to the natural disturbance regimes of these grasslands, including herbivory, fire, and rainfall variability, losses in species diversity, especially forb diversity, are incurred. Forb species are therefore of central importance to the conservation of the biodiversity within and the functioning of the Grassland Biome. This has been true since the Pleistocene as discovered by Bråthen *et al.* (2021). A 20-year grassland community study in Brazil found similar changes in plant richness, diversity, cover and composition (de Souza *et al.*, 2022). The grasslands in this study were also found to be on different trajectories mainly due to encroachment (de Souza *et al.*, 2022). It is important to note the multifaceted nature of grassland community trajectories when facing environmental change.

Disturbance in grasslands can either maintain or cause a loss of diversity, as evidenced by the distinguishment of disturbances as either exogenous or endogenous (Buisson *et al.*, 2019). The present study has demonstrated that drought acted as an agent of change, that is, the drought brought about shifts in abundance (cover) and occurrence. It thus paradoxically maintained diversity. This was evidenced by the species evenness in the open community that was restored to pre-drought numbers and the gains and losses of species occurrence and abundance over the course of the drought. Grasses have been found to tolerate drought better than forbs due to their

C_4 photosynthetic pathways (Ward *et al.*, 1999), while forbs flourish occasionally due to their ability to avoid drought by making use of deep rooting systems (Nippert & Knapp, 2007). While woody plants have been found to increase in dominance and/or biomass in grassland ecosystems as the climate changes due to higher atmospheric CO_2 concentrations (Bond & Midgley, 2012; Higgins & Scheiter, 2012; Moncrieff *et al.*, 2015). In other words, natural disturbance regimes are necessary to restore balance within systems so that some species do not become dominant over others. Natural change or endogenous change may result in fluctuations in species richness and diversity but, ultimately, the grasslands seem more adaptable to these changes. Anthropogenic change or exogenous change results in species loss that affect the grassland composition. This was evidenced by the land-use change as a result of which some plant species were completely lost from the grasslands. For these systems to be saved and protected, restoration efforts will be required. These should involve the reintroduction of species with below-ground storage organs and bud banks. These species are lost from the system when it is transformed, by exogenous disturbances, and are often unable to return to the system naturally (Buisson *et al.*, 2019).

Grasslands are more resilient to endogenous than to exogenous disturbances. They evolved in the presence of endogenous disturbances and are therefore adapted to them (Buisson *et al.*, 2019; Silveira *et al.*, 2020; Veldman *et al.*, 2015b). Therefore, plants are resilient and able to adapt to natural changes in disturbance regimes such as occasional severe droughts. The severe drought that lasted from 2013 to 2016 did not affect grassland diversity and composition as much as might be expected. In contrast, and in general, exogenous disturbances in the form of land-use change lead to a loss of species as the plants are unable to adapt to the abrupt changes. Land-use change has caused dramatic declines in the world’s old-growth grasslands (Buisson *et al.*, 2019). Plants obtain their resilience from the traits they possess that enable them to withstand endogenous disturbance events. Some very important plant traits are related to the well-developed belowground structures from which they resprout after natural disturbances. Forbs in grassy systems are very resilient to frequent fires due to their belowground storage organs (Zaloumis & Bond, 2016). These structures also enable the storage of carbon belowground, which is relocated aboveground after disturbances and drought (Buisson *et al.*, 2022). This is especially important since a great driver for the planting of trees in open ecosystems is that they would store more carbon than open ecosystem species.

Woody plants do occur in grasslands (Chapters 4 & 5). The abundance and occurrence of these woody species are however determined by other factors. Although the cause of the woody encroachment in the closed community is unknown, a shift was observed. Encroachment is a complex and multifaceted phenomenon, and understanding its ecological consequences requires a holistic view that will take into account the multiplicity of organisms and ecosystem processes and services it affects (Maestre *et al.*, 2016). It is also important to note that the impacts of shrub encroachment are context-dependent and that a one-size-fits-all paradigm does not exist (Maestre *et al.*, 2016).

Woody species are increasing in abundance and occurrence in the HNR. Management should therefore decide on whether to leave this process and manage whatever grassland state emerges or manage the system to maintain the open physiognomy. This is especially important since there has been a gain in woody species abundance and occurrence observed in the open community. It is possible that the entire WGG system is shifting to an alternative state due to external changes. The decision that needs to be made should therefore be based on whether ecosystem change is natural and should be allowed to continue, or whether the change will result in the loss of ecosystem function and ecosystem services, which will negatively influence humans. Another point to consider is the positive effects of encroachment. There is increasing evidence that encroachment might have positive effects that outweigh the perceived negative effects (Eldridge *et al.*, 2011; Maestre *et al.*, 2016). Plant diversity and ecosystem functions have been found to be maximized under moderate levels of woody plant cover (Barbosa da Silva *et al.*, 2016; Maestre *et al.*, 2016).

The grasslands studied in this thesis were often small and isolated due to changes in land-use. But conservation of these remnants should be continued since they still house high diversity of species and functions that ultimately provide us with ESs. These grasslands can also act as steppingstones or corridors between larger intact grasslands. It is also important to know that if the management of these grassland remnants is abandoned, a time delay in the extinction of species often results due to plant population inertia (Johansson *et al.*, 2011). This means that the populations will decline, but this decline will be slow and occur over several decades.

Grasslands, as open ecosystems, need to be conserved for the high diversity that they host and the multiple ecosystem services they provide to people. Grasslands should not simplistically be viewed as degraded forests: as indicated a grassland with high woody biomass loses species diversity (Chapter 5). Grasslands should therefore not be targeted for ‘reforestation’ campaigns, but rather be kept intact and conserved in their ‘open’ state.

To conclude, results obtained from this thesis confirm that grassland communities’ diversity, structure, and functioning are shaped by climate variability, land-use management, and encroachment. These findings expand our understanding of the effects of natural and anthropogenic change on grassland ecosystems and should contribute to their sustainable management and conservation.

7.3 Conservation and management implications and future research

From a biodiversity-conservation perspective, the transformation of grasslands either through land-use change or encroachment and the consequent loss of grassland-associated diversity are of great concern. The loss of especially forb diversity is worrying, since many forb species are found exclusively in grassland habitats and may be difficult to restore. The loss of plant species impacts ecosystem functioning, which holds conservation implications for plant and animal species. Ideally, there should be a mosaic of forest and grassland patches to promote heterogeneity and maintain regional diversity.

The present project elucidates the response of South African grasslands to change, whether natural or anthropogenic. However, a need arises to understand the effect of such changes in other grassland systems, such as arid grasslands. This will further broaden our current understanding of grassland vegetation dynamics. Furthermore, studies that link changes in grassland dynamics to animal occurrence and behaviour are necessary. For example, the critically endangered Wolkberg Zulu butterfly (*Alaena margaritacea*) is endemic to the WGG and would likely go extinct if this vegetation type disappears. Therefore, it would be valuable to understand how woody encroachment would influence this species. The effects of encroachment on fauna are less clearly defined and highly variable, since some species have been found to positively respond to encroachment, while others decline (Eldridge *et al.*, 2011). It would also be valuable to quantify the carbon sequestration potential of different grassland systems in comparison with more wooded or encroached systems so as to counter tree planting initiatives that motivate these carbon saving potential.

Grasslands host a variety of species that, in their cumulative effects provide crucial ecosystem services to humans. It would therefore be advisable that transformed grasslands be restored to a grassland state closely resembling the original one. It is however important to note that the restoration of grassland ecosystems is not as simple as allowing herbaceous species to recolonise, supplementing the process with native seed, re-establishing appropriate grazing and fire disturbance regimes, and controlling ruderal, exotic, or woody species (Buisson *et al.*, 2022). It has been estimated that secondary grasslands can take a century or millennia to recover their former species richness (Nerlekar & Veldman, 2020). It is therefore unlikely that secondary grasslands will ever be able to be fully restored to the original old-growth grasslands state. Even so, there is a suite of characteristics one can aim for in the restoration process, namely long-lived perennial species, diversity of belowground structures that enable resprouting after aboveground disturbances, and large belowground carbon stores (Buisson *et al.*, 2022). It is vital that existent old-growth grasslands be protected, especially from threats affecting belowground processes and structure, as restoration cannot guarantee complete recovery after degradation (Buisson *et al.*, 2022).

The open and closed communities focussed on within the present project burned the same number of times in the last ten years, and yet one community became more ‘closed’ and the other not. Instead of focussing on the presence or absence of fire, experimenting with the fire regime characteristics is therefore a management option when it comes to the control of woody encroachment (McGranahan & Wonkka, 2018).

Fire characteristics that have been experimented with to control woody encroachment in grasslands and savannas include the type of fire (head or backfires), return interval of fire, season (summer versus winter, for example), severity, intensity, structural heterogeneity, and burn area (Scholtz *et al.*, 2022; Trollope & Potgieter, 1985). It was established that high-intensity fires were able to return an encroached area back to or close to the original community (Scholtz et al 2022). One way in which this might be done is by burning the closed community in summer, which would result in more intense fires due to the heat. Fire in the dormant season of plants has been found to result in higher resprouting rates, in contrast with fires during the growing season (Geldenhuys, 1977; Robertson & Hmielowski, 2014; Scholtz *et al.*, 2022). Further studies can determine the effect of different fire characteristics on the woody encroachment observed in the closed community of the WGG.

A further management option is to reintroduce large mammalian herbivores to the HNR or introduce livestock, thus restoring grazing regimes. This can be explained by the fact that the grazing and trampling of herbivores facilitate the reach of sunlight to the ground level by preventing competitive plant species from growing too tall (Bråthen *et al.*, 2021). Trampling would, in turn, push litter into the soil and generate gaps for the establishment of other species (Bråthen *et al.*, 2021). Furthermore, urination and defecation return nutrients to the soil that are readily available for plant uptake, thus increasing nutrient cycling rates (Bardgett & Wardle, 2003).

The removal or reduction of woody species is also a management option. The former procedure was found to increase herbaceous cover (19%) and density (35%) (Ding & Eldridge, 2019). Even so, the removal of woody species through cutting, herbicide application, or ploughing very often have negative consequences for the remaining biodiversity (Buisson *et al.*, 2022). This option should therefore only be applied if extensive research has been done on the possible positive or negative outcomes of the procedures.

Careless and poorly planned tree-planting efforts in the name of restoration should be prohibited, as this often leads to the establishment of forests in natural grassland ecosystems (Buisson *et al.*, 2022). This is especially concerning in light of the fact that 1 million km^2 of Africa’s grassy biomes are targeted for tree planting by 2030 (Bond *et al.*, 2019). Trees should be planted to restore forests and, in urban areas, for shade and enjoyment, and distinctly not in Africa's savannas and grasslands (Bond *et al.*, 2019). The key message here is that humans should only plant trees where they belong and not in open ecosystems.

The Grassland Biome of south Africa should be continuously studied and monitored to fully understand its dynamics and responses to change. A particular focus area should be to study the changes in plant functional composition as a result of a drought, as this could affect the resistance to drought and the recovery after the drought of grassland ecosystems (Stampfli *et al.*, 2018). This is important because a change in resistance, recovery and resilience will ultimately affect the ecosystem services provided by grasslands (Stampfli *et al.*, 2018).

Old-growth and secondary grasslands of South Africa should be conserved, even if it is on privately owned or working land. In this regard, South Africa can follow in the footsteps of Namibia by implementing communal conservancies (Dinerstein *et al.*, 2017) where landowners are actively involved in the protection of natural systems and animals. One such area that is in the process of being created in South Africa is the Grasslands National Park in the Eastern Cape. This national park will go a long way towards showing the possibilities of what can be done if different sectors and stakeholders work together for one common goal. Furthermore, monitoring of the WGG in the HNR should be continued by SAEON so as to better understand the changes occurring in this system, especially considering future climate change that could contribute to encroachment. Results from the HNR could also be used to guide future long-term monitoring studies in other grassland systems in South Africa and in other countries around the world.

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