UNIVERSITY OF KWAZULU-NATAL

THE INFLUENCE OF DROUGHT AND ABIOTIC DRIVERS ON A MESIC GRASSLAND

by

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Doctor of Philosophy

In

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In

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College of Agriculture, Engineering and Science

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Dedication

This body of work is dedicated to my late mother Nombulelo Sweetness Maziko, uMagatyen'omhle, my late father Zandile Elliot Gqasana uMmngwevu, and my late nephew Sisipho Mgijima.

Even though you're not with us anymore but your love carries me through life.

To my dad, I wish you could have held on just a little bit longer for this moment!

ABSTRACT

A significant percentage of southern African grasslands have undergone deteriorative transformation due to climate-related changes. This PhD research study investigated the impacts of drought on species composition dynamics in a South African mesic grassland with data collected over a period of 3 years. **Chapter 3** investigated the effects of a short-term drought on plant species composition under different burning management regimes on the long-term veld burning experiment at Ukulinga Research Farm, University of KwaZulu Natal, South Africa. The results showed that annual burning produced higher species diversity and richness while no burning promoted the development of woody plants such as *Vachellia nilotica* and forbs. The short-term drought did not have a significant impact on the species composition of the grassland. **Chapter 4** assessed the influence of drought on grass-on-grass interactions in pot trials at the Neil Tainton Arboretum greenhouse and the study found that the presence of competition reduced phytometer biomass, but at varying levels with different species. Broad-leaved species had a stronger competitive effect over narrow-leaved species as they reduced the number of tillers, biomass production and tuft size of narrow-leaved species. The competitive strength within broad-leaf species was species-specific, with *Megathrysus maximus* being more competitive than *Cymbopogon excavatus*. Palatable species had a dominant competitive effect over unpalatable species without exposure to defoliation and *Tristachya leucothrix* had a stronger competitive effect over *Themeda triandra*. Drought did not have any significant impact on final dry biomass, but significantly reduced the tiller numbers for palatable species as well as tiller numbers and tuft circumference for broad-leaved species. **Chapter 5** focused on how the grass sward composition, biomass production as well as root biomass and length were affected by different moisture levels, including drought at 50% rainfall reduction on the Drought Net Experiment at Ukulinga Research Farm. Species diversity, richness and aboveground biomass were significantly affected by varying weather patterns, while all were not changed by moisture manipulations. Species diversity and richness increased in 2021/2022 while the biomass was greater in 2020/2021. On the contrary, high moisture levels reduced root biomass and this can be attributed to resources being allocated to greater above-ground biomass during moisture availability. Greater root biomass was found in the top 16cm of soil. **Chapter 6** assessed the influence of nutrient addition (nitrogen, phosphorus, potassium) on species composition, diversity and biomass production under different moisture levels including drought (50% rainfall reduction) over 3 years on the Drought Net Experiment at Ukulinga Research Farm. Interannual climate variability significantly increased species richness and diversity by 2021/2022. An interaction between fertilization and years shows a significant difference in response to interannual climate variability across fertilizer levels. Annual weather patterns refer to the differences in each 12-month cycle, centred around the growing season The unfertilized plots were unaffected by annual climate fluctuations and the fertilized plots showed significantly greater diversity in 2021/2022, the growing season with the highest rainfall. Species evenness fluctuated annually in the high and ambient rainfall treatments while it remained unchanged in the drought treatment. *Tristachya leucothrix, Themeda triandra* and *Cymbopogon excavatus* were abundant in both unfertilized and fertilized sites but most species increased in abundance in the fertilized areas. High moisture levels significantly increased plant biomass production while drought had the opposite effect. The findings from this study have shown that interannual climate variability and fertilization had a significant impact on species composition and productivity. The length of drought is a key factor that determines if a drought causes significant impacts on a plant community and long-term experimental applications such as fire, have more pronounced implications on a grassland community. Lastly, the competitive effect of plants is species-specific, and influenced by plant morphological traits. The results of this research study emphasize the importance of long-term experimental research for varying levels of drought and fire on mesic grasslands.

PREFACE

The research contained in this dissertation was completed by the candidate while based in the Discipline of Grassland Science, School of Life Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg campus, South Africa, under the supervision of Doctor Michelle Tedder, Professor Kevin Kirkman and Doctor Terry Everson. This thesis is the result of the author's original work except where acknowledged or specifically stated to the contrary in the text. It has not been submitted for any degree or examination at any other university or academic institution.

Signed [redacted]

Yonela Maziko (candidate)

Date 08/07/2024

Signed ………………………

Dr Michelle Tedder (supervisor)

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Prof Kevin Kirkman (co-supervisor)

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Signed ………………………

Dr Terry Everson (co-supervisor)

Date ……/07/2024

DECLARATION 1: PLAGIARISM

I, Yonela Maziko, declare that:

(i) the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

(ii) this dissertation has not been submitted in full or in part for any degree or examination to any other university;

(iii) this dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

(iv) this dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

> a) their words have been re-written but the general information attributed to them has been referenced;
>
> b) where their exact words have been used, their writing has been placed inside quotation marks, and referenced;

(v) where I have used material for which publications followed, I have indicated in detail my role in the work;

(vi) this dissertation is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included:

(vii) this dissertation does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections.

[redacted]

Signed: Yonela Maziko

Date 08 July 2024

DECLARATION 2: PUBLICATIONS

Chapter 4 ("The influence of drought on grass-to-grass interactions in pot trials") has been submitted for publication at the Agronomy Journal. My three supervisors have been included as co-authors because of their significant scientific input.

The conferences at which this certain chapters of the thesis have been presented at are also shown below:

Chapter 3: Maziko Y, Tedder M, Kirkman KP, Everson T. 2020. The influence of drought and fire on grass sward composition and structure in a mesic grassland. Poster presentation 2020 at the British Ecological Society conference.

Chapter 3: Maziko Y, Tedder M, Kirkman KP, Everson T. 2021. The influence of drought and fire on grass sward composition and structure in a mesic grassland. Platform presentation at the Grassland Society of Southern Africa.

Chapter 5: Maziko Y, Tedder M, Kirkman KP, Everson T. 2022. The influence of different moisture levels on grass sward composition and biomass production in a mesic grassland. Platform presentation at the BioAfrica Converntion.

Signed [redacted]

Yonela Maziko

Date 08/07/2024

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- I would like to thank the technical team at Ukulinga Research Farm that helped us set up the Drought Net experiment.
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For emotional support, I always turned to a dear friend Dr Sokfa John, without fail, he provided assistance whenever needed, for that I am thankful. For continued strength and mentorship, I thank Dr Xolisa Melamane. I would like to thank my friends Mr Lwando Zumani, Dr Lerato Phali, Ms Pilela Majokweni, Ms Nolubabalo Ketani, Mrs Ntomboxolo Mamayo, Dr Regis Wilson, Luhlumelo Mva and the late Ms Akhona Ndandani for their unwavering support during this time.

Finally, I would like to thank my sister, Mrs Kholeka Makinana-Ndwayana, the consistent love and support she pours on me gives me life.

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

1.1. Background

Grasslands are estimated to cover around 50 million square kilometers, comprising 37 % of the earth's surface, worldwide (Loveland et al., 2000). They dominate a substantial percentage of the central and eastern regions of southern Africa. The value of such lands lies in their biodiversity, livelihoods and ecosystem services (SANBI, 2014). The ecosystem services provided by grasslands include carbon sequestration, habitat and forage for domestic and wild herbivores and food security by sustaining the livelihood and economy of over a billion people under the low-income belt (Bengtsson et al., 2019). Grasslands also contribute to the conservation of water and soil resources (Carlier et al., 2009) and support plant and animal biodiversity (SANBI, 2014). During the last century, grasslands have been reported to be in decline, worldwide (Egoh et al., 2016), mainly due to conversion to arable land for production of crops, urbanization, mismanagement (Queiroz et al., 2014) and the impacts of climatic change. About 60% of the South African grassland biome is estimated to have undergone permanent transformation, with only 15% in a natural state, 25% considered to be degraded to some extent and only 2% formally conserved (Carbutt et al., 2011). Worldwide, 20% has been converted to croplands (Ramankuttu et al., 2008), and with the increasing human population, this is likely to increase, decreasing the availability of natural grazing land and increasing the pressure on the remaining grasslands. A vast majority of the remaining natural grassland is degraded, as a result of overgrazing, land mismanagement, woody encroachment and climate change poses a threat to the already degraded grasslands.

For centuries, fire has shaped plant communities, including those of grasslands (Scott, 2000). One of the most significant ecological variables influencing vegetation communities is fire, which changes the competitive relationships between species and eradicates woody and herbaceous species that are intolerant of fire (Briggs et al., 2002). The composition, structure, and patterns of vegetation are all impacted by the changes in ecosystems that fire brings about (Neary et al., 2005). Fire can induce large shifts in the vegetation and changes to the plant diversity (Pyke et al., 2010). However, depending on the ecosystem type and location, the responses of vegetation to fire in grassland and savanna ecosystems are highly diverse. High-frequency prescribed fires can suppress shrubs and trees in the tallgrass prairie of North America, allowing C4 grasses and forbs to establish and grow in infertile and mixed tree-grass vegetation systems (Peterson and Reich, 2008; Reed et al., 2005). Recurrent fires in Texas, USA, decrease C3 grass cover and enhance C4 grass cover regardless of season (Ansley et al., 2010). Grasslands in southern Africa are home to a broad variety of forbs, but relatively few grass species (Bond and Parr, 2010). This suggests that forb species are more resilient to seasonal variations and fire frequency than dominating grasses (Uys et al., 2004). Mesic grasslands are frequently referred to as being derived from or maintained by fire. This is because fire specifically influences the species richness, heterogeneity, and diversity of the vegetation (Bond and Keely, 2005; Pyke et al., 2010), both alone and in conjunction with other elements such as precipitation (Sankaran et al., 2005) and grazing (Bond and Keely, 2005). A low fire frequency decreases species richness in South African grasslands (Kirkman et al., 2014). Additionally, fire has an impact on soil and water resources, which are essential to ecosystem processes (DeBano et al., 1998). The susceptibility of grasslands to fire depends on environmental conditions such as wind and drought (Noble, 2008), hence understanding the relationship between grasslands and fire as well as climatic changes is integral for grassland management.

The current climatic changes include erratic weather events such as drought (Thomas et al., 2007). Drought is defined as temporary periods of dry soil caused by a decrease in current wet seasons rainfall (Dai, 2011) and it accelerates desertification. Since the 1950s, drought events have intensified in southern Africa (Thomas et al., 2007). Elevated temperatures and the reduction of rainfall are the main driving factors of drought. Increased temperatures actively increase the frequency and intensity of droughts due to increased evaporation rates which reduce soil moisture, creating very dry conditions. An increase in seasonal fluctuations and intensity of extreme rainfall events may also be expected (Porter and Samenov, 2005), specifically in South Africa, where droughts are expected to become more frequent (Reed et al., 2007). Drought has numerous impacts on the environment, such as desertification, loss of water and agricultural damages which include crop failure and food shortages.

Plant and soil nutrient relationships can also be significantly impacted by drought. For instance, plant tissue under drought stress had lower concentrations of nutrients such as nitrogen and phosphorus (He and Dijkstra, 2014). The two most significant limiting elements in grasslands are phosphorus (P) and nitrogen (N), both of which are essential to plant development (Fay et al., 2015). In grasslands, competitive relationships are also influenced strongly by selective and non-selective defoliation where selective defoliation gives a competitive advantage to unpalatable species and non-selective defoliation to palatable species (Fynn et al., 2005). High nitrogen has been shown to lower species diversity and dramatically raise above-ground biomass (Gao et al., 2021; Zhao et al., 2019). Nonetheless, it has demonstrated that while adding nitrogen decreased species diversity, it boosted functional richness (Mao et al., 2017; Niu et al., 2014). Nitrogen fertilization also has the ability to control the impact of phosphorus fertilizer on the ecosystems (Bracken et al., 2015). Phosphorus fertilizer enrichment either increases or has no effect on species diversity and ecosystem productivity (Gao et al., 2021; Menge and Field, 2007). In general, nutrient enrichment leads to reduced species diversity and compositional changes, which eventually affect the functioning and resilience of the ecosystem (Harpole et al., 2016). Drought limits the availability of water and as a result plant nutrient uptake, thus influencing biomass production and ultimately plant species composition (Valliyodan and Ngoyen, 2006). While the functional structure and diversity of communities have been evaluated in the past in response to drought (Carmona et al., 2015; Nunes et al., 2017) or reduced nutrient limitation (Helsen et al., 2014; Suding et al., 2005), the simultaneous effects of both are still unknown. One study found that the interaction of drought with nutrient enrichment changed the dominance of plant traits, decreasing plant biodiversity (Nogueira et al., 2018). In terms of plant competition, trade-offs in leaf width, specific leaf area, and height result in species differentiation in competitive ability along a fertility gradient (Fynn et al., 2005).

Drought also affects root density. The size, length and depth of roots determine the plant’s ability to absorb nutrients and water, influencing plant growth (Franco et al., 2011), but further understanding of how water stress affects root depth is required. The fluctuation of rainfall had a greater effect on species abundance compared to grazing (O'Connor, 1994). In the following dry years, plant density increased due to tuft fragmentation and recruitment, however, in a severe final drought year, density declined due to plant mortality (O'Connor, 1994). Considering the accelerated frequencies and intensities of drought, insight into the effect of drought on species composition and ecosystem function is required to improve the management of grasslands and introduce climate change mitigation strategies. This study seeks to investigate the changes in species composition and species functional traits brought about by drought in South African mesic grassland.

1.2. Problem statement

Even with the range of ecosystem services provided by grasslands, this biome is under massive threat from global environmental change (Piao et al., 2006). African grasslands are extremely vulnerable to the current rate of environmental change, and since grasslands are highly sensitive to anticipated changes, they have limited capacity for adaptation (Piao et al., 2006). Studies considering the sensitivity of grasslands to factors such as fire and nutrient status are important in light of the current fluctuations in climate. Recent climate change projections predict an increased impact on terrestrial ecosystems over time (Piao et al., 2006), however, vegetation responses differ with each biome (Piao et al., 2006). The changes in environmental conditions, specifically drought, limit resource availability, affecting the performance of individual plant species. Drought has significant effects on species composition and the general plant community (White et al., 2000). There is limited information available on the effects of drought on plant species dynamics in mesic grasslands in southern Africa. Given the global importance of grasslands, we must advance our understanding of plant community responses to environmental change. More information on the effects of multi-year droughts on South African mesic grassland is necessary to understand the extent and pace of changes in species composition and functional traits to improve management regimes and mitigate the effects of drought.

1.3. Justification

Environmental changes can result in grassland degradation which translates into economic losses (Wessels et al., 2008). Grassland degradation includes loss of productivity, quality, biological diversity and economic potential (Zhou et al., 2015). The anticipated impacts of climate change warrant this research study on grasslands and their response to environmental variability. More research is still necessary to provide management techniques and mitigation strategies on the impacts of climate change and for the creation of solutions that promote the sustainable use of grasslands. It is specifically important to investigate the influence of drought on ecosystem dynamics such as species composition, structure, productivity and root depth to provide insight into drought resistance strategies. Additionally, fire has been a vital management component that has been used for many years for the maintenance of grassland diversity (Boval and Dixon, 2012; Lunt and Morgan, 2002). Henceforth, it is important to establish the interactions of fire and drought on plant communities. The management and conservation of grasslands are crucial to the balance and production of food demands and other ecosystem services (Boval and Dixon, 2012) and mitigate the potential threat to food security caused by drought. Furthermore, the results of this study can contribute to grassland management strategies that promote the success of post-disturbance recovery. The project has the potential to contribute to knowledge of the impacts of drought on grasslands on a global scale through the Drought-Net Research Collaboration Network (DroughtNet, 2024), which is an international network that has various projects that assess terrestrial ecosystem sensitivity to drought in various areas around the globe.

1.4. Research questions

- What effect does a short-term drought have on species composition under different burning regimes without grazing?
- How do multi-year rainfall reduction and addition influence species composition, biomass production and grass species functional traits in a mesic grassland sward?
- What influence does drought have on grass species interactions?
- What is the response of plant communities to nutrient addition (nitrogen, phosphorus, potassium and micronutrients) under 50% rainfall reduction and 50% water addition?

1.5. Objectives

The main aim of the study is to investigate the impacts of multi-year above- and below-average rainfall and single-year drought on South African mesic grassland ecosystem dynamics therefore contributing to the understanding of drought effects worldwide through the Drought-Net initiative. This may be achieved through the following primary objectives:

- To investigate the effects of multi-year, severe drought and above-average rainfall on species composition and biomass production of plants in a mesic grassland.
- To quantify the effects of drought, above-average rainfall and nutrient addition on plant community species composition (diversity, richness and evenness) and biomass.
- To determine the influence of a single-year drought on species composition under different burning management regimes regimes exclusive of grazing.
- To investigate the effect of drought on grass-grass interactions.

1.6. Outline of dissertation/thesis structure

Chapter 1 is the general introduction with research questions, problem statement, justifications and objectives of the thesis.

Chapter 2 comprises the literature review of this study.

Chapter 3 investigated the effects of a short-term drought on vegetation structure, inclusive of plant species composition and diversity on the mesic grassland under different burning management regimes. This chapter also focuses on the interaction between drought and burning management.

Chapter 4 assessed the influence of drought on grass-on-grass interactions in pot trials.

Chapter 5 focused on how the grass sward composition, biomass production as well as root biomass and length were influenced by drought (50% rainfall reduction).

Chapter 6 assessed the influence of nutrient addition (nitrogen, phosphorus, potassium) on species composition and biomass production under different moisture levels in a mesic grassland.

Chapter 7 is the final chapter, which provides the general discussion and conclusions as well as recommendations and future research possibilities.

CHAPTER 2: LITERATURE REVIEW

1.7. Introduction

Drought is a period of moisture deficit compared to the normal state of that given environment based on the local climate (Dai, 2011; IPCC, 2014; White et al., 2000). Drought can be categorized as hydrological, agricultural and meteorological drought (Dai, 2011). A meteorological drought results from a lack of rainfall, a hydrological drought from a lack of surface and sub-surface water and agricultural drought from a lack of soil moisture availability (Dai, 2011). Global climate models predict reduced rainfall frequency, leading to more frequent and longer droughts (IPCC, 2013; Knapp et al., 2001; Meehl et al., 2000; Schar et al., 2004). An increase in drought frequency is attributed to a decrease in rainfall and increased evapotranspiration from elevated temperatures (Trenberth et al., 2014). This is likely to alter the structure and function of grassland ecosystems (Fay et al., 2001; Knapp et al., 2001). The drought period and its impacts on agriculture, plant communities and ecosystems may last for a season or might persist for many years, with varying chances of recovery. Drought is the most expensive environmental hazard globally and influences water resources, agricultural productivity and natural ecosystems (Mishra and Singh, 2010). Depending on the duration and intensity of the drought, coupled with the vulnerability of the system in question, drought can have detrimental consequences for natural systems, which may include damaging wildfires, high plant mortality rates and low vegetation productivity (Cook, 2019). The investigation of grassland responses to erratic rainfall events and prolonged drought is important in understanding how mesic grasslands respond, survive and possibly recover from extreme environmental conditions in the future.

1.8. Grassland biome in South Africa

Grasslands are open canopy ecosystems with extensive grass cover (Blair et al., 2014) . The South African grassland biome covers 29% of the country, occurs in eight provinces and is mostly situated on the high central plateau commonly known as the Highveld (Figure 2.1). Compared to other biomes, it is the second largest and second most diverse in South Africa (SANBI, 2013). Species composition is dominated by hemicryptophytes of the Poaceae family (O'Connor and Bredenkamp, 1997). Despite the relatively uniform structure of grasslands, there is a wide range in the floristic composition and functional characteristics of plant species, and vegetation dynamics as influenced by abiotic environmental factors like mean annual precipitation, temperature, fire and soil type (O'Connor and Bredenkamp, 1997), and altitudinal gradient (Mucina and Rutherford, 2006).

There are two main types of grasslands in South Africa, fire climax (mesic) and climatic climax (dry) grasslands are defined by annual rainfall (Tainton, 1999). This study is based on mesic grassland defined as receiving >500 mm of rainfall annually (Ellery et al., 1995). The stable rainfall in the grassland biome results in grasslands being a centre for livestock production, particularly cattle grazing (Palmer and Ainslie, 2005). South African grasslands have experienced extensive human impacts. Most of these impacts have been due to grassland conversion to cropland and forestry plantations, but also include urban growth and mining (Mucina and Rutherford, 2006). In South Africa, grasslands are one of the most extensively modified biomes, with 30% of that change being irreversible. Poor agricultural management has partially degraded another 30% of South African grasslands, also causing woody species encroachment, as a result, South African grasslands have become extremely fragmented (Oudtshoorn et al., 2011).

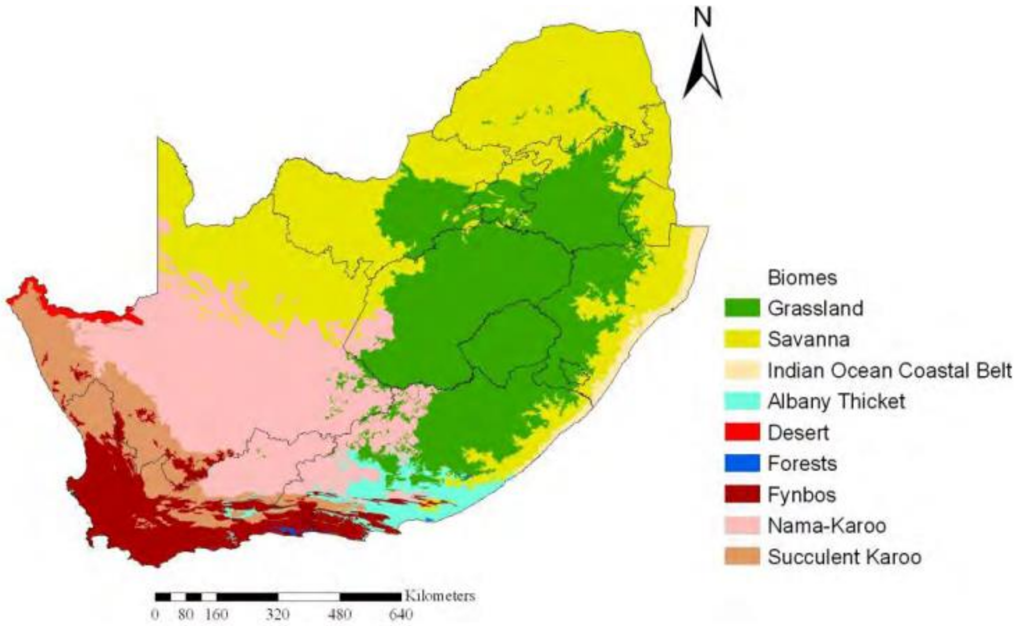

Figure 2.1: Map of South Africa with the grassland biome in green (Mucina and Rutherford, 2006).

1.9. Mesic grasslands

South African mesic grasslands are seasonal, having an annual rainfall above 500 mm that predominantly falls in the summer months (Ellery et al., 1995). Mesic grasslands are usually prevented from transitioning to savanna and woodlands by regular fires (Wills, 2015). The mesic grasslands of KwaZulu-Natal experience high precipitation during the growing season and frequent fires during the dry season. Long-lived tufted sourveld grasses and geophytic and bulbous forbs with a restricted ability to rebound from severe disturbance are their defining characteristics (Camp and Smith, 1997). This biome is also home to many rare, endangered and endemic plant and animal species (SANBI, 2013). The rich population of herbaceous perennial forb species found in South Africa's mesic grasslands outnumbers grass species by up to 5.6:1, offers a range of ecosystem services in addition to serving as forage, and is vulnerable to overgrazing (Morris and Scott-Shaw, 2019). Healthy grassland systems provide forage, habitats and water for humans, livestock and wildlife. In these systems, the herbaceous vegetation is rarely stressed because of high soil moisture during the growing season and because plant dormancy occurs as a mechanism to survive winter low rainfall and temperatures (Wills, 2015). The increased seasonal and annual minimum and maximum temperatures, as well as altered precipitation patterns, are some of the effects of climate change on grasslands (Houghton et al., 2001; IPCC, 2014). During water-limited periods, warming increases water stress and negatively affects the net primary productivity of these grasslands (Grant et al., 2015). The projected intense and infrequent precipitation events increase the variability of the soil water content and place the vegetation under stress (Grant et al., 2015). Therefore, it is important that grassland vegetation responses to drought are routinely assessed.

1.10. Climate change

The United Nations Framework Convention on Climate Change (UNFCCC) defines climate change as “a change of climate which is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and which is in addition to natural climate variability observed over comparable time periods” (IPCC, 2013). Global warming occurs when the sun’s rays hit the earth’s surface and only 30% of the heat waves are reflected in the atmosphere (Shahzad, 2015). The earth warms because of the excess heat being absorbed by the land, air, and ocean when harmful ultraviolet radiation penetrates the ozone layer (Shahzad, 2015). As a result, increased concentrations of greenhouse gases, such as carbon dioxide, methane, and nitrous oxide, disrupt the electromagnetic energy transfer equilibrium in the atmosphere, leading to global warming. Currently, the atmospheric concentrations of greenhouse gases have increased due to human activities such as the burning of fossil fuels, cement production, and deforestation (IPCC, 2013).

The mean surface air temperature of the earth increased by 0.6 to 0.9°C globally between 1906 and 2006 (Grant et al., 2015; Shahzad, 2015), with more rapid increases of between 1.1 to 2.6°C from 1986 to 2005 (Collins et al., 2013; Engelbrecht et al., 2015). A key consequence of global warming is the accelerated hydrological cycle which has increased climate variability (Giorgi et al., 2011). This has led to changing precipitation patterns and more frequent and intense weather events, such as extreme drought and heavy rainfall (Collins et al., 2013; Cook, 2019; Giorgi et al., 2011). The high temperatures also support high evaporation rates which reduce soil moisture levels and promote the occurrence of droughts (White et al., 2000). Climate change can have detrimental impacts on natural and agricultural ecosystems.

1.11. Climatic conditions leading to drought

The IPCC (2007), predicts higher temperatures and more frequent droughts induced by climate change throughout the globe. Several climate prediction models also suggest a gradual temperature increase and greater frequencies of heat waves and drought (Fischer and Schar, 2010; Meehl et al., 2000; Schar et al., 2004). Intensified and frequent rainfall promotes the reduction of soil-water infiltration and increased runoff, with longer periods between such events, thus exacerbating drought (Cook, 2019). Climate change is predicted to have a considerable influence on evapotranspiration, soil moisture content and rainfall (Knapp et al., 2001). Limited moisture availability is also expected to reduce relative humidity (Berg et al., 2016), and increase temperatures which accelerate vegetation curing (Williams et al., 2013). Since grasslands are dependent on the availability of water, the ongoing global change threatens the sustainability of grassland ecosystems through chronic alterations in weather patterns and resource accessibility (Zhou et al., 2022).

1.12. The impacts of drought on the ecosystem

Climatic factors, mainly temperature and rainfall are the primary determinants of vegetation distribution (Bond et al., 2003). Rising temperatures, erratic rainfall patterns and higher frequencies of extreme weather events (Meehl et al., 2000; Schar et al., 2004), lead to accelerated ecological degradation, which has been widely reported in grasslands (Yin et al., 2014). Ecological vulnerability to climate change is dependent on the capability of natural ecosystems to cope with stress. Dry conditions hinder photosynthesis, and increase wildfire occurrence (Abatzoglou and Williams, 2016) as well as pests and plant diseases. These factors lead to high plant mortality rates, and low plant production and threaten regional biodiversity (Wang et al., 2010), negatively affecting the entire ecosystem.

A combination of different climatic variables such as temperature, relative humidity, evaporation and rainfall have a strong influence on vegetation (Kutiel et al., 2004) . The impact of each variable differs to an extent that some variables may even be limiting factors to the plant community of a given vegetation type. Water stress pushes organisms to the brink of their physiological threshold, hence plant communities may be left vulnerable to disturbances, reducing the resilience of the vegetation to environmental change (Gutschick and BassiriRad, 2003). Water limitation is a threat to global food security as it significantly contributes to land degradation (Tigkas et al., 2019), a process that is compounded by unsustainable land use such as overgrazing and land transformation (Emadodin and Reinsch, 2018).

Grass species distributions may change in response to climate change as the local environmental conditions diverge from those optimal for the species. Changes in the local environment can lead to species reordering or extinction, ultimately altering long-term community composition within grassland plant communities (Jones et al., 2017). For example, the loss of perennial grasses can trigger ecological regime shifts, perhaps promoting the dominance of annual grasses or shrubs (Baez et al., 2013), or the transition from native to invasive grasses (Abatzoglou and Kolden, 2011; Munson et al., 2013). These transitions are frequently accompanied by losses in ecosystem services, such as reduced forage availability (Izaurralde et al., 2011), declining soil carbon and nutrient availability (Meisser et al., 2019) increased bare ground, and ultimately greater soil erosion rates (Tigkas et al., 2019).

Drought can strongly affect ecosystem function by changing the relationship between plants, microbes and soil chemistry, eventually impacting nutrient cycling (Meisser et al., 2019), given that internal nutrient cycling in plants is a crucial biological process that will affect plant growth and productivity (Wright et al., 2002). Although there is limited data available on root trait responses to drought, root traits are important indicators of various ecosystem processes including carbon and nutrient cycling and biomass accumulation (Bardgett et al., 2014). The lack of root trait literature hinders our ability to predict grassland ecosystem functioning and respond to climate change drivers, like drought episodes. Understanding the response of grassland ecosystems to drought is vital in assessing how vegetation responds and survives under extreme climatic conditions and later contributes to our preparedness for projected drought occurrences and the formulation of mitigation strategies in future.

1.13. The effects of drought on plant biomass production

Water deficit is one of the fundamental consequences of drought and has a major influence on plant growth and yield (Jaleel et al., 2009). Drought is a key environmental factor that impairs various plant physiological and metabolic processes including respiration, ion uptake, photosynthesis, translocation and nutrient metabolism (Farooq et al., 2009) which may result in the suppression of plant growth and productivity, or even lead to plant mortality (Chen et al., 2015). As a reaction to increased transpiration rate under limited water availability, plant stomata often close (Manzoni et al., 2011). Stomatal closure prevents water loss from transpiration pathways, but stomatal closure not only depends on loss of water through the leaves but is directly sensitive to soil dehydration (Hoshika et al., 2013). Stomatal closure reduces net photosynthetic activity which ultimately causes a reduction in biomass production (He and Dijkstra, 2014; Pirasteh-Anosheh et al., 2016; Zhou et al., 2015). It is not clear if high moisture deficits, warming or the two combined have the greatest effect on the decline of plant productivity but when water loss becomes severe, photosynthetic activity may be completely arrested, disturbing plant metabolism and again ultimately causing plant death (Zhao et al., 2015).

Plant community response to drought can be framed as the sensitivity of dry matter productivity to water limitations (Knapp et al., 2015a). The extent to which the biomass yield of grasses is affected by drought is difficult to predict even though biomass loss due to drought has been reported in various studies (Gibson-Forty et al., 2016; Jaleel et al., 2009). The magnitude of loss is variable because of various factors such as plant diversity and management regime (Grant et al., 2014; Zwicke et al., 2013). Several studies have established that the variations in precipitation have been associated with changes in plant community especially the primary productivity of plants (Reynolds et al., 2015). In grasslands, net primary production (NPP), and biomass allocation are all impacted by extreme drought. A 4-year experimental drought treatment on alpine grassland, for example, found that drought did not impact total net primary production (NPP) but instead moved more net primary production belowground (Liu et al., 2018).

A severe drought reduces the ratio of leaves to stems and increases the concentration of fibre by inhibiting growth, accelerating the death of tillers and causing leaf senescence, which eventually reduces the biomass production of a plant (Bruinenberg et al., 2002; Ren et al., 2016). However, moderate drought stress usually results in distinct morphological responses, such as delays in plant growth and maturation, particularly for perennial species, and relatively mild to moderate leaf loss (Buxton, 1996). Annual biomass was reduced by a quarter under mowing and grazing in a severe drought treatment of 74% (Deleglise et al., 2015). In the same study, in the months following the drought, biomass remained in decline in the temperate grassland, which shows that the impacts of drought and that the mowing and grazing also influence biomass production when combined with drought (Deleglise et al., 2015).

Yield losses have been documented in numerous studies as a result of droughts; however, their extent can vary greatly because of a variety of biotic and abiotic factors (such as plant diversity, productivity, altitude, and management) that may interact (Bruinenberg et al., 2002; Gilgen and Buchmann, 2009; Grant et al., 2014; Ren et al., 2016; Vogel et al., 2012; Zwicke et al., 2013). Additionally, a decrease in the diversity of herbaceous plant species results in a less effective use of soil nutrients and a reduction in biomass production (Hooper et al., 2005). In another study, plant productivity was maintained throughout a drought in both Kansas and Nebraska natural grasslands during the 1930s, but it was only maintained by a shift in species composition to more drought-tolerant species (Weaver, 1968). This illustrates that plants differ in drought resistance and response depending on the plant growth stage and the intensity and duration of the drought (Jaleel et al., 2009). Hence it is important to understand how various plant traits, such as productivity respond to drought. An improved understanding of vegetation sensitivity to climate change is essential for better response predictions.

1.14. Species composition: diversity, richness and basal cover

Changes in species composition are expected with the reported global climate change (IPCC, 2007; Smith, 2011). Grassland vegetation is more susceptible to changes caused by water deficit and elevated temperatures than other biomes (Xu et al., 2018). This can be attributed to herbaceous species in grasslands having low carbon and water storage capacity compared to woody species (Craine et al., 2013). Experiments that simulate the effects of extreme rainfall or drought and warming have been established in various ecosystems (Fay et al., 2008; Smith, 2011), but one that is of immediate interest is that of a mesic grassland in this study. Extreme climatic events may cause a shift in the population of local species, causing changes to the plant community and its ecosystem. One of the main environmental risks is droughts, which can limit the geographic distribution of plant species and lower plant productivity as well as cause widespread plant death (Tilman and Haddi, 1992; Zhao and Running, 2010). Droughts can therefore be considered a disruption to the structure and functioning of ecosystems.

Over 11 years, extreme temperatures and prolonged rainfall deficits reduced basal cover and altered species composition, particularly that of perennial grasses, in a semi-arid rangeland in Arizona (Bodner and Robles, 2017). Other studies have reported similar declines in vegetation cover in both the savanna and grassland biomes (Bodner and Robles, 2017; Hamerlynck et al., 2013; Knapp et al., 2015a). Drought drives strong changes in species diversity (Gilgen et al., 2010; Joseph et al., 2012; Jung et al., 2014; Stampfli and Zeiter, 2004). Species diversity can be divided into two main components: richness, representing the number of species in that given area, and evenness, which is the variability in species abundance (Magurran, 2004). Some theoretical studies have suggested that there is a consistent positive relationship between evenness and richness (Alatalo, 1981; Hill, 1973), while more recent empirical studies suggest that the relationship between species richness and evenness is not constantly positive and may differ according to local environmental factors (Manier and Hobbs, 2006; Stirling and Wilsey, 2001). An extreme climatic event such as drought can act as an environmental filter by allowing only certain species to persist, often leading to decreased biodiversity (Grime, 2006; Whitney et al., 2019).

Radical changes in species composition are only likely to take place under severe drought conditions (Stampfli and Zeiter, 2004). In their Switzerland study, these authors found that an extreme drought induced a shift in the species' relative cover, resulting in a reduction in grass cover and an increase in forb cover. This could be caused by the abundant grasses failing to re-colonise due to few, short-lived seeds compared to forbs or other grasses which were better equipped to germinate. No new species colonised the area after the drought, but the success of existing plants with high regenerative capacity indicated that drought leaves plant communities susceptible to colonisation (Stampfli and Zeiter, 2004). To assess physiological drought tolerance and the role of leaf functional traits, over 400 grass species were collected from six continents and grown at Kansas State University under high-intensity lighting and stimulated sunny conditions to create elevated temperatures (Joseph et al., 2012). After six weeks, watering was stopped, and it was found that drought-resistant grasses are fairly well distributed across the world. Plants tolerate drought through their ability to access stable or stored water and by accelerating reproduction. This study also showed that diversity increases the chances of survival of individual plants in a grassland because species-rich areas are more likely to be composed of drought-tolerant plant species (Joseph et al., 2012). Drought changes the composition of species in a community as it has been found to increase grass mortality over time (Browning and Archer, 2011). Dry conditions tend to favor the dominance of woody shrub species as the high grass mortality rate creates available niches for the woody species (Winkler et al., 2019).

1.15. The impact of nutrient addition and drought on grassland plant communities

The nitrogen, phosphorus or potassium nutrients are known to cause some limitations to the terrestrial productivity of grasslands and cause changes in the plant community. Furthermore, these nutrients were often co-limiting, limiting grassland productivity together more than the sum of their individual limitations (Fay et al., 2015). However, the implications of nutrient addition, coupled with drought stress are still poorly understood in the grassland systems. The uptake, transport and partitioning of nutrients such as nitrogen, phosphorus, potassium and other important minerals are all affected by drought stress, which can inhibit plant growth and reproduction (Gessler et al., 2017; Hu and Schmidhalter, 2005). The reduction in soil moisture may constrain plant nutrient uptake by reducing nutrient availability (Alam, 1999). For instance, rainfall reductions of 50 mm reduced carbon uptake by approximately 55 gm^{-2} (Raz-Yaseef et al., 2015). Drought decreases soil nutrient availability by reducing nutrient uptake and nutrient transportation from plant roots to shoots which inevitably decreases leaf size and photosynthetic activity. Low soil water content also reduces the nutrient diffusion rate in the soil to the absorbing root surface (Chaves et al., 2003). This means that even though nutrients may be available through fertilization, insufficient water availability will still hinder plant growth and productivity.

Plant growth is heavily dependent on the availability of nitrogen which is important for the development of chlorophyll and photosynthetic enzymes. Drought causes a substantial drop in soil nitrogen concentration (Rennenberg et al., 2009). However, after several years of drought, other research (Johnson et al., 2008) reported no effect of drought on total nitrogen. Drought also promotes the reallocation of nutrients to younger leaves (Chaves et al., 2003). The length and severity of droughts contribute to the discrepancy (Hartmann et al., 2013). Both organic matter decomposition and microbial activities are highly sensitive to changes in temperature and moisture (Nielsen and Ball, 2015) and the availability of nitrogen is not only affected by plant organic matter input (Gavrichkova et al., 2019), but also by microbial activities (Chen et al., 2017). The knowledge about the effects of drought on available nutrient dynamics is essential for the prediction of the ecosystem response to the increasing frequency and intensity of extreme climate events (IPCC, 2013).

This study specifically focused on the addition of nitrogen, phosphorus and potassium to determine how the grassland plant community is affected. The addition of fertilizers has an important effect on plants in general, especially plant productivity, thus, adding nutrients may either promote or reduce plant resilience during a drought, depending on the amount of water available. Future droughts will cause structural and functional changes in a variety of ecosystems; thus, it is critical to comprehend how the climate may interact with drought characteristics as well as nutrient addition to affect the species composition and productivity of grasslands. Phosphorus is required for energy transfer, enzyme regulation, transportation of carbohydrates and carbohydrate storage. However, the addition of phosphorus can considerably improve plant growth in drought conditions, through increased stomatal conductance and photosynthetic activity (Garg et al., 2004). Inversely, drought stress can reduce both plant nitrogen and phosphorus (He and Dijkstra, 2014). Various studies have shown that the addition of potassium mitigates the devastating effects of drought by increasing the resistance of plants through improved stomatal regulation and protein synthesis (Garg et al., 2004; Hu and Schmidhalter, 2005; Sangakkara et al., 2001).

1.16. Effects of drought and burning management on species composition

Fire is a key factor in grassland vegetation dynamics and is responsible for sustainable ecosystem function (Scheintaub et al., 2009). Fire has shaped the evolution and appearance of many plants in grassland ecosystems and directly influences the structure of plant communities. For example, fire-tolerant perennial forbs with underground storage organs and perennial tussock grasses are characteristics of often burned grasslands (Parr et al., 2014). Mesic grasslands are prone to fire and depend on it for the maintenance of biodiversity (SANBI, 2014). However, since the projected high drought frequencies may affect biodiversity, this research aimed to provide insight into the response of species composition to short-term drought and various fire regimes. Climate change affects fire behavior in two ways, by increasing temperature and dry weather conditions, particularly in the months before the burning season. The reduced fuel moisture content increases the likelihood of ignition and fire spread. Areas may have the same climatic conditions but have different natural fire regimes and that may be attributed to variations in extreme weather conditions, fuel properties or the position of natural fire breaks such as rivers (Bond and Wilgen, 1996). Natural fires are also occurring at higher frequencies due to the changing climate (Rowland et al., 2010). Plant water uptake is limited in dry soils, hence live fuel moisture is reduced during drought (Westerling et al., 2002).

The influence of fire on species composition has been well documented (Everson and Everson, 2016; Parr et al., 2014; SANBI, 2014; Tainton and Mentis, 1984). Withholding fire may shift the plant composition of grassland towards woody encroachment (SANBI, 2014) and may cause a reduction in grass biomass production and forage quality overall (Everson and Everson, 2016). Excessive fire may also become problematic to species composition, specifically for forbs, as more frequent burns may destroy the mesic grassland's natural pollinators (SANBI, 2014). Excessive fire can cause direct pollinator mortality (Love and Cane, 2016), which affects plant species composition by reducing the reproductive success of flowering plants and seed production (Rodger et al., 2021). Additionally, frequent fires kept the grassland species composition mostly unchanged for over 30 years while the unburned veld had lower veld condition scores in the montane grasslands of KwaZulu-Natal (Everson et al., 1985a). In addition to the removal of moribund grass material, fire is sometimes used to get a nutrient-rich green flush of new growth (Scholes and Archer, 1997). In both Ukulinga and Konza, frequent fires promoted a grass dominated grassland and fire exclusion incited bush encroachment (Kirkman et al., 2014).

Areas with fire exclusion at a mesic site in the Kruger National Park were colonized by firesensitive forest species (Bond et al., 2003). The absence of fire also caused a decline in important dominant grasses. *Themeda triandra* cover decreased from 70% to less than 10% within three years in fire exclusion plots in the Midlands of Kwa-Zulu Natal (Tainton and Mentis, 1984). In the Cathedral Peak area of the Drakensberg Mountains, KwaZulu-Natal, the basal cover was found to be higher in areas with higher fire frequencies (Gordijn et al., 2018). This is due to the ability of grasses to recover rapidly after burning, with the removal of aerial cover that shades tillers, promoting grass growth. With long fire return intervals, grass biomass or moribund vegetation accumulates and shades out the short plants, while the growth of taller plants may exceed grass canopy cover, increasing their chances of survival. Under shorter fire return intervals, grasses that are strong competitors are favored, hindering the recruitment of other plants (Lunt and Morgan, 2002).

The amount of available precipitation modifies the impacts of fire in grasslands (Anderson et al., 2007; Porensky et al., 2013). For instance, soils are usually drier in burned sites, and differences in soil moisture are emphasized in low-rainfall years (Knapp et al., 1998). Ecologists are mostly concerned with the changing climate in relation to both grazing and fire and how the grasslands will be affected. For example, the interactive effects of varying precipitation, grazing, and fire management could significantly influence plant community composition (Anderson et al., 2007; Porensky et al., 2013) and grassland biomass production (Frank, 2007). One study found that drought decreased species diversity at a high burning frequency (Koerner and Collins, 2014). However, there is a knowledge gap in the relationship between fire and drought, which this study has the intention to fill. The information from this research is particularly important for the management of grasslands as well as for future predictions of fire behavior influenced by the weather. Anticipating the implications of both historic and future droughts on fire regimes is a critical element of adaptation.

1.17. Influence of drought on species interaction and competition

Plants compete for nutrients, light, and water in a community, and the coexisting species have different environmental needs (Grant et al., 2015). The interaction is facilitative when plants improve the environment for another plant (Grant et al., 2015). Legumes, for instance, have nodules that fix atmospheric nitrogen and, through increased soil nitrogen, encourage the growth and establishment of the surrounding plants. Some plants shade nearby plants, which reduces transpiration and increases the amount of water available in the soil (Grant et al., 2015). Variability in temperature and precipitation disturbs patterns of cooperative interaction amongst plant communities (Grant et al., 2015; Scholes and Archer, 1997).

The interactions between plants are key determinants of the structure and functioning of grassland ecosystems (Hautier et al., 2009). Such interactions affect the distribution and abundance of species (Grant et al., 2015). For instance, stress-tolerant species usually persevere during extreme environmental conditions, given their competitive abilities over stress-intolerant species (Grant et al., 2015). This means that climatic conditions can change the species composition of a given area, based on the competitive abilities of a given species. Environmental stress affects competitive species more than stress-tolerant species (Grant et al., 2015). Facilitation and competition in semi-arid areas are important in determining ecosystem stability. In observational studies, it was shown that plant-plant interactions may shift from facilitation to competition, depending on water availability (Butterfield et al., 2016). In a simulated environment investigating drought and an increase in precipitation, the response of interspecies interactions depended on diversity and competition between species (Jentsch et al., 2009).

The variations in response to water stress are species-specific. For instance, the same morphological traits responded differently to water deprivation. An example was observed in *Pastinaca sativa*, which due to its deep roots had better tolerance to drought by extracting nutrients and water from deeper soil depths as opposed to other plants (Tutin, 1980). Also, annuals are more likely to disappear during prolonged drought while perennials have a higher survival rate because germination is water-limited (Bosy and Reader, 1995). Facilitation still prevails under moderate water stress but declines with extreme stress levels (Butterfield et al., 2016). Facilitation may sustain the productivity of all plants and maintain species diversity during periods of stress while negative interactions reduce diversity, possibly leading to extinction (Hautier et al., 2009). Another competitive trait is rapid plant growth for the acquisition of light and space, especially as a means to recover from drought (Gonzalez and Loreau, 2009). Higher photosynthetic rates are essential for rapid growth, but so is the capacity to draw nutrients and water from the soil (Grime, 1979).

A list of some critical plant traits which are related to a plant's competitive ability in different environments was provided by (Goldberg, 1996). The characteristics that predict the competitive ability of a plant include larger leaf size and greater plant height, as well as a greater accumulation of above-ground biomass (Frecketon and Watkinson, 2001; Trautz et al., 2017; Warren et al., 2002). The plant traits that create a strong competitive ability include the ability of a plant to take up more space. The plant must be able to avoid being shaded by other plants by growing taller or tolerating shade with broad leaves and a high accumulation of nitrogen in the leaves. There is evidence that shows the combination of being tall and broad-leaved exerts dominance, but mostly in fertile and resource-rich environments (Keddy et al., 2002; Leps, 1999; Tilman et al., 1994). On the contrary, species with narrow leaves were found to be more drought-tolerant than species with broad leaves (Craine et al., 2013; Hacke et al., 2000). Additionally, narrow-leaved grasses such as *Eragrostis racemosa* and *Eragrostis capensis*, were found to be strong competitors at low nutrient levels (Fynn et al., 2011). Although a large resource allocation to height and above-ground biomass may give a competitive advantage in resource-rich habitats, it may hinder competition in less fertile and drought-stricken habitats (Tilman, 1988). The impacts of drought on grass-grass interactions remains a subject that needs to be explored further, as there is limited information available on the subject and this study aimed to fill this gap. In summary, developing an understanding of the relationship between plant traits as well as their interactions has the potential to predict species' response to dry environmental conditions.

1.18. Root responses to climatic variations

Roots play an integral part in plant growth, and it is important to understand their response to dry conditions. Plant characteristics such as roots are increasingly being used to forecast and explain ecosystem functioning (Vries et al., 2016). Unlike leaves, roots are in direct contact with the soil, modifying the soil environment by penetrating the soil and absorbing water and nutrients (Bardgett et al., 2014). Because of this, root functional features may be more accurate predictors of ecosystem and soil processes than leaf traits and root responses to climate change are critical indicators of ecosystem functioning (Bardgett et al., 2014; Vries et al., 2016). Drought can change the phenotypic root features and affect the functioning of the root system (Borken and Matzner, 2009). Long-term precipitation deficiencies affect the growth and even increase the death of plants by reducing the amount of water that is available in the root zone (Seleiman et al., 2021).

Drought significantly reduced plant and root growth in grassland species (Vries et al., 2016). Root systems change physiologically in adaptation to dry conditions, with the root biomass of biennial and perennial species increasing after drought (Dreesen et al., 2012). Biennials and perennials often accumulate belowground reserves for regrowth the following year towards the end of the growing season. The type of roots can determine the defence mechanism a plant has against dry conditions. Plants may develop traits such as high root tissue density or root dry matter content which are linked to drought resistance. Unlike grasses. woody species that are adapted to dry climatic conditions normally have a high root-shoot ratio because of deeper root systems (Hartmann, 2011). However, many herbaceous species also have thin roots, elongated deeply into the soil, allowing them to acquire soil resources during dry periods (Zwicke et al., 2013). Plants may also respond to drought by allocating some resources to the root, such as carbon, and by adjusting chemical or morphological traits (Poorter et al., 2012). Data available on root trait responses to drought are limited to a small number of plant species and in most cases, are related to fertilization. For instance, under drought conditions, fertilization promotes root elongation and improved water and nutrient extraction from the deeper soil layers (Hu and Schmidhalter, 2005). A few studies report plant species producing thinner roots with high specific root lengths in response to drought, a strategy for water acquisition with low investment (Comas et al., 2013; Debinski et al., 2010). On the contrary, other research suggests that certain plant species generate thicker roots with a low specific root length (Zimmermann, 1983; Zufferey et al., 2011). More recently, drought has been found to decrease root length and increase root diameter (Zhou et al., 2019). This study attempted to bring forth more information on the response of belowground root biomass and length to different moisture levels.

1.19. Impacts of varying water levels on the plant community

Moisture limitations are a major limiting factor for the productivity of terrestrial plants (Weltzin et al., 2003). Severe droughts are thought to lower evapotranspiration by limiting the amount of water available, which lowers plant transpiration and soil evaporation (Seneviratne et al., 2010). Different responses to low soil water levels were observed from various plant species, but stomatal regulation, with lower stomatal conductance under high temperatures, was uniform throughout the species (Boeck et al., 2006; Lemmens et al., 2006). In addition, a distinct trend of low water efficiency caused by higher temperatures was also observed under high temperatures (Boeck et al., 2006; Weltzin et al., 2003) . Mesic grasslands are highly sensitive to moisture availability hence it is vital to understand how the grassland plant community responds to moisture variations. A higher rate of vegetation mortality can also be brought about by severe dryness due to lower photosynthesis and increased autotrophic respiration (Silva et al., 2009). Therefore, over time, the phytosociology of entire plant communities might change as a result of increasingly frequent and severe drought events and the resulting extinction of some plant species (Silva et al., 2009).

Drought triggers a variety of plant reactions. It can cause decreased height, smaller leaves, fewer leaves overall, and modifications to the reproductive phase (Ajum et al., 2011). Reducing the water supply at the plant establishment stage leads to dehydration and even death because water is the most crucial ingredient from germination and seedling formation to the establishment in the field (Jaleel et al., 2009). To promote plant growth in height and width, water is necessary for processes like germination, cell division, and elongation. Water is also necessary for metabolic activities like the synthesis of organic compounds, photosynthesis, respiration, and several other physiological and biochemical processes (Taiz and Zeiger, 2006). Therefore, all the molecular, biochemical, physiological, and morphological characteristics of plants change as the supply of water diminishes. Throughout their life cycle, plants require a lot of water and nutrients, and a decrease in the amount of water in the soil has an impact on every part of a plant's development.

Plant physiological and biochemical activities are impacted by the physical environment alterations brought about by this decrease in soil moisture (Silva et al., 2009). Even in fertilized soils, drought can result in nutritional deficits because it decreases the mobility and absorption of individual nutrients, which slows the pace at which minerals diffuse from the soil matrix to the roots (Silva et al., 2009). Drought is, therefore, without a doubt the main stressor limiting plant life. Plants may be able to survive drought with small, frequent rainfall events by remaining in an almost dormant state during dry spells until proper rainfall is received (Vandegeer et al., 2020). However, with extreme and infrequent rainfall events, which are predicted to increase with climate change, grasses may be negatively affected.

1.20. Plant adaptations to drought

How plants respond to drought stress depends on the duration and intensity of drought together with the stage of growth. Plants vary in their tolerance to drought. Some plants modify their traits to acquire more water whilst others have a greater water-use efficiency and are more drought tolerant (Taiz and Zeiger, 2006). Plants can tolerate environmental stress by accessing stored water resources or by accelerating reproduction to avoid drought-induced mortality (Craine et al., 2013). These drought-adapted plants are likely to survive these conditions, but only if it does not occur too frequently (Lemoine et al., 2018). Adaptation may be measured by comparing plant responses to a second drought against the first event, or by comparing plants exposed to a single late drought event to multiple drought events (Lemoine et al., 2018).

Repeated drought events may also threaten the resistance of plant communities by weakening biomass production (Zavalloni et al., 2008). One study recorded no evidence of plant acclimation, specifically in terms of water potential, stomatal conductance and photosynthesis in *Elymus elymoides* (C_3) and *Bouteloua gracilis* (C_4) grasses to up to two sequential drought occurrences (Lemoine et al., 2018). Increased temperatures due to climate change, specifically in humid regions, tend to benefit grasslands in conventionally colder areas by increasing the yield of both shrubs and C_4 grasses (Xu et al., 2012). On the initial days of an experiment, the *Brachypodium distachyon* grass showed reduced water potential, stomatal conductance and photosynthetic rate but under sustained drought, the grass adapted to water deficits by recovering in the above-mentioned parameters to similar levels as those of plants with adequate water levels. However, the recovery of these parameters was not matched by biomass as plants had a 50% reduction in biomass production compared to well-watered plants. Low water volume leads to reduced biomass but fewer significant impacts on biomass were recorded on treatments with low rainfall in different grasses namely *Cynodon dactylon, Lolium. perenne, Plantago lanceolate*, *Eragrostis leptostachya* and *Ornithopus compressus* (Gibson-Forty et al., 2016). Thus, the precipitation magnitude may have a greater effect on plant physiology than precipitation frequency on the productivity of grasslands (Gibson-Forty et al., 2016).

Since drought and its effects are quite a complex phenomenon, various theories have been presented to understand how plants respond to rainfall variations. The pulse reverse hypothesis describes the response of annual plants to drought and states that pulses of precipitation promote plant biomass production, including the allocation of resources to reserve structures (Noy-Meir, 1973; Ogle et al., 2015). This model is used to determine whether plant production depends more on the duration or frequency of rainfall specifically in arid rangelands. The model is easily applied to annual species as they often respond quicker to rainfall events. The ecological memory hypothesis, states that the length, strength and temporal patterns of preceding environmental and physiological conditions influence plant responses to ecological events (Ogle et al., 2015). An example of this is that rainfall patterns in previous months or years can change stomatal conductance, affecting the productivity of plants (Sala et al., 2012). This is ecological memory, which may result in positive or negative drought legacy effects where drought may continue to make changes to plant or ecosystem functions even after precipitation has stabilised (Sala et al., 2012). The deficit in soil water decreases plant water potential and increases leaf abscisic acid content (Acharya and Assmann, 2009). These changes to carbon and water relations can ultimately lead to increased water-use efficiency contributing to drought adaption (Vandegeer et al., 2020). One of the current study's objectives was to test the plant community response to a previously occurred short-term drought in different burning treatments, which relates to the ecological memory hypothesis. Severe drought causes mortality to an established plant community which allows for regeneration of perennial grasses. The success of regeneration is dependent on the growth rate of vegetation, tolerance of storage organs to drought, and the recruitment success of the resulting seedlings (Grime, 2001). The survival of plant communities is highly influenced by the availability of resources post-drought (Davis et al., 2000).

1.21. Plant responses to increased temperature

Due to high global greenhouse gas concentrations in the atmosphere, temperature is projected to increase gradually around the world (Houghton et al., 2001) and high temperatures are usually a contributing factor to drought (White et al., 2000). A generally warmer climate can be beneficial for plant productivity, as temperatures are closer to the growth optimum and the growing season is extended (Walther, 2003). On the other hand, extremely high temperatures accelerate evapotranspiration which lowers plant productivity. This leads to a decline in stomatal conductivity and photosynthesis, limiting plant production (Chaves et al., 2003).

An understanding of how both temperature with varying moisture availability affect plants is vital. The co-occurrence of these two factors can have a detrimental effect on plant physiology and productivity, more destructive than that of heat and drought in separate events (Boeck et al., 2011; Xu and Zhou, 2006). Overall, plant responses to climatic extremes vary according to the stage of development, which means the timing of a climatic event is of high importance. The interaction between high temperatures and drought has an impact on plant species richness (Boeck et al., 2008). The depletion of soil moisture through drought also amplifies heat extremes by decreasing evapotranspiration, which further reduces the chances of precipitation (Seneviratne et al., 2010).

In a study to quantify the impacts of a combination of drought and heat waves and to determine how both factors exacerbate or counteract with each other on the photosynthetic rate of plants, as well as biomass production, Dreesen et al. (2012), found that these factors were not affected by drought exposure without heat (Dreesen et al., 2012). Similarly, a rapid increase in photosynthetic activity as compensation after exposure to drought extreme has been observed (Mirzaei et al., 2008). Conversely, several studies are arguing that drought has limited implications on photosynthesis and productivity (Boeck et al., 2011; Kahmen et al., 2005; Llorens et al., 2003). These unexpected results could be attributed to the rapid recovery of photosynthesis after exposure to the drought period. Most literature though has recorded an increase in belowground biomass due to drought and lower rates of photosynthesis and biomass production (Carter et al., 1997; Chaves et al., 2003; Kahmen et al., 2005). Another possible explanation for the increased biomass after drought would be the shifting of species dominance, provided that the drought extremes were in favour of highly productive species in general (Dreesen et al., 2012). Biomass seemed not to be negatively affected by heat as it increased in heat-induced, drought. Temperature was recorded throughout the current study but was not considered as part of the objectives. It is, however, important to note that temperature due to warming may influence the observed results.

This study aimed to address the research gaps outlined in the research questions. It specifically addressed the effects of both short and long-term drought on species composition and aboveground and belowground biomass production of a mesic grassland. The sensitivity of drought-stricken grasslands to different burning regimes as well as nutrient enrichment were factors that were also considered in the research study.

CHAPTER 3: THE INFLUENCE OF A SHORT-TERM DROUGHT ON SPECIES COMPOSITION OF A MESIC GRASSLAND UNDER VARYING FIRE FREQUENCY TREATMENTS

Abstract

Climate and fire are key drivers of mesic grassland communities. With global climate change causing frequent drought events, understanding how grasslands respond to changes in fire and drought combined may aid in projecting future changes in grassland plant communities. The aim of this study was to determine the response of the mesic grassland plant community, under different prescribed burning regimes, to a single drought event. Plant community structure and composition were investigated in unburned, annually burned and triennially burned treatments before and after a single drought event that occurred in 2014/2015. Percentage cover of each species was visually estimated during the growing season of 2020 and compared with species composition data collected in 2005 and 2010, before the drought event. The results showed that burning regime significantly impacted species composition and that the single drought event did not have any significant impact on the species composition of the mesic grassland. Annual burning consistently increased species diversity and richness, while fire exclusion had the lowest values. Frequent burning promoted the abundance of palatable grasses, while fire exclusion led to a dominance of woody species, forbs and low palatability grasses. Triennial burning resulted in a more balanced mixture of grasses and forbs. In the burning treatments, fire demonstrated a noticeable trend in the species richness, variety, and abundance. Frequent fires maintained a rich, palatable grassland community, which is an advantage for livestock production. Furthermore, grasslands with frequently prescribed fire may be more resilient in surviving the impacts of short-term drought.

Keywords: *plant community, grasses, forbs*

3.1. Introduction

Fire is an important ecosystem driver in grasslands, which can alter the relative abundance and richness of both grasses and forbs, and the vegetation structure (Bond and van Wilgen, 1996). It is a frequent and consistent cause of change in a wide range of biomes and ecosystems worldwide. Particularly in grassland systems, fire impacts species composition and vegetation structure (Bond and van Wilgen, 1996). Fire frequency and intensity can be a major determinant of woody and herbaceous species abundance (Bond and Keely, 2005; Cavender-Bares and Reich, 2012). The use of fire as a management strategy can enhance ecosystem benefits for the advantage of grazers, by improving grassland health by removing moribund and unpalatable grasses, (Bond and Archibald, 2003), controlling invasive alien species (Dew et al., 2017) and reducing bush encroachment (Smith et al., 2016). Episodic releases of soil nutrients caused by frequent fire are likely to promote the growth of both annual and perennial grasses (Ignace et al., 2007). Thus, the frequency of fire can be an important regulator of ecosystem resilience to drought (Smith et al., 2016).

The challenge is that little is known about the interaction between burning management and drought and how both interactively impact grassland communities. It is also noted that grazing is also an important and influential factor to grassland communities in relation to fire and drought as well, however this chapter focuses specifically on fire and drought only. This research attempts to fill that knowledge gap. Grasslands with regular fires and reasonably consistent annual rainfall offer data that can be utilized to study how disturbance and resource availability affect different plant communities (Dukes et al., 2005). Rainfall availability is not always consistent due to climate change. Hence the experimental research of drought is imperative amid unpredictable rainfall due to climate change. The economic, social and environmental losses imposed by drought are of increasing concern at local and international levels. As a result, there is a pressing need to understand how water deprivation influences grassland responses. Since fire is a natural component of mesic grasslands (Dukes et al., 2005), it is especially important to understand how the relationship between drought and fire affects grassland communities. It is for this reason that this study investigates the impacts of a single drought event under prescribed, periodic fire management on grassland species composition. This is crucial for grasslands which are often limited in meeting multifunctional objectives, and sustainable agricultural production against the everchanging environmental conditions due to global warming (Fridley et al., 2016; Newbold et al., 2015).

Objective

To investigate the impacts of a short-term drought superimposed on annual, triennial and no-burn treatments, without grazing, on species composition dynamics such as diversity and richness of species and their functional groups.

3.3. Materials and methods

3.3.1. Study site

The study was conducted at Ukulinga Research Farm, University of KwaZulu-Natal, (29°24' E, 30°24' S), Pietermaritzburg, South Africa. It has an altitude of approximately 840 m above sea level and receives an average annual rainfall of 838 mm (Ward et al., 2017). The summers are warm (mean monthly maximum 26.4°C) and winters are mild (mean monthly minimum 8.8°C) with occasional frost. The soil is generally considered to be infertile and acidic, derived from shales, with a fine texture, falling under the Westleigh classification. The vegetation consists of a closed canopy, forb-rich grassland, classified as Moist Midlands Mistbelt Grassland. The vegetation is described as KwaZulu-Natal Hinterland Thornveld (Mucina and Rutherford, 2006), dominated by C4 grasses, such as *Heteropogon contortus* and *Themeda triandra* (Fynn et al., 2005).

3.3.2. Site selection and experimental layout

A long-term grassland fire experiment was established at Ukulinga Research Farm in 1950. The experiment is arranged in a split-plot randomized block design with 12 subplot burning or mowing treatments and three replicates (n = 3 plots per fire frequency) (Morris and Fynn, 2001). The blocked design accounts for changes in soil depth across the trial. In this study, a subset of treatments on the trial was sampled to assess the individual and interactive effects of fire frequency and year on plant community composition, structure and dynamics. Three fire frequencies from the long-term experiment were used, which were every year (annual), every three years (triennial) and the control (no burn), with all burns taking place in spring. All burn treatments were replicated three times. The same sampling procedure and site selection that was used by (Kirkman et al., 2014) was applied for this study, and for this reason, the biennial burn was omitted. Two 2 x 2m subplots were marked in each replicate of the annual, triennial and no-burn plots. Each of the 2 x 2m subplots was divided into four 1 x 1 m quadrats (Figure 3.1). In the previous study, the treatments were initially surveyed annually for species composition in the early and late growing season from 2005 to 2010 and a significant effect of burning frequency was found on species composition with no effect of year (Kirkman et al., 2014) During this time the mean annual rainfall was 677mm ± 30.23. The motivation for following the site selection and methodology of (Kirkman et al., 2014) is that the region experienced a short-term drought with total rainfall falling below 600mm during the growing seasons of 2013/2014 and 2014/2015. Following this short-term drought, we resurveyed species composition during the early and late growing season of 2020. Therefore the data used for the statistical analysis includes the data collected at the beginning (2005) and end (2010) from the (Kirkman et al., 2014) study and the resurveying year of 2020. There has been no grazing on the experimental site since 1950.

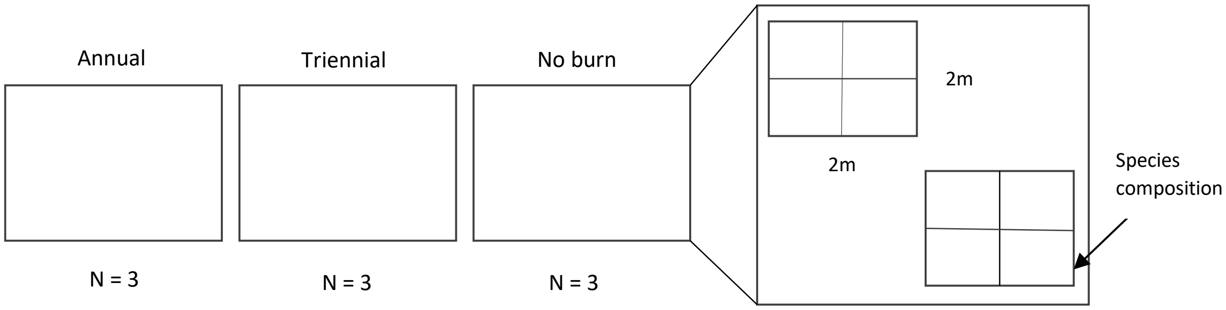

Figure 3.1. Experimental design of plant community response to annual, triennial and no-burn fire frequencies at the Ukulinga Research Farm, Pietermaritzburg, South Africa.

3.3.3. Vegetation assessment

The 2 x 2 m plots were permanently marked in 2005 and were resurveyed for species composition assessment in the growing season of 2019/2020. Canopy cover (to the nearest %) of each species rooted in each quadrat was visually estimated during the early and late growing season following a modified (Daubenmire, 1959) procedure. The greatest cover values for each species from each season were averaged across the four quadrats to produce the average cover of each species in each 2x2m plot per replicate under each burning treatment. The same was done for the different functional groups, grasses and forbs, in each plot.

3.3.4. Rainfall

Total rainfall (mm) was recorded over a period of 17 growing seasons, from 2005 to 2022 (Figure 3.2). Mesic grasslands generally receive high annual rainfall of >600 mm per year (Palmer and Ainslie, 2005). For most growing seasons, the total rainfall fell within the average range for a mesic grassland, except for 2014 and 2015, a regional drought, where both growing seasons fell below 600mm. This was the motivation behind resurveying the plots in 2019/2020, to determine if any changes to plant community properties might have been caused by the short-term drought.

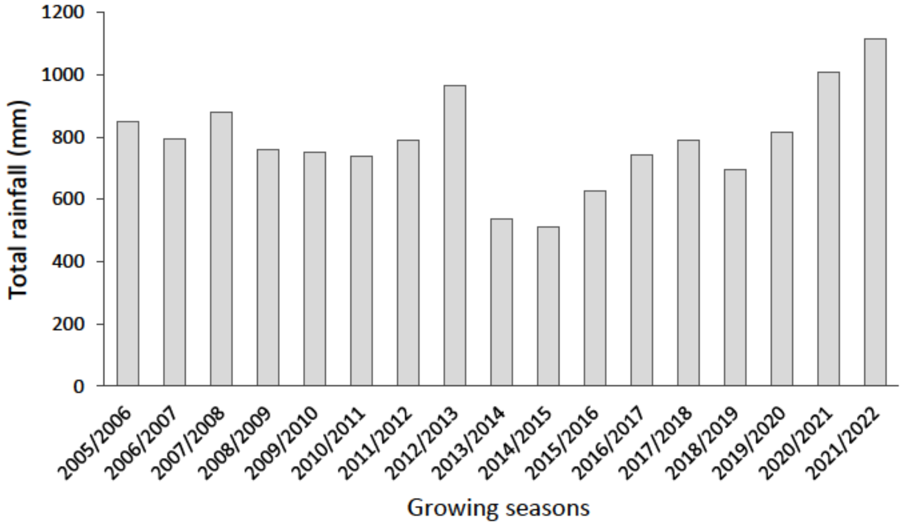

Figure 3.2: Growing season rainfall from 2005 to 2022 at Ukulinga Research farm, Pietermaritzburg, South Africa.

3.5. Statistical analysis

Differences in species composition were identified using direct gradient analysis. To avoid pseudo-replication, average values for each species in each plot were calculated for each of the survey years. The influence of a single drought event and burning frequency on species composition was assessed with a Canonical Correspondence Analysis (CCA). The canonical correspondence analysis was performed using R Studio (version 4.0.2), using the packages "vegan" for the analysis and "ggplot2" for plotting the ordination graphs (RStudio team, 2020). Plant community variation is explained by the selected environmental matrix in the eigenvalue ordination analysis and the categorical factors were separated into specific levels using the ellipses function from the "vegan" package. Permutation tests were used to determine the significance of the CCA models. Significant effects were graphed and species with an occurrence of <15% were excluded from the ordination plot for the reduction of clutter.

Where the CCA revealed significant differences, further investigation took place using PERMANOVA, a multivariate analysis of variance technique appropriate for use with abundance data. This determined the overall main and interactive effects of a single drought event and fire frequency on species composition using data from 2005, 2010 and 2020. The PERMANOVA was run using a Bray–Curtis dissimilarity matrix with 999 permutations, using type III sums of squares to test for the significance of factors (Clarke and Warwick, 2001). An analysis of similarity (ANOSIM) was then run to determine levels of community similarity among treatment groups using permutation procedures. Furthermore, a SIMPER analysis was used as a post hoc test to determine which species contributed the largest amount to the differences in composition between burning treatments. Species composition data was used to calculate diversity and richness for all species, grasses only and forbs only. Species richness (S) and Shannon–Wiener diversity index (H’) are metrics to measure functional diversity (Keylock, 2005). Before the ANOVA was performed, the diversity indices were calculated using the following method:

The Shannon–Wiener diversity index (H’) was calculated using the following equation:

$H' = SUMpi \log(b)pi$

where, pi = proportional abundance of species, b = base of logarithm (Keylock, 2005).

Finally, an analysis of variance (ANOVA) was carried out, which specifically examined the effects of year and the different burning frequencies; namely no burn, annual and triennial burn on the Shannon–Wiener diversity (H’) and species richness (S), for grasses only and forbs only. When ANOVA revealed significant differences, means were separated using Tukey’s post hoc test. This statistical analysis was performed using R Studio (version 4.0.2) with the following packages: “vegan”, “tidyverse”, and “pairwiseAdonis”. All variables were checked for normality and homogeneity of variance prior to analysis.

3.6. Results

According to the ordination analysis, annual burning favours the abundance of grasses more than that of forbs. Long-term burn exclusion favoured woody encroachment with species such as *Vachellia nilotica* and the growth of more forb species such as *Cheilanthes viridis* and *Passiflora suberosa* with fewer grass species (Figure 3.3). There was more species variation in the triennial burn treatment with a high species richness of both forbs and grass species (Figure 3.3). Only the burning treatments and grass species (Figure 3.3). Only the burning treatments were statistically significant (p<0.05), while the year was not.

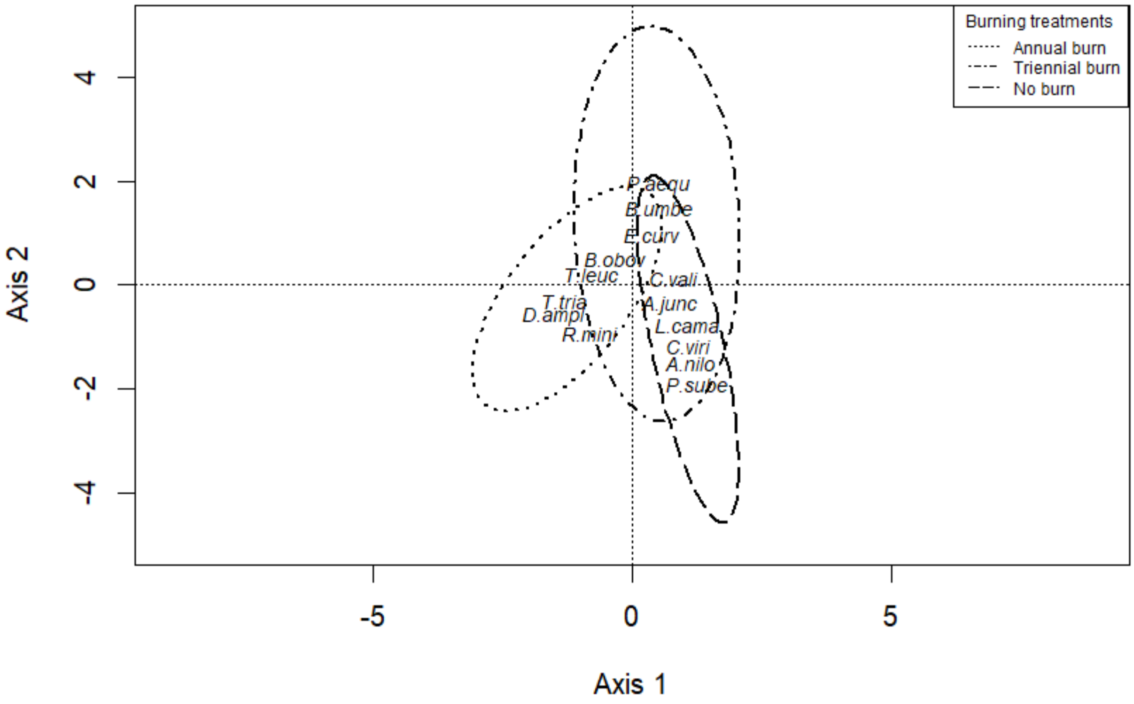

Figure 3.3: Canonical correspondence analysis ordinations of plant community composition between the annual, triennial burn and no-burn treatments in 2005, 2010 and 2020 at Ukulinga Research Farm, Pietermaritzburg, South Africa. *T. leuc - Tristachya leucothrix, T. tria - Themeda triandra, D. ampl - Diheteropogon amplectens, A. junc - Aristida junciformis, C. vali - Cymbopogon validus, R. mini - Rhynchosia minima, B. obov - Becium obovatum, E. curv - Eragrostis curvula, B. umbel - Berkheya umbellata, P. aequ - Panicum aequinerve, A. nilo - Acacia nilotica (Vachellia nilotica), C. viri - Cheilanthes viridis, L. cama - Lantana camara, A. macu - Aloe maculata, P. sube - Passiflora suberosa.*

The annually burned treatment was dominated by grasses, such as *Tristachya leucothrix* and *T. triandra* which were the most dominant species from 2005 to 2020 (Figure 3.4), as well as *H. contortus*, which is a grass known to be resistant to burning (Fish et al., 2015). The canopy cover of *Diheteropogon amplectens* increased from 35% in 2005, to 55% in 2010, and then significantly reduced to 30% in 2020. Unlike the preceding years, 2020 had a spike in the dominance of the perennial forb, *Thunbergia atriplicifolia*, increasing up to 65% in cover from a low 20% in 2005 and 12% cover in 2010 (Figure 3.4). Triennial burning promoted a dominance of unpalatable grasses such as *Aristida junciformis* and *Cymbopogon validus* (Figure 3.5). The forb *Berkheya umbellata* was dominant in 2005 and 2010 at 65% cover but decreased to 45% in 2020. *H. contortus* showed the opposite trend, increasing from 30% cover in 2005 to 46% in 2010 and 2020 (Figure 3.5). The absence of fire promoted the dominance of woody plants e.g. *V. nilotica* and forbs, such as *P. suberosa* and *C. viridis* while there were more unpalatable grasses such as *A. junciformis* (Figure 3.6).

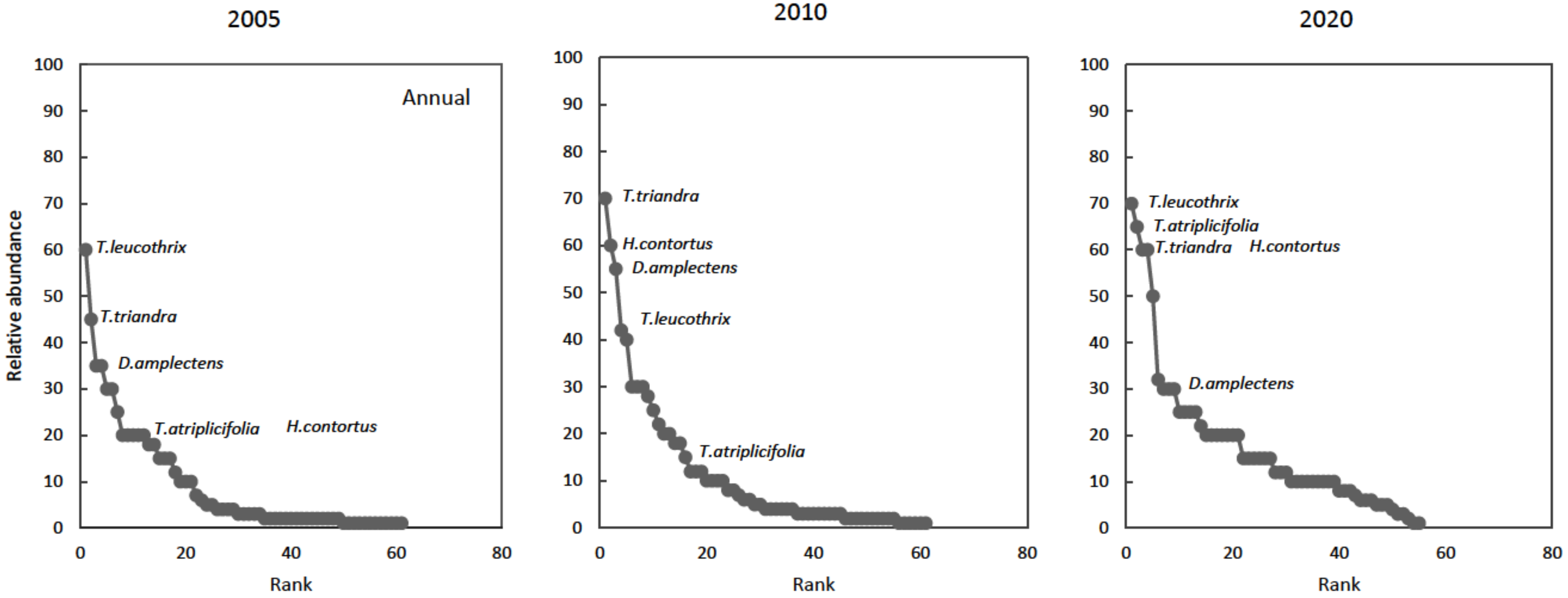

Figure 3.4. Species rank abundance curves for the annual burn treatments between 2005, 2010 and 2020 at Ukulinga Research Farm, Pietermaritzburg. *T. leucothrix* - *Tristachya leucothrix*, *T. triandra* - *Themeda triandra*, *D. amplectens* - *Diheteropogon amplectens*, *H. contortus* - *Heteropogon contortus*, *T. atriplicifolia* -*Thunbergia atriplicifolia*.

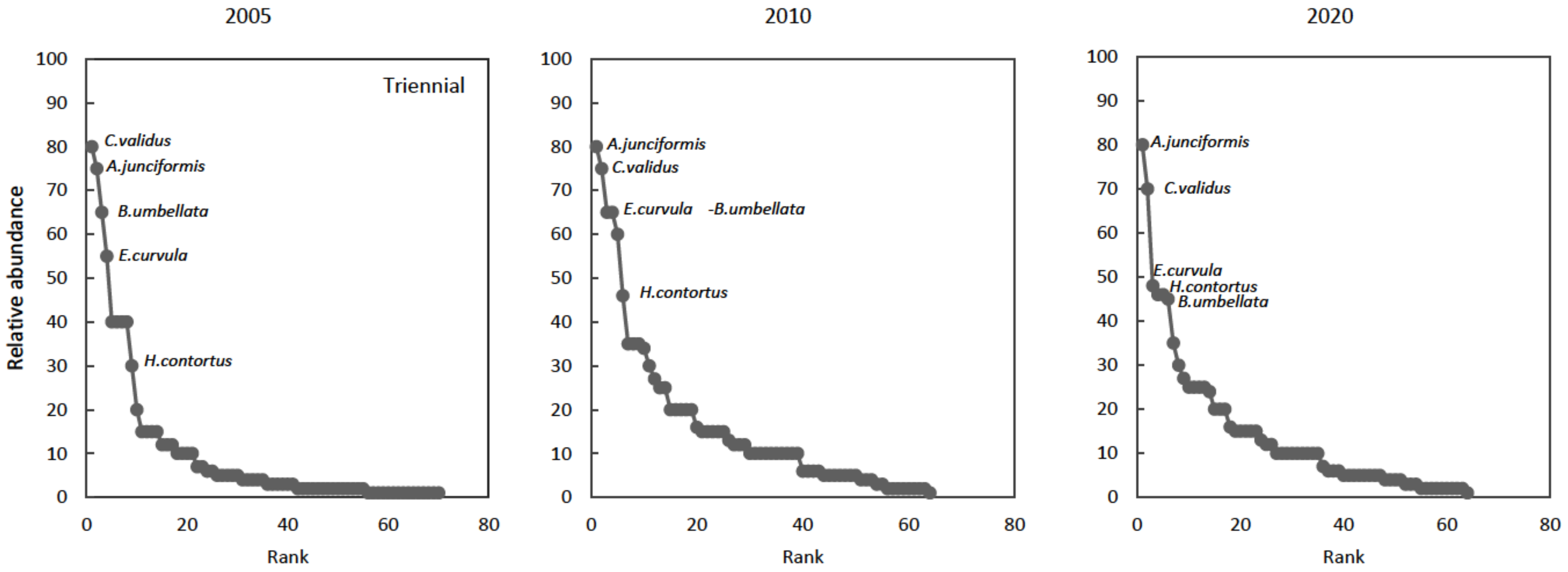

Figure 3.5. Species rank abundance curves for the triennial burn treatments between 2005, 2010 and 2020 at Ukulinga Research Farm, Pietermaritzburg. *C. validus* - *Cymbopogon validus*, *A. junciformis* - *Aristida junciformis*, *B. umbellata* - *Berkheya umbellata*, *E. curvula* - *Eragrostis curvula*, *H. contortus* - *Heteropogon contortus.*

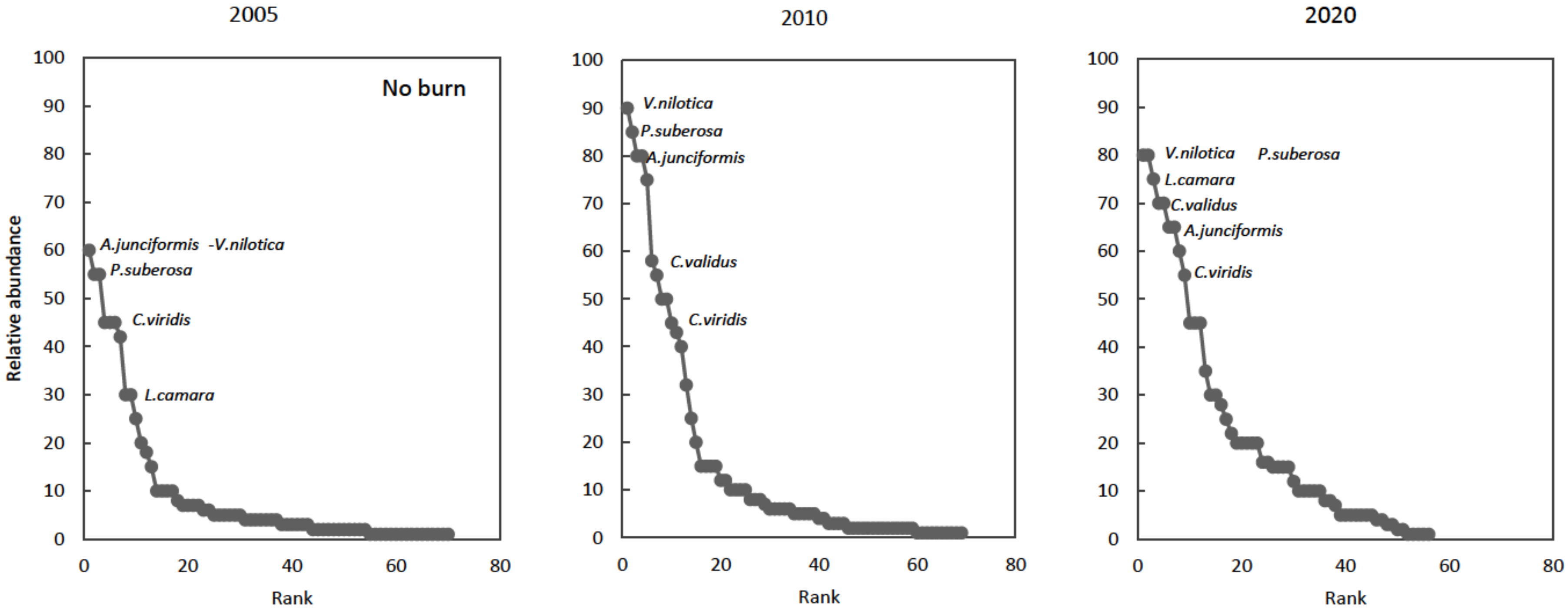

Figure 3.6. Species rank abundance curves for the no-burn treatments between 2005, 2010 and 2020 at Ukulinga Research Farm, Pietermaritzburg. *V. nilotica* - *Vachellia nilotica*, *A. junciformis* - *Aristida junciformis*, *P. suberosa* - *Passiflora suberosa*, *C. validus* - *Cymbopogon validus*, *L. camara* - *Lantana camara*, *C. viridis* - *Cheilanthes viridis*.

The dominant species were selected to illustrate the direction of species dominance between the years in the three burning treatments (Figure 3.7). There was a significant decline in the dominance of *C. excavatus* from 45% in 2005 to 10% in 2010 and 16% in 2020, while the opposite was observed for *L. camara* which completely disappeared in 2010 from 5% cover in 2005 and increased to 75% in 2020 (Figure 3.7). The grasses, *T. leucothrix* and *T. triandra* dominated the annual burn treatment. The cover of *H. contortus* was low (25%) in 2005 and spiked to 60% cover in both 2010 and 2020, while *A. junciformis* had a gradual increase from 15% in 2005 to 25% in 2010 and 32% in 2020. There was an increase in *D. amplectens* from 35% in 2005 to 55% in 2010 and then dropping to 30% in 2020 (Figure 3.7). The triennial burning treatment was dominated by the grasses, *A. junciformis*, *C. validus* and *E. curvula*. The forb *B. umbellata* was dominant in 2005 and 2010 at 65% coverage and decreased to 45% coverage in 2020. The opposite was observed for *H. contortus* which increased from 30% cover in 2005 to 46% in 2010 and 2020. *Pentanisia angustifolia* started at a low 10% cover in 2005, increased to 60% in 2010 and then decreased to 46% in 2020 (Figure 3.7).

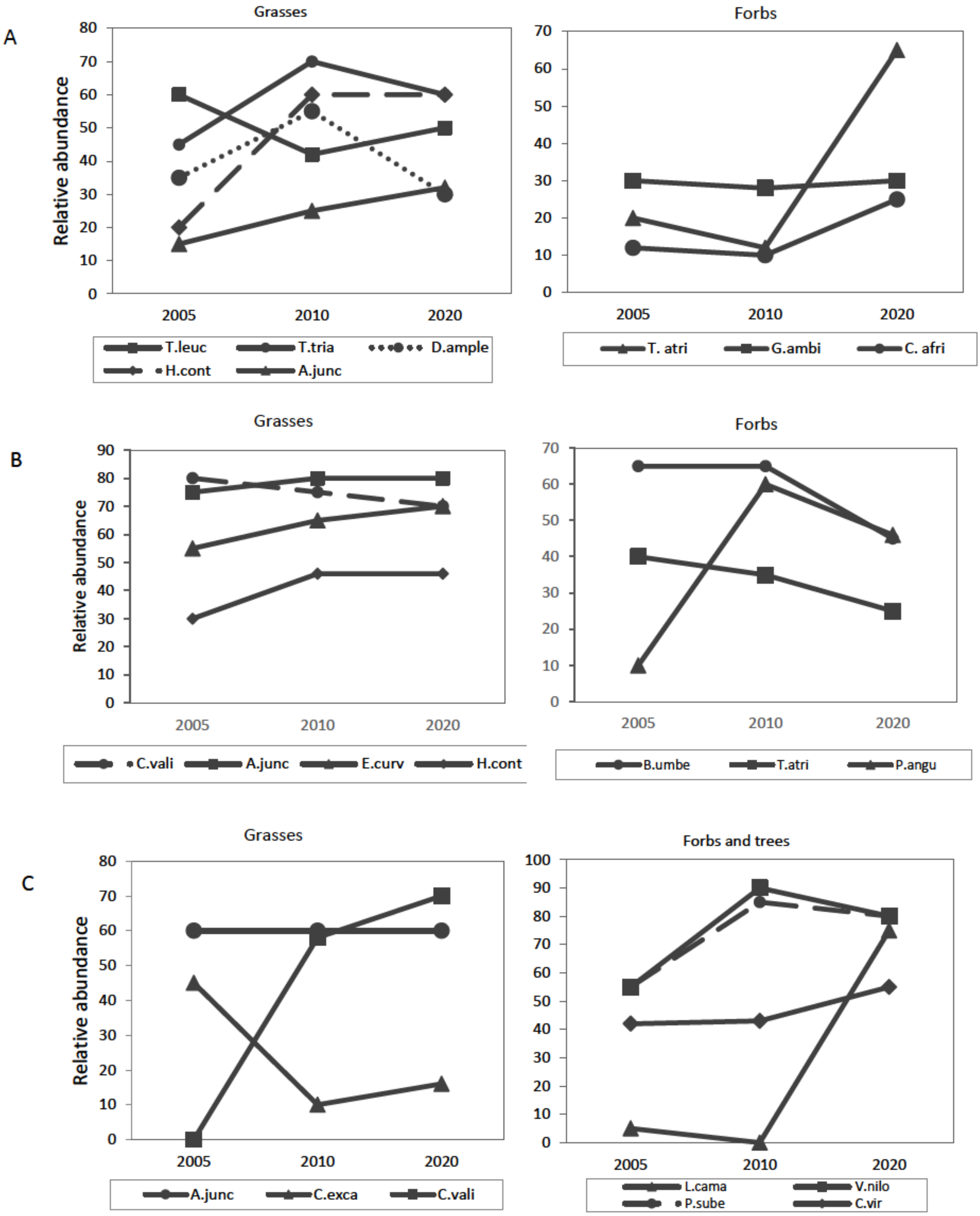

Figure 3.7. Patterns of mean relative abundance for the most dominant species on the (A) annually burned, (B) triennially burned and (C) no-burn treatments (2005-2010-2020) at Ukulinga Research Farm, Pietermaritzburg. Grasses: *A. junc* - *Aristida junciformis*, *C. exca* - *Cymbopogon excavatus, C. vali* - *Cymbopogon validus*, *H. conto* - *Heteropogon contortus, E. curv* - *Eragrostis curvula T. leuc* - *Tristachya leucothrix*, *T. tria* - *Themeda triandra*, *D. ample* - *Diheteropogon amplectens*, Forbs: *P. sube* - *Passiflora suberosa*, *C. viri* - *Cheilanthes viridis, L. cama* - *Lantana camara*, *B. umbel* - *Berkheya umbellata, T. atri* - *Thunbergia atriplicifolia, P. angu* - *Pentanisia angustifolia, G. ambi* - *Gerbera ambigua, C. afri* - *Commelina Africana*, woody species: *V. niloa* - *Vachellia nilotica*.

The most abundant grasses: *A. junciformis*, *T. leucothrix*, *T. triandra, E. curvula, H. contortus* and *D. amplectens* were identified by the SIMPER analysis to be driving the differences between the burning treatments (Table 3.1). According to the SIMPER analysis, *A. junciformis* is the main species contributing to the differences between the burning treatments at 16% dissimilarity (Table 3.1). In general, *T. leucothrix* and *T. triandra* were significantly more abundant in the annually burned areas, whereas *A. junciformis* and *C. viridis* were abundant in the fire-excluded areas (Table 3.1). *Cymbopogon viridis, P. suberosa and T. atriplicifolia* are forbs that mostly influenced the differences between burning treatments. The abundance of *T. leucothrix, E. curvula, T. triandra H. contortus* and *D. amplectens* increased with higher burning frequency (Table 3.1).

Table 3.1: SIMPER analysis for species abundance between the years in the no burn, annual and triennial burning treatment using Bray-Curtis dissimilarities test showing average dissimilarity, percentage contribution (%), cumulative percentage (%), and mean abundance

Taxon	**Av. dissim**	**Contrib. %**	**Cumulative %**	**Mean Annual**	**Mean Triennial**	**Mean NoBurn**
Aristida junciformis	16.17	16.56	16.56	1.23	6.45	18.2
Tristachya leucothrix	6.903	7.07	23.63	4.2	1.79	0
Eragrostis curvula	6.862	7.029	30.66	0.769	4.11	1.55
Cheilanthes viridis	6.547	6.705	37.36	0	0	7.93
Themeda triandra	6.387	6.542	43.9	6.38	0	0
Heteropogon contortus	6.105	6.253	50.16	5.51	0	0
Thunbergia atriplicifolia	5.822	5.963	56.12	1.38	3.15	0.517
Cymbopogon validus	5.686	5.824	61.95	0	3.47	3.97
Diheteropogon amplectens	4.403	4.51	66.45	3.26	0	0
Gerbera ambigua	3.153	3.229	69.68	1.18	0.823	0
Setaria nigrirostris	2.33	2.387	72.07	0.662	1.18	0
Cymbopogon excavatus	2.221	2.274	74.35	1.62	0	0
Hypoxis colchicifolia	2.041	2.09	76.44	0.431	0.935	0
Passiflora suberosa	1.906	1.953	78.39	0	0	3.28
Becium obovatum	1.898	1.944	80.33	0.769	0.452	0
Helichrysum nudifolium	1.759	1.801	82.13	0	0.984	0.517
Acalypha angustata	1.385	1.418	83.55	0	0.823	0
Hibiscus aethiopicus	1.316	1.348	84.9	0	1.1	0
Pentanisia angustifolia	1.307	1.338	86.24	0	1.1	0
Setaria pallida-fusca	1.288	1.319	87.56	0	0.242	1.03
Elionurus muticus	1.176	1.204	88.76	0.231	0.806	0
Tagetes minuta	1.14	1.168	91.11	0	0.806	0
Spermacoce natalensis	1.085	1.111	92.23	0	0.758	0
Aloe maculata	1.058	1.084	93.31	0	0	1.72
Rhynchosia minima	0.9855	1.009	94.32	0.385	0.258	0
Hypoxis hemerocallidea	0.9098	0.9318	95.25	0	0	1.16
Berkheya umbellata	0.8126	0.8323	96.08	0	0.968	0
Hyparrhenia hirta	0.641	0.6565	96.74	0.538	0	0
Brachiaria serrata	0.4902	0.5021	97.24	0.308	0	0
Gnidia caffra	0.4902	0.5021	97.74	0.308	0	0
Berkheya setifera	0.4779	0.4895	98.23	0	0.29	0
Eriosema cordatum	0.4628	0.474	98.71	0.277	0	0
Hyparrhenia dregeana	0.4288	0.4392	99.15	0	0.242	0
Senecio coronatus	0.4169	0.427	99.57	0.231	0	0

The PERMANOVA revealed that the application of fire had a significant impact on species composition (Table 3.2). However, the annual weather patterns did not have a significant impact on the species composition. Therefore, in the long-term drought in 2014/2015 did not influence any significant changes in the species composition of the grassland and any changes to plant composition were only inflicted by fire (Table 3.2). A *post hoc* analysis was done to further investigate the differences between burning treatments, and it was found that the species composition between all burning treatments was significantly different. Again, the year had no significant impact on the diversity and richness of species, while fire had a significant impact on both (Table 3.3). There was no significant impact of the interaction between year and fire.

Greater burning frequency increased both the diversity and richness of species, while the absence of fire significantly reduced species diversity and richness.

Table 3.2: Permutational multivariate analysis of variance (PERMANOVA) for years (2005, 2010 and 2020) under the undefoliated, annual and triennial burning regimes, showing *P*-values with significant effects (*P* < 0.05) in bold at Ukulinga Research Farm, Pietermaritzburg, South Africa

Source	Sum of squares	df	Mean square	F-value	P-value
Year	859,12	2	989,12	0,70567	0,6153
Fire	13675	2	6437,6	4,6529	**0,0001**
Y*F	-12884	4	-7942	-5,8433	0,2201
Residual	1,0357	36	1339,2		
Total	1,0357	40			

Table 3.3: Analysis of variance for Shannon's diversity index and species richness between years 2005, 2010 and 2020 on the no burn, annual and triennial burning treatments showing total the *F*-value and *P*-value with significant effects (*P* < 0.05) in bold

		Diversity		**Richness**	
Source	**Df**	**F-value**	**P-value**	**F-value**	**P-value**
Year	2	4.223	0.431	29.328	0.062
Fire	2	0.449	**0.023**	1.234	**<0.001**
Y * F	4	3.565	0.215	2.746	0.078
Error	24				
Total	36				

The canonical correspondence analysis ordination for grass community composition showed a high grass variability in the annually burned site with the no burn site being the least variable and dominated by species such as *A. junciformis, C. validus* and *M. maximus* (Figure 3.8). Frequent burning promoted greater diversity and richness of grasses and diversity decreased with the absence of fire (Figure 3.9). The relative cover of forbs and grasses was significantly changed by burning (Table 3.4). There was no significant difference between the grass richness in the triennial and no burn areas (Figure 3.10A), while the forbs from the annually burned site were not significantly different from the triennially burned area (Figure 3.10B). Annual burning had fewer forb species, while the triennially burned site had a larger number of forbs such as *Z. capensis*, *T. asperata*, *P. hottentotta*, *S. coronatus*, *A. setosa*, *T. atriplicifolia* and *B. setifera* to name a few (Figure 3.11). The diversity of forbs was high in both the annual and triennially burned sites, while the absence of fire decreased forb species diversity (Figure

3.12A). Forb richness was the greatest under triennial burning and there was no significant difference between forb richness in the unburned and annually burned areas, but both sites had comparatively lower forb richness to the triennial burn treatment (Figure 3.12B).

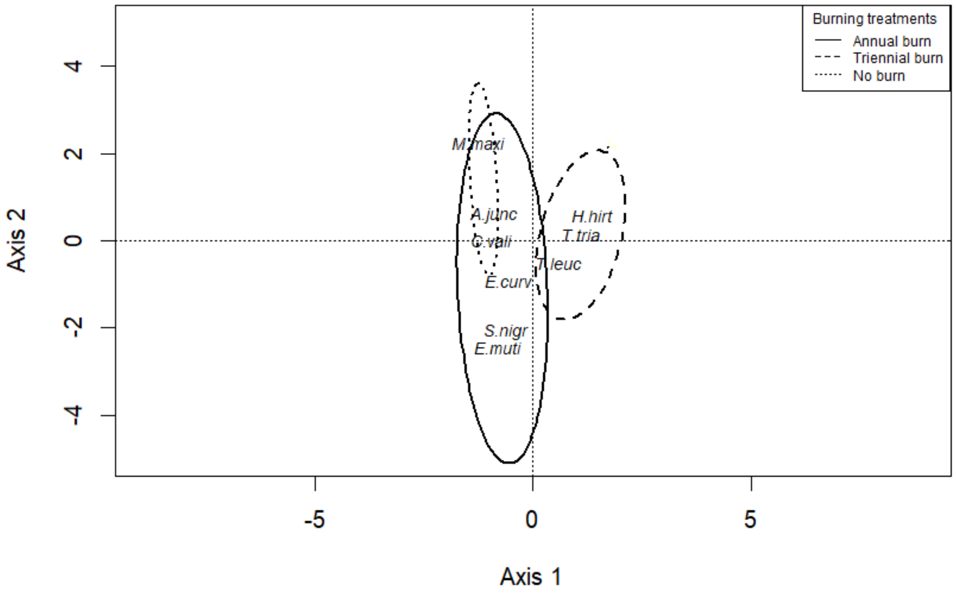

Figure 3.8: Canonical correspondence analysis of grass community composition between the annual, triennial burn and the no-burn treatments from Ukulinga Research Farm, Pietermaritzburg, South Africa. Species with an occurrence of <15% were omitted from the ordination diagrams to reduce clutter. *T. leuc - Tristachya leucothrix, T. tria - Themeda triandra, A. junc - Aristida junciformis, C. vali - Cymbopogon validus, E. curv – Eragrostis curvula, T. leuc - Tristachya leucothrix, S. nigr - Setaria nigrirostris, H. hirt - Hyparrhenia hirta, E. muti - Elionurus muticus, M. maxi – Megathrysus maximus*.

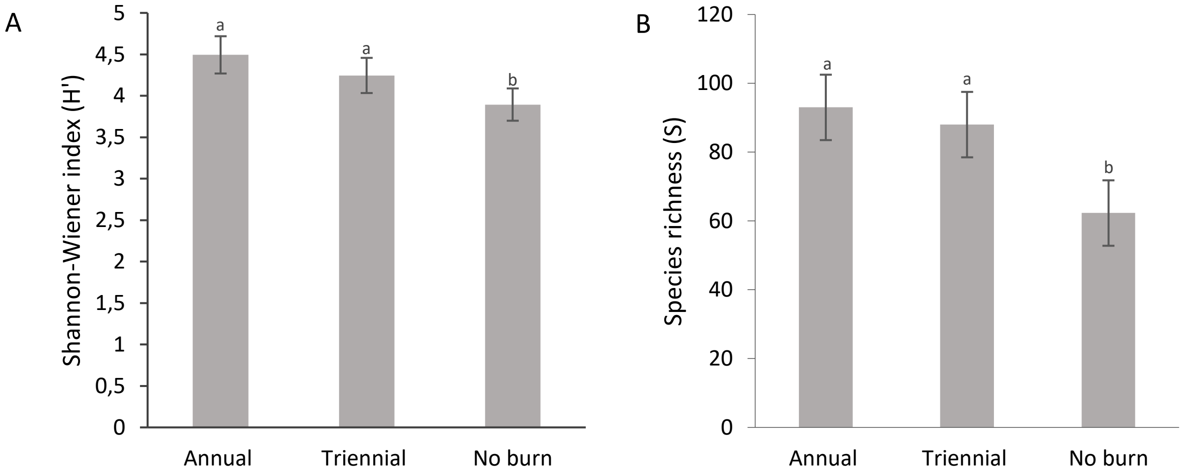

Figure 3.9: Mean species diversity (H') - A, and mean species richness (S) - B, in response to annual, triennial and no burn fire frequency from data collected in 2005, 2010 and 2020 at Ukulinga Research Farm, Pietermaritzburg, South Africa. Superscripts showing different letters are significantly different ($P < 0.05$).

Table 3.4: Analysis of variance for relative grass and forb cover between the years 2005, 2010 and 2020 under no burn, annual and triennial burning treatments showing total the *F*-value and *P*value with significant effects (*P* < 0.05) in bold.

		Relative grass cover		Relative forb cover	
Source	Df	F-value	P-value	F-value	P-value
Year	2	2.251	0.089	0.7685	0.765
Fire	2	0.852	**0.042**	3.6475	**0.003**
Y * F	4	2.247	0.108	0.7534	0.868
Residual	18				

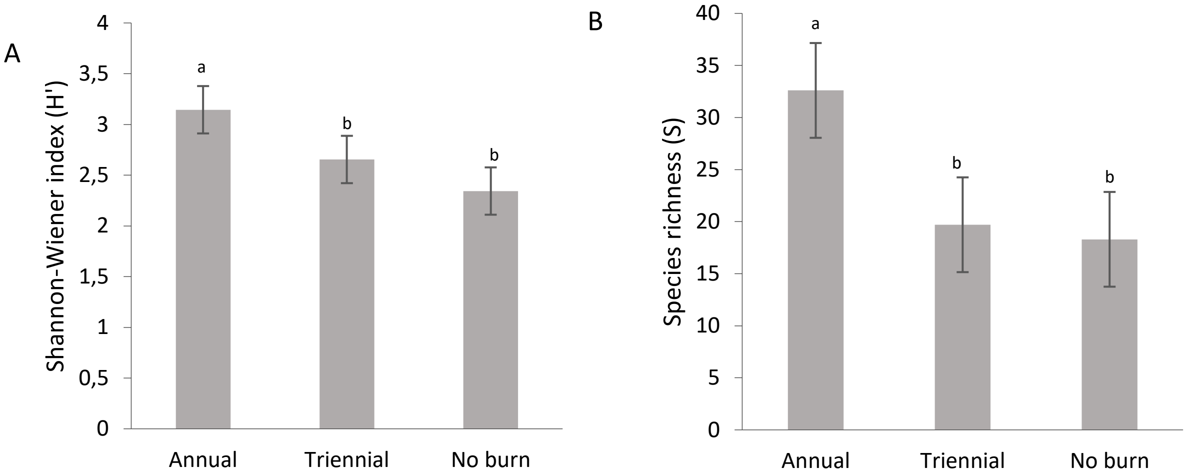

Figure 3.10: Mean grass diversity (H') - A, and mean grass richness (S) - B, for no-burn, annual and triennial burning treatments from data collected in 2005, 2010 and 2020 at Ukulinga Research Farm, Pietermaritzburg, South Africa. Superscripts showing different letters are significantly different (*P* < 0.05).

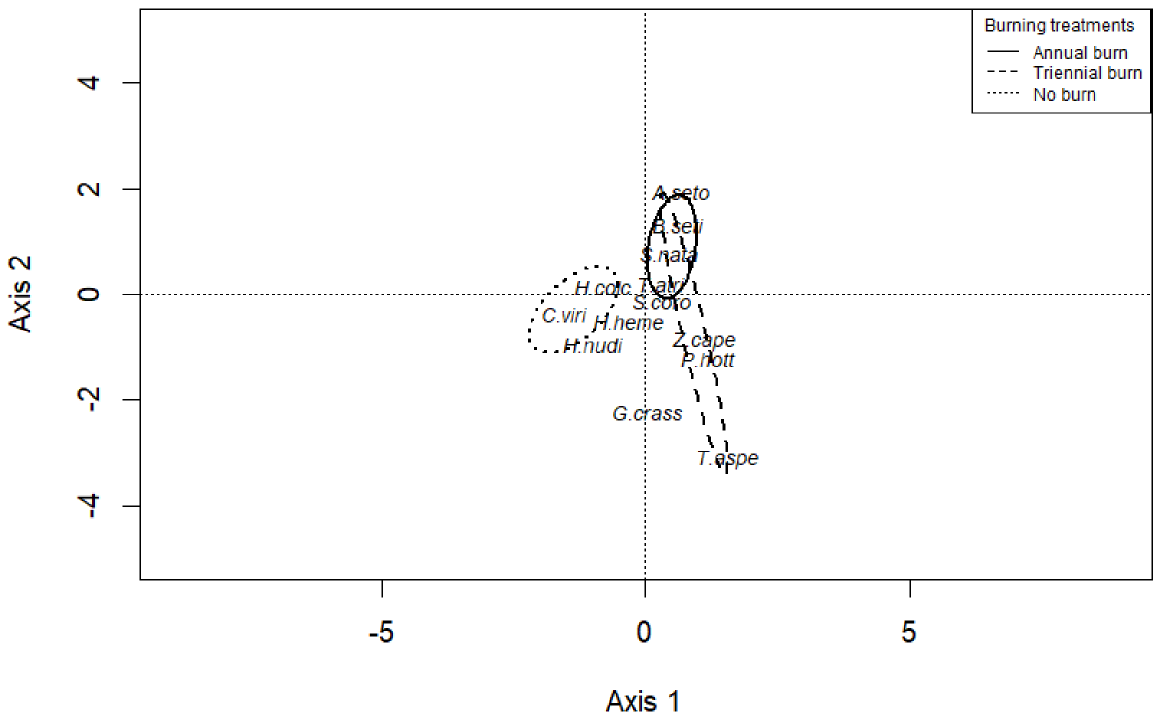

Figure 3.11: Canonical correspondence analysis of forb community composition between the annual, triennial burn and the no-burn treatments from Ukulinga Research Farm, Pietermaritzburg, South Africa. Species with an occurrence of <15% were omitted from the ordination diagrams to reduce clutter. *H. heme - Hypoxis hemerocallidea, H. colc - Hypoxis colchicifolia, H. nudi - Helichrysum nudifolium, B. seti - Berkheya setifera, S. coro - Senecio coronatus, S. nata - Spermacoce natalensis, Z. cape - Zonia capensis, T. atri - Thunbergia atriplicifolia, C. viri - Cheilanthes viridis, G. crass - Gladiolus crassifolius, A. seto - Albuca setosa, P. hott - Polygala hottentotta, T.aspe - Trachyandra asperata*.

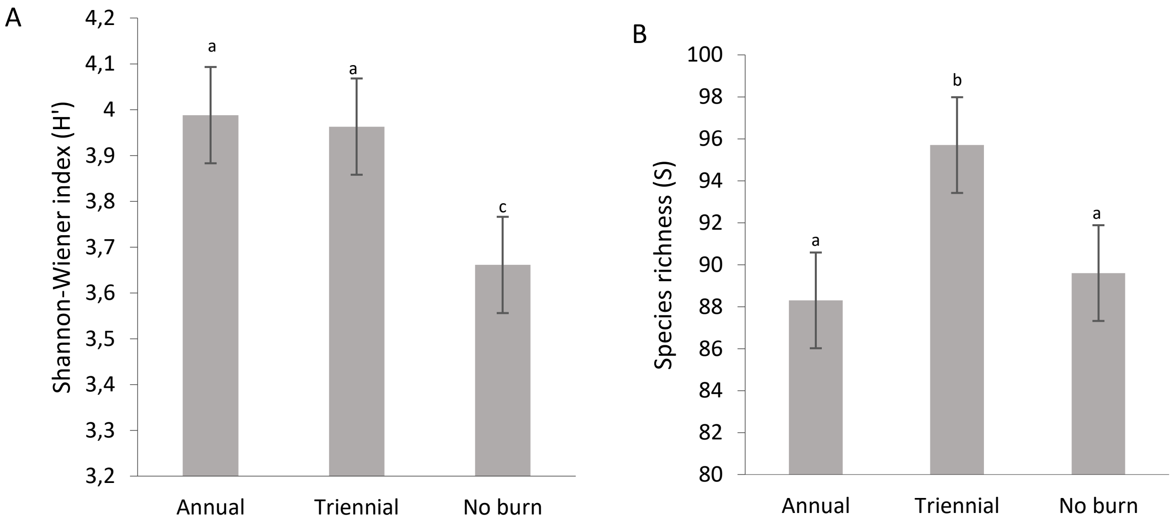

Figure 3.12: Mean forb diversity (H') - A, and mean forb richness (S) - B, for the no-burn, annual and triennial burning treatments from data collected in 2005, 2010 and 2020 at Ukulinga Research Farm, Pietermaritzburg, South Africa. Superscripts showing different letters are significantly different ($P <$ 0.05).

3.7. Discussion

3.7.1 Effects of drought and fire on species composition and diversity

This study investigated the impacts of fire and drought and their interactions on the grassland community, specifically species diversity. This interaction is mostly unexplored in field-based studies, as most research has focused on the impacts of drought or fire as separate entities. Drought, symbolized by year, did not have a significant impact on species composition and diversity, while prescribed burning had a highly significant impact on the grassland community. With that being said, it must be noted that the second set of data was collected in 2019/2020, a few years after the 2014-2015, which might have affected the accuracy of drought impacts on species composition, or given the grassland some time to recover from the drought. There was a greater species diversity in treatments with shorter burning intervals, in this case, the annually burned areas. The annually burned treatment was dominated by *T. triandra* which is a decreaser species that does not thrive under heavy grazing, but benefits from frequent burning (Male et al., 2022). The palatable grass, *T. leucothrix* was also one of the most dominant species throughout the study. Frequent fire can, therefore, produce and maintain a plant community, suitable for healthy livestock grazing by promoting the dominance of palatable grass species. Annual burning has been shown to increase biodiversity and species diversity, which in turn has been found to increase plant communities' resistance to the effects of disturbances influenced by climate change. This resistance is often measured based on the rate at which a diverse plant community recovers following disturbances (Gunderson, 2000). It is now generally acknowledged that species diversity influences ecosystem services, and the stability of ecosystem processes (Gross et al., 2014; Tilman et al., 2006). Numerous long-term fire experiments have shown a positive relationship between fire frequency and plant diversity (Glitzenstein et al., 2003; Palmquist et al., 2014; Uys et al., 2004).

The triennially burned treatment presented more species variation with an increase in the abundance of forbs, compared to the annually burned treatment. Annual burning favoured a high abundance of grasses, while long-term burn exclusion favoured encroachment of *V. nilotica,* as well as the dominance of forbs such as *C. viridis* and *P. suberosa*, with less grass cover. Similar results were reflected by Zouhar (2021), where extremely infrequent and no burning stimulated the abundance of woody species in grassland and prairie ecosystems. The primary consequences of fire are the extensive and sporadic destruction of aboveground biomass eradicating the growth of woody species (Bond and Wilgen, 1996). The absence of fire suppressed palatable grass abundance and promoted unpalatable grasses such as *Aristida* *junciformis* and *C. validus.* These findings are similar to those of Munyai et al. (2023), who found less palatable grasses in unburnt areas, replaced by unpalatable grasses that thrive under shady conditions. As a consequence, the absence of fire promotes woody encroachment (Bond and Wilgen, 1996), which may be influenced by elevated litter levels on the soil surface (Fynn et al., 2003), contributing to diminished grass seed germination (Jutila and Grace, 2002). Frequent fire encourages grass tillering by destroying moribund material (Tainton and Mentis, 1984), hence eliminating bush encroachment. Fire and grazing are highly influential in plant community structures, having a significant impact on the amount and quality of fodder (Twidwell et al., 2021). While grazing is more selective and affects grasses, forbs, and shrubs at the species level, burning assists in the clearance of both live and dead standing biomass (Bond and Keely, 2005). Therefore, fire is essential for the maintenance of grasslands with palatable grasses.

Additionally, tall-grass species such as *A. junciformis and C. validus* and trees such as *V. nilotica*, that unburned sites, may have shaded out and excluded the shorter grass species, that were either scarce or non-existent in undisturbed sites. One of the reasons for the invasion of *A. junciformis* is that it has thin, pointed leaves which allow for penetration through the accumulated moribund biomass in the absence of burning. The absence of fire also promoted large, tussock grasses such as *C. validus* which readily outcompete other grasses with no burning. Given that an abundance of *C. validus* can cause a build-up of moribund material that stunts the growth of other species, therefore, the dominance of *C. validus* is an indicator of community degradation. These grasses suppress fires due to their tufted nature and the durable thatch qualities of *C. validus* (Rungqu et al., 2016), resulting in extensive biomass that deprives other plant species of sunlight and other resources (Munyai et al., 2023). *Cymbopogon validus* is an Increaser I species with a strong competitive response (Fynn et al., 2011), which normally increases in underutilized veld (Fish et al., 2015).

The results of this study concur with those of Uys et al. (2004), in a long-term burning experiment across montane, mesic and semi-arid South African grasslands, where it was shown that frequent fire promotes the dominance of grasses and some forbs. In the same areas, the exclusion of fire for up to 10 years showed a high replacement of grasses and a few forbs by woody plants, which ultimately led to decreased species diversity (Uys et al., 2004) Similar results were found in our study where the unburnt treatment had the lowest concentration of grasses and a higher woody species dominance compared to other burning treatments. Again, on an Australian grassland, it was found that some native species tend to die out in the absence of fire (Morgan and Lunt, 1999). This could explain the findings of this study, where the no burn treatment, had almost an entirely different species composition from the rest of the plots which were burned. (Uys et al., 2004). found that the plots which were never subjected to burning were composed of species which were almost completely different from all the plots that were burned, implying that fire, depending on the rate and season of application, does have a significant impact on species composition and diversity.

Although fire is a key driver in grassland vegetation, fire operates within the limitations of varying moisture conditions (Collins et al., 2012) and the state of the vegetation (Thonicke et al., 2010). Growing season rainfall limits net primary production in grasslands, predicting total aboveground net primary production (Robinson et al., 2013). The lack of response to drought, in terms of species composition, may be explained by the fact that grasses, particularly C4 species, are adapted to tolerate drought, while forbs eliminate the risk of drought impacts by having deeper rooting profiles (Nippert and Knapp, 2007). Globally, C4 grasses dominate in most grasslands with warm climates (Vendramini et al., 2023). These species are adaptable to dry conditions as they can reduce their water loss through controlled transpiration by closing their stomata during photoperiods (Kazemi et al., 2023). In this study, there was a greater percentage of C4 and perennial grasses in general. Although both C3 and C4 plants are susceptible to drought, C4 grasses inhabit drier habitats than their C3 relatives and as such, C4 plants display advantages in hydraulic performance in dry environments (Taylor et al., 2014).

3.7.2 Effects of drought and fire on species richness

The results found in this study suggest that burning significantly affects species composition while a single-year drought does not. The responses to burning remained unchanged from those reported in (Kirkman et al., 2014). The species richness from the annually and triennially burned sites was significantly different from the unburned area. The overall species and grass species diversity was different under all three fire treatments and richness lower in unburned sites. Forb diversity under burning was generally higher than the unburned treatment, whereas richness was higher under triennial burning, possibly due to the high abundance of both forbs and grasses and the annual and unburned sites were the same. A similar study determined that the the absence of fire reduced grass species richness by more than 50% and that annual burning resulted in 22% greater species richness relative to triennial burning at Ukulinga Research Farm (Fynn et al., 2004). Several studies have also found species richness to increase with fire frequency (Mehlman, 1992; Tester, 1989). Disturbance by fire is necessary to achieve maximum grass species richness by removing litter and increasing the availability of light for plants (Fynn et al., 2004). Frequent fire provides a green flush of nutrient-rich grass by eradicating moribund material and litter build-up on the soil surface (Bond and van Wilgen, 1996), and as a result, the surface soil's microclimate and nutrient levels changes (Lavorel et al., 1999). Fire functions as a natural selective factor, promoting the dominance of grasses by boosting plant nutrient availability in the soil (Snyman, 2003).

There was no significant difference between forb species richness in the annually burned and unburned areas, but the triennially burned area had a greater species richness than the other sites. Although forb richness remained unchanged between annually burned and unburned sites, species composition was altered because species that were excluded by fire or a lack of it, were replaced by others better adjusted to those conditions. However, in a southern African study (Uys et al., 2004), no clear trends in species richness in response to the frequency of fire were found in mesic grassland. One of the possible reasons most forb species endure a wider range of burning seasonality and frequencies might be because forbs dominate grasslands worldwide in terms of species richness and thus occur across a wide climatic range with variable disturbance regimes (Seabloom et al., 2013). Many forbs have short life cycles with shallow roots, making them transient for much of the year (Pokorny et al., 2004), therefore able to avoid environmental disturbances and persist in the grassland.

Species richness is a strong forecaster for recovery from disturbances (Ruijven and Berendse, 2003). In the current study, drought had no significant impact on species richness. Similar to diverse plant commuinities, species rich plant communities are expected to be more resilient to the impacts of extended disturbances such as drought. In addition, a more diverse plant community is anticipated to be more stable against perturbations and extreme environmental events (Gunderson, 2000). The impact of fire on species diversity and richness could be attributed to the fact that the fire treatment has been applied for more than 70 years, while the drought occurred for a comparatively shorter period. Long-term disturbance may lead to changes in the environment, such as vegetation structure, soil organic matter, and nitrogen availability (Fynn et al., 2003), which may affect species richness. For this reason, long-term experiments are useful for studying the effect of disturbance on species richness. Conversely, as species richness may only respond temporarily to short-term disturbances like the current drought, this could result in findings about the impact of disturbance that are not authentic (Wilson and Tilman, 2002).

3.7.3 Grassland survival/resistance to drought

Grasslands thrive across a wide range of climatic conditions and extremes. This suggests that specific plant strategies and ecological processes aid grasslands to develop, endure, withstand, recover from or adapt to this wide range of environmental conditions (Lüscher et al., 2022). There are two major plant response tactics under moderate and severe water shortage which are ‘drought resistance’, referring to the maintenance of leaf growth and biomass production and ‘drought survival’, which is, the plant’s capacity to survive after growth cessation due to severe drought (Volaire, 2018). While drought survival is crucial under severe drought stress, drought resistance is more important under moderate drought stress, however, both improve the plant communities' resilience by promoting post-drought recovery. The fact that the mesic grassland in this study remained unchanged after the short-term drought suggests that this grassland benefits from the aforementioned drought survival or drought resistance strategies. High species richness and diversity may contribute to this drought survival. In addition, the rainfall patterns experienced over the last 20 years indicate that the grassland is adapted to rainfall variability, which may have increased the grassland’s capacity for drought resistance.

3.8 Conclusions

The short-term drought did not have a significant impact on the grassland community. The different burning frequencies resulted in significant changes to the plant community in terms of diversity and richness. The various burning treatments had very distinct differences in terms of species composition, for instance, the absence of fire clearly diminishes the abundance of grasses and favors the growth of forbs and woody species. The absence of fire led to a significant decline in the grass species richness, but not forb species. Thus, it seems that elements associated with occasional disturbance, including decreased light availability and elevated litter levels, regulate grass-species richness. Since forbs have greater phylogenetic variety than grasses, disturbance has a smaller impact on the richness of forb species than grass species. The species that were more resilient to the sward structural and microclimatic characteristics connected with unburned sites replaced the species that vanished with a lack of disturbance. The length of the drought can be a contributing factor to the results. It is also important to consider that there were a few years after the drought of 2014/2015 that had normal to high rainfall before the data collection in 2020, therefore the grassland may have had a few years for recovery. Fire is important for the survival and maintenance of a grassland community, because the fire exclusion areas were eventually invaded by woody species. Annual burning on the other hand, promotes a diverse, species-rich plant community, dominated by palatable grasses such as *T. leucothrix* and *T. triandra*. Fire had more visible impacts on the plant community than drought because of its consistent application over more than 70 years in comparison to the short-term drought.

3.9 Recommendations

Based on the results of the study, frequent burning in mesic grasslands is highly recommended for the maintenance of a grass-rich plant community that contains highly palatable grass species. The length of fire return intervals depends on the end goal and purpose of burning management. Triennial burning creates a forb rich species composition. For grasslands, the absence of fire can be detrimental to livestock grazing as it promotes woody species encroachment. In the wake of urbanization and climate change, fire is a necessary management tool for the maintenance of highly diverse and species-rich mesic grasslands. Grasslands under frequent prescribed fire may be able to withstand short-term drought. For further research, the biomass production of the plant community must be measured, to assess whether it is affected by short-term drought or if it contributes to the survival of a plant community against drought. Additionally, lengthy periods of drought in a grassland managed with prescribed fire may have a different vegetative response.

CHAPTER 4: THE INFLUENCE OF DROUGHT ON GRASS-GRASS INTERACTIONS IN POT TRIALS

Abstract

Plant communities are influenced by water availability and display physiological trade-offs through competitive traits, such as resource attainment strategies. This study explores the competitive interactions between broad vs narrow-leaved grasses as well as palatable vs unpalatable grasses and their effect on plant traits under drought conditions. The adequate moisture treatment was irrigated twice a week with 500ml water, while the drought treatment was irrigated once a week with the same volume. Six grass species were collected from a mesic grassland at Ukulinga Research Farm and transplanted into pots with a 25cm diameter, in the greenhouse at the NM Tainton Arboretum. The palatable grass species were represented by *Themeda triandra* and *Tristachya leucothrix* and the unpalatable species by *Aristida junciformis* and *Cymbopogon excavatus*. *Aristida junciformis* and *Melinis nerviglumis* represented narrow-leaved grasses while *Megathyrsus maximus* and *Cymbopogon excavatus* represented broad-leaved species. The experiment was conducted using the phytometer method where there is a central focus tuft (phytometer) surrounded by four competitor tufts. Each species was alternated, serving as both a phytometer and a competitor and all species were also grown individually without competitors. Total biomass, height, tiller numbers, tuft circumference and average leaf area of all phytometers were measured. The study's overall conclusions suggest competition reduces phytometer biomass. Broad-leaved species were highly competitive compared to narrow-leaved species. Broad-leaved competitors reduced phytometer tiller numbers, biomass, and tuft size and showed a level of species specificity, with *M. maximus* being more competitive than *C. excavatus*. No species showed any significant effects of competition on specific leaf area. Surprisingly, drought did not have any significant impact on final dry biomass, but significantly reduced the tiller numbers of palatable species as well as tiller numbers and tuft circumference of broad-leaved species. Palatable species had a general competitive effect over unpalatable species, while broad-leaved species had a stronger competitive effect over narrow-leaved species. This study found that narrow and broad-leaved grasses were unaffected by drought and this could be due to the short duration or severity of the drought. Both unpalatable and palatable species were unaffected by drought, possibly due to the absence of grazing. Palatable species, like *T. leucothrix*, had a higher capacity for competition. There may be limitations to the intensity of the drought treatment applied as it did not impact grass biomass production. However, most species showed a competitive effect during the period of moisture limitation. Grasses are generally better able to deal with low to moderate drought but more sensitive to longer and severe periods of water stress.

Keywords*: Drought, competition, phytometer, biomass*

4.1. Introduction

Climate models predict an increase in the probability of extreme weather as a consequence of global warming, such consequences include frequent and severe droughts (IPCC, 2018). Under water deficit, some grass species reveal mechanisms to combat dehydration while others may not be as well adapted to long-term water deficit (Farooq et al., 2009). Drought may alter plant traits that are linked with biomass allocation and reproduction, which can affect how a plant accesses water resources and may reduce competitive ability (Potts et al., 2019). The onset of water limitation usually increases competition between plant species but may also encourage facilitation among some plants as environmental stress progresses (Butterfield et al., 2016). Plants generally compete for nutrients, light, space, pollinators and moisture, but at the same time co-existence can create favourable microclimates conducive to facilitation (Brooker et al., 2007). The intensity and importance of facilitation may be higher under moderate stress and eventually decreases, sometimes collapsing under severe resource limitation conditions and increasing competitiveness among grass species (Castanho et al., 2015).

Competitive interactions have been extensively researched in ecology, as they impact the distribution, abundance, and traits of coexisting species (Clements et al., 1929; Gause, 1934; Goldberg and Barton, 1992; Trinder et al., 2013). Their relationship to environmental heterogeneity (Fransen et al., 2001), disturbance frequency and intensity (Connell and Slatyer, 1977), and resource availability (Goldberg and Barton, 1992; Grime, 1979) are all significant. They are most closely linked to the identification of below or above ground limiting resources (Tilman, 1982). Some authors state that limiting resources can lead to intense competition among plants (Butterfield et al., 2016; Chapin and Shaver, 1985). By contrast others contend that greater competition is the outcome of an increase in resources (Corcket et al., 2003; Kadmon, 1995). However, competition remains an important ecosystem interaction, particularly in systems with water limitation (Fowler, 1986).

Plant interaction theory predicts that superior competitors will eventually drive an inferior competitor to a lower abundance or in extreme cases, extinction (Grime, 1973; Tilman, 1985). Despite this, species with different competitive abilities, often coexist in natural communities (Tilman, 1994) although sometimes with large differences in abundance. The degree of intraspecific competition may be a crucial factor in determining whether competing species can coexist (Chesson, 2000; Tilman, 1988). Since members of the same species will share the same resource requirements, theory predicts that intraspecific competition will usually be strong. As some species limit themselves more than others, niche differences, also known as stabilizing forces can encourage coexistence (Chesson, 2000). To avoid competitive exclusion, common species, in particular, need stronger self-limitation than uncommon species (Chesson, 2000).

Besides plant differing traits, such as palatability and leaf size, previous studies generally show a positive relationship between abundance and competitive ability and in some cases, strong competitors in greenhouse experiments may be highly abundant in the field (Aplet and Laven, 1993). Therefore, the most abundant species in the local environment were considered to represent the local vegetation in the experiment to assess the effect of drought and competition on various grass species traits. Palatability is also an influential factor in plant competition. For instance, the response of grasses to grazing pressure is predominantly explained by selective grazing (Anderson and Briske, 1995). Palatable grasses are generally leafy, less hairy, more tender because of lower lignin content with more sugar content and usually grow in fertile soil and have a high nutritional value (Tainton, 1999).

Palatable species may be quickly eliminated during drought if there is herbivory due to selective grazing. Unpalatable species have a competitive advantage because they are subjected to low herbivory frequency and intensity, giving them a competitive advantage over other species in the community, however in the absence of grazing, the competitive ability of the palatable species is greater than unpalatable species (Anderson and Briske, 1995; Briske, 1991). Grasses with broader leaves might have a photosynthetic competitive advantage due to their larger surface area and stomata, but, these exact structural elements may cause the leaf to lose a significant amount of water through transpiration compared to plants with smaller leaves (Buckley et al., 2017). This means plants with narrow leaves are expected to have a better chance of survival during dry conditions. There is limited literature available on grass competitive responses to soil moisture deficits. One of the palatable species used in this study, *T. triandra* is dominant throughout grasslands in Australia, the Middle East, India, East Asia and eastern and southern Africa (Bews, 1929). As a result, the grasslands that are dominated by *T. triandra* are of significant ecological and economic value (Bews, 1929). In response to moisture deficit, palatable species such as *T. triandra* and *T. leucothrix* are likely to be replaced by unpalatable grasses, such as *A. junciformis* and *C. excavatus* (O'Connor et al., 2003). To adapt to dry conditions, plant leaves often become thicker, but smaller in leaf area with higher leaf tissue densities (Werner et al., 1999), which has a direct impact on plant photosynthesis and biomass production.

Leaf size can be used as an indirect method for selecting for increasing tiller (and growing point) density (Kemp and Culvenor, 1994). Tall plants with large leaves generally have a competitive advantage over small plants when competing for aboveground resources (Weiner and Damgaard, 2006), as the plants with larger leaves can accumulate higher biomass. Increased height enhances the plant's competitive advantage by providing greater absorption of light and shading out shorter competitors (Huber et al., 1998). Tall plants with large leaf size, have greater biomass production rate per unit leaf mass because tall plants with broad leaves have a high photosynthetic capacity (Pallardy, 2008). Since plant photosynthetic capacities are directly correlated with leaf area (Tessmer et al., 2013), small plants with larger leaf area can have a high photosynthetic capacity. One of the leaf characteristics that most accurately reflects plant growth and production is specific leaf area (SLA), which is defined as the ratio of total leaf area to total leaf dry mass (Evans, 1972). SLA describes the distribution of leaf biomass relative to leaf area and, hence, corresponds to carbon gain relative to water loss within a plant canopy (Pierce et al., 1994). It is influenced by environmental factors and has been shown to be positively correlated to precipitation (Hovenden, 2001; Loveys et al., 2002). Factors including nutrient enrichment (nitrogen and phosphorus) and disturbance such as grazing can change the dominance of a community toward species with greater SLA. More shading and high water availability generally cause a rise in SLA in herbaceous plants (Galmes et al., 2005). Experimental drought offers important information about community changes at finer temporal scales. Comprehending plant adaptive strategies and community resilience is an important area of ecological research (Kooyers, 2015).

Access to and use of water strongly influences a plant's competitive abilities and abundance (Kooyers, 2015; Ogle and Reynolds, 2004). Biomass is often used as a measure of ecological status in a given biome or vegetation type, as it reflects the amount of energy stored in the vegetation (Ramoelo et al., 2015). It can therefore be assumed that the biomass production of different plant species gives us the best measure of these species' chances of survival in the event of resource limitation and competition. Practically, biomass can be used as an indicator of the quality of a given plant community, as well as the quantity of a given species in relation to others (Ramoelo et al., 2015). The information acquired by measuring biomass could assist in the application of management practices that counteract the effects of drought. This study gives insight to the competitive abilities of the selected grasses in times of moisture limitations and the information serves as a reflection of the mesic grassland's ability to withstand moisture depletions to a certain degree. The dominant species at Ukulinga Research Farm were selected to link the pot trial to the field trials and to give an indication of responses that might be seen in the field. Experiments can provide a valuable tool to assess plant–plant interactions under changing environmental conditions. Experimental moisture manipulation pot trials were therefore selected to determine the impact of drought responses on important grass species.

4.2. Aims and research questions

This study aims to investigate the competitive effects of grasses with broad leaves against those with narrow leaves, as well as palatable grasses against unpalatable species, on plant growth, represented by aboveground biomass production, height, tuft circumference, specific leaf area and tiller numbers under drought conditions. The following questions were addressed:

(1) What are the effects of grass-grass competition on the growth of six locally common grass species?
(2) Does water limitation have a relative effect on competitive interactions between the six grass species?
(3) How does the combination of water limitation and competitive interactions affect biomass, height, tiller count, specific leaf area and tuft size of grasses?
(4) Does the palatability or leaf size of grasses have an effect on grass adaptability to drought and competitive interactions?

4.3. Methodology

4.3.1. Study site and experiment

The experiment was conducted in the greenhouse at the NM Tainton Arboretum, University of KwaZulu-Natal, Pietermaritzburg, South Africa. The region experiences hot summers and mild winters with a mean monthly maximum of 26.4°C in February and minimum of 8.8°C in July. The greenhouse has a relative humidity of 82.7%. The grass species were collected from Ukulinga Research Farm, University of KwaZulu-Natal, Pietermaritzburg, South Africa, an area with mild winters, with a mean minimum temperature of 8.8°C in July, and hot summers, with a mean monthly maximum of 26.4°C in February.

Six common grass species were collected from a mesic grassland at Ukulinga Research Farm, planted in pots with a 25cm diameter in an open shade house and allowed to establish for a period of two weeks before the experiment began. The selected species represented palatable and unpalatable groups as well as broad leaf and narrow leaf groups. The plant's palatability can be an indication of the veld condition. The palatable grass species used were *Themeda triandra* and *Tristachya leucothrix* and the unpalatable species were *Aristida junciformis* and *Cymbopogon excavatus*. *Aristida junciformis* and *Melinis nerviglumis* represented narrow-leaved grasses while *Megathyrsus maximus* and *Cymbopogon excavatus* represented broad-leaved species. The six grass species were selected because of their dominance in the field site where they were collected and their widespread distribution in eastern South Africa (Oudtshoorn, 2002). Characteristics of these species are described in Table 4.1.

Table 4.1: Characteristics of phytometer/competitor grass species (van Oudtshoorn 2002)

Species	**Lifespan**	**Tillers**	**Height (cm)**	**Leaf blade length (mm)**	**Leaf blade width (mm)**	**Leaf blade shape**	**Palatability**
A. junciformis	Perennial	Many	20-75	Up to 300	Up to 3	Roll	Low
C. excavatus	Perennial	Few	50-150	50-300	5-14	Flat	Low
M. maximus	Perennial	Few	60-200	60-400	4-20	Flat	High
M. nerviglumis	Perennial	Many	25-60	40-200	2-11	Roll	Average
T. triandra	Perennial	Average	3-15	150-300	1-8	Flat	High
T. leucothrix	Perennial	Many	15-90	50-400	2-6	Flat	High

The experiment was conducted using the phytometer method where there is a central focus tuft representing the phytometer, surrounded by four competitor tufts of a different species to the focal plant. Each species alternates and serves as both a photometer and a competitor. Intraspecific competition was not assessed, but all species were grown in the absence of competition to provide a control measure of growth. To investigate the effect of drought on grass-on-grass interactions, grass combinations were transplanted into pots with a 25 cm diameter, filled with coarse, nutrient-poor sand and allowed a two-week establishment period. Tufts were standardized to four tillers per tuft. All pots were fertilized with 100ml of 80% Hoagland's nutrient solution (N = 172, P = 25, K = 188 mg L^{-1}) (Hoagland and Arnon, 1950) once a week. All tufts were cut to a uniform height of 10cm to help survive the transplant stress and to also establish a base height for when the treatments were applied. Cutting the tufts to a uniform height was done when the tufts were transplanted, at the beginning of the official treatment application.

Plants under the adequate moisture treatment were irrigated twice a week with 500 ml municipal water, while the drought treatment was only irrigated once a week with the same amount. The plants were irrigated directly into the pot trays to allow for water absorption through the roots. The trial was arranged in a randomized block design with 6 replications, resulting in a total of 264 pots, where 132 pots were induced with the drought treatment and the remainder received the adequate moisture treatment. Each phytometer species had 44 pots. At the beginning of the experiment, tuft basal circumference was measured. The study was conducted over a period of 20 weeks, during the growing season (October – February). At the end of the experiment, the final height and tuft circumference of all phytometers was measured, and the tiller numbers were counted. To determine the specific leaf area (SLA) of each plant, 9 leaves were selected from the top, middle and bottom sections of the stems within each tuft (3 from each section). For clustered tufts such as those of *T. triandra*, leaves were picked directly from the middle cluster through to the surrounding leaves. The collection of leaves from different regions was to ensure that the average SLA is a direct representation of leaves from the whole plant, not just one specific region of a plant. Only fully expanded leaves were collected. An LI-3000C portable leaf area meter (Li-Cor Bioscience) was used to measure the leaf area, then the leaves were oven dried for 48 hours at 60ºC and weighed to determine specific leaf area to 2 decimal points of a gram. The phytometer grasses were then harvested at the soil surface and oven-dried for 48 hours at 60°C before weighing to determine the final dry biomass.

4.3.2. Statistical analysis

Generalized linear models were used to assess the main effects and interactions of competition and water levels on the mean biomass of all phytometers with binomial distribution, after the data violated the assumptions of ANOVA. Analysis of variance was used to assess the main effects of water level, phytometer species and competition for plant height (cm), tiller numbers, tuft circumference (cm) and specific leaf area (SLA) after all variables met the assumptions of normality. When the analysis of variance (F-test) revealed significant differences, further analysis was conducted using the Tukey's test to separate means ($\alpha = 0.05$). This analysis of variance and post-hoc data analysis was performed using SPSS 26.0 (IBM Systat Software), and SigmaPlot 12.5 (Systat Software) for visual representation of results. The generalized linear models were also conducted using SPSS 26.0 (IBM Systat Software).

4.4. Results

Palatable phytometers

The generalized linear models investigating the effects of drought and competition on grass biomass revealed that the differences in water level did not have any significant impact on the final dry biomass of palatable species (Table 4.2), but biomass was significantly affected by the presence of competitors ($P<0.05$). The presence of competition significantly reduced the biomass production of palatable grasses (Table 4.2). The amount of biomass reduction was dependent on the specific competitor plant in question. For instance, *Cymbopogon excavatus* had a greater competitive effect than the other unpalatable species, *Aristida junciformis*. *C. excavatus* reduced the biomass and tuft circumference of the palatable phytometers more (Figure 4.1A & 4.1B). This may be explained by the physiological differences between the two competitors, given that *C. excavatus* is a generally larger and taller plant than *A. junciformis* which is comparatively shorter, with narrower leaves. The individually planted grasses consistently had a higher biomass production compared to plants with competitors (Figure 4.1A).

Table 4.2: Results for generalized linear models of the main effects and interactions of species status and water level on mean dry biomass, for palatable phytometers

Source of variation	**df**	**X^2**	**P-value**
Phytometer (P)	1	0.532	0.466
Competition (C)	2	13.136	**0.001**
Water Level (WL)	1	1.013	0.314
P * C	2	0.956	0.620
P * WL	1	0.681	0.409
C * WL	2	1.592	0.451
P * C * WL	2	0.512	0.774

*Table shows Wald Chi-squares (X^2), degrees of freedom (df), and p-value, significant effects ($P<0.05$) in bold.

The palatable phytometers, *T. triandra* and *T. leucothrix*, differed in terms of their number of tillers (9.68 ± 0.628 vs. 9.54 ± 0.579) and tuft circumferences (11.15 ± 0.752 vs. 8.51 ± 0.619) with *T. triandra* consistently being larger. Competition reduced tuft circumference (Figure 4.1B), however, the mean height, tiller numbers and SLA for *T. triandra* and *T. leucothrix* remained unaffected by the presence of competition (Table 4.3). Interestingly, the average number of tillers for palatable phytometers was the only physiological trait affected by differences in water levels (Table 4.3; Figure 4.2). In both species, drought significantly reduced ($P<0.05$) the number of tillers. There was a common outcome throughout all treatments in terms of specific leaf area, where no significant effect was recorded for all species (Table 4.3).

Table 4.3: Results for analysis of variance of the main effects and interactions of species and water level for mean final height (cm), tiller numbers, tuft circumference (cm) and specific leaf area (SLA) on palatable phytometers

		Height		**Tiller numbers**		**Tuft circumference**		**SLA**	
Source of variation	**d.f**	**F-ratio**	**P-value**	**F-ratio**	**P-value**	**F-ratio**	**P-value**	**F-ratio**	**P-value**
Phytometer (P)	1	0.634	0.429	740.227	**<0.001**	14.021	**<0.001**	0.357	0.552
Competition (C)	2	2.865	0.065	0.047	0.830	28.063	**<0.001**	0.520	0.597
Water Level (WL)	1	0.016	0.901	16.770	**<0.001**	0.012	0.912	1.472	0.230
P * C	2	0.979	0.382	0.204	0.653	1.018	0.367	0.077	0.926
P * WL	1	0.032	0.860	2.207	0.119	0.490	0.487	0.572	0.452
C * WL	2	1.026	0.365	0.065	0.799	0.807	0.451	1.861	0.164
P * C * WL	2	0.307	0.737	0.350	0.706	1.063	0.352	0.736	0.483
Error	60			0.068	0.935				
Total	72								

*significant effects ($p<0.05$) in bold.

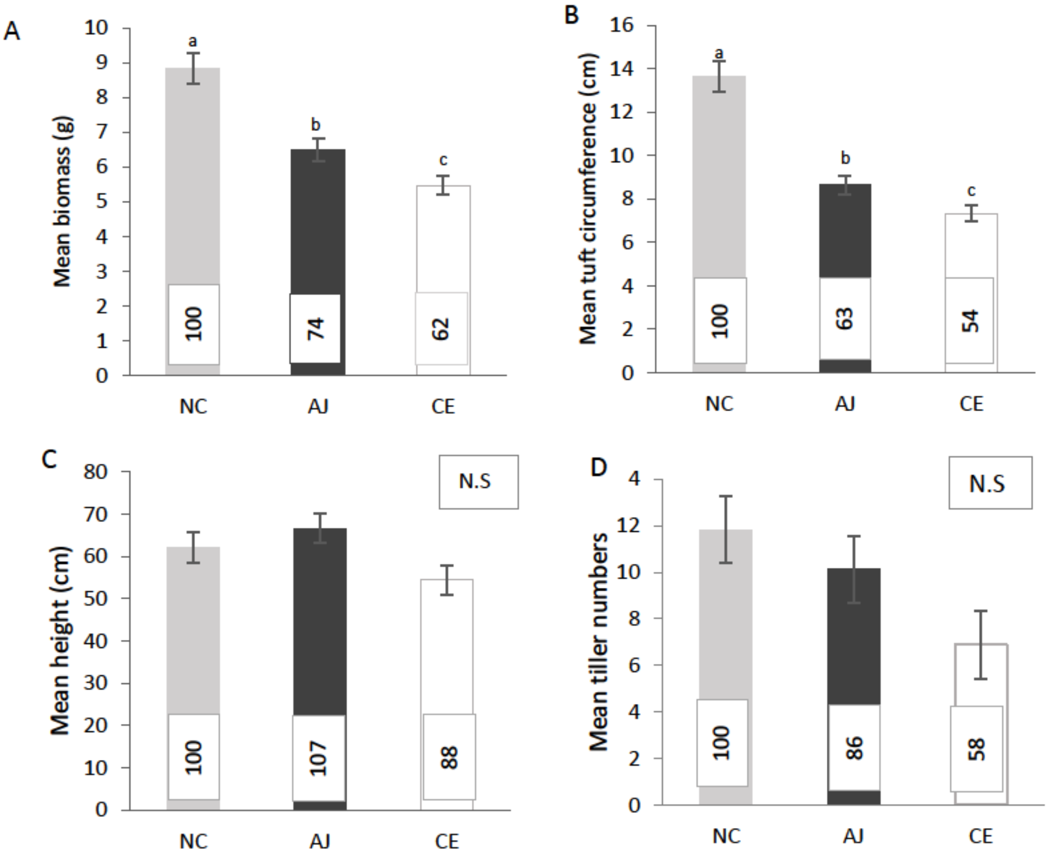

Figure 4.1: Mean (± SE) biomass (g) - A, mean tuft circumference (cm) - B, height (cm) – C and mean tiller numbers – D for palatable phytometers *Themeda triandra*, *Tristachya leucothrix* (combined) with unpalatable competitors, *Aristida junciformis* – (AJ), *Cymbopogon excavatus* (CE) and no competition (NC). Treatments with letters in common are not different (P > 0.05). Graphs with N.S are non-significant.

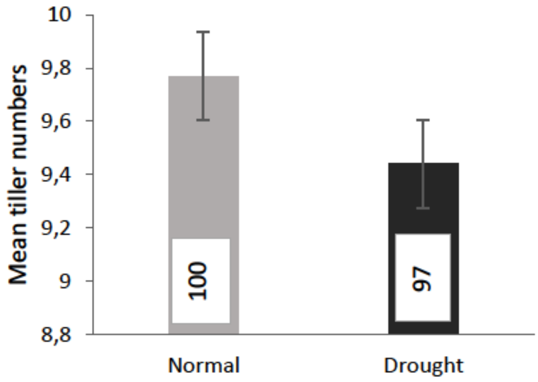

Figure 4.2: Mean (± SE) tiller numbers for all palatable phytometers under normal and drought water levels.

Unpalatable phytometers

There was a general reduction of biomass in the presence of a competitor but there was also a species-specific competitive effect. The biomass of plants growing with *T. triandra* was significantly greater (P<0.05) than those growing with *T. leucothrix* (Table 4.4, Figure 4.3A). *Tristachya leucothrix* also had a stronger competitive effect than *T. triandra* on the height of the unpalatable species (Table 4.5, Figure 4.3). These results signify that *T. leucothrix* had a stronger competitive effect on unpalatable species than *T. triandra* in the reduction of biomass and height of the phytometer. In addition, tiller number and tuft circumference were reduced by the presence of competitors but there were no species-specific differences in the reduction of these traits between competitors (Table 4.5, Figure 4.3B, 4.3D). There were no interactive effects on final dry biomass, tiller number, tuft circumference and SLA in the unpalatable phytometers.

Table 4.4: Results for generalized linear models of the main effects and interactions of species status and water level on mean dry biomass, for unpalatable phytometers

Source of variation	**df**	**X^2**	**P-value**
Phytometer (P)	1	10.323	**0.001**
Competition (C)	2	20.823	**<0.001**
Water Level (WL)	1	0.053	0.818
P * C	2	2.829	0.243
P * WL	1	0.397	0.529
C * WL	2	5.955	0.051
P * C * WL	2	2.760	0.252

Table 4.5: Results for analysis of variance of the main effects and interactions of species and water level for mean final height (cm), tiller numbers, tuft circumference (cm) and specific leaf area (SLA) on unpalatable phytometers

	Height			**Tiller numbers**		**Tuft circumference**		**SLA**	
Source of variation	**df**	**F-ratio**	**P-value**	**F-ratio**	**P-value**	**F-ratio**	**P-value**	**F-ratio**	**P-value**
Phytometer (P)	1	10.858	**0.002**	128.114	**<0.001**	15.501	**<0.001**	1.922	0.171
Competition (C)	2	16.307	**<0.001**	3.846	**0.027**	9.455	**<0.001**	0.456	0.636
Water Level (WL)	1	1.929	0.170	0.222	0.639	0.164	0.687	1.202	0.277
P * C	2	3.645	**0.032**	1.528	0.225	0.237	0.790	0.969	0.385
P * WL	1	0.019	0.890	2.002	0.162	0.262	0.611	0.908	0.344
C * WL	2	1.364	0.263	1.070	0.350	0.189	0.828	0.559	0.575
P * C * WL	2	0.553	0.578	0.327	0.722	0.885	0.418	0.458	0.634
Error		60							
Total		72							

*significant effects (p<0.05) in bold

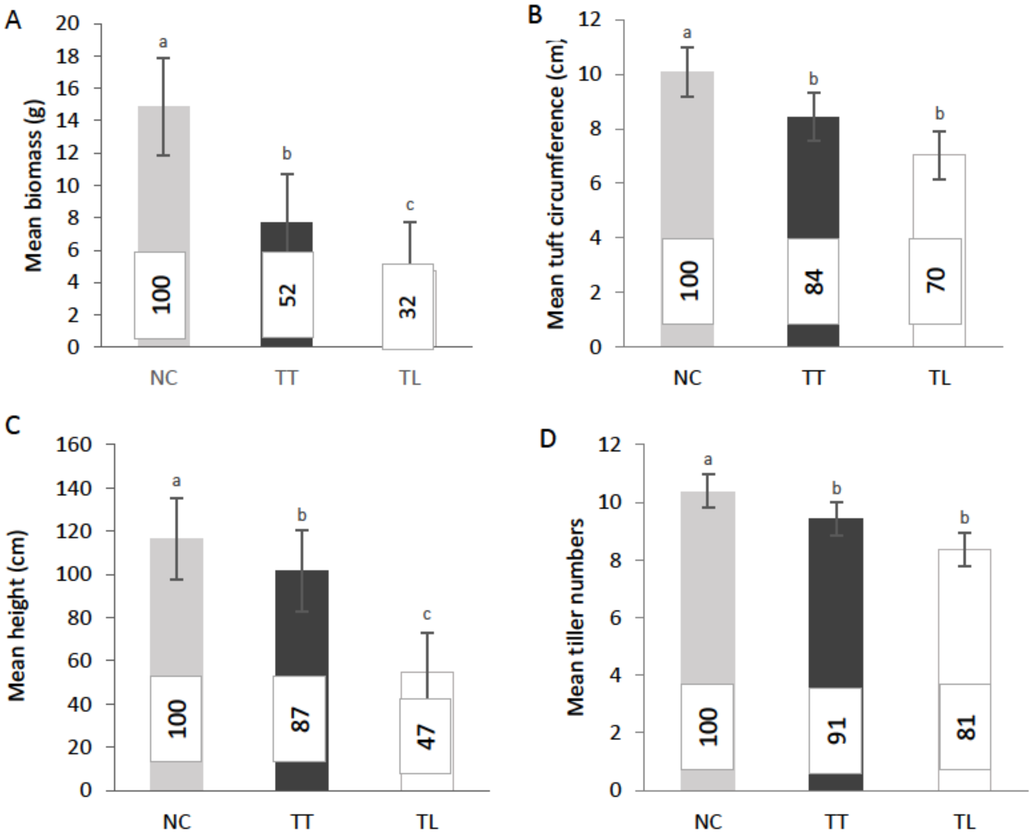

Figure 4.3: Mean (± SE) biomass (g) - A, mean tuft circumference (cm) – B, mean height (cm) - C and mean tiller numbers – D for unpalatable phytometers *Aristida junciformis*, *Cymbopogon excavatus* (combined), with palatable competitors, *Themeda triandra* (TT), *Tristachya leucothrix* (TL) and no competitor (NC). Treatments with the same superscripts are not significantly different ($P > 0.05$).

Cymbopogon excavatus phytometers were larger than *A. junciformis* in terms of biomass (11.91g ± 1.551 vs.6.33g ± 0.613), height (106cm ± 10.374 vs. 75.56cm ± 4.612) and tuft circumference (9.64 ± 0.542 vs. 7.37 ± 0.308) (Table 4.3). A significant interaction between phytometer species and competitor species on height was observed. Generally, *C. excavatus* phytometers were taller than *A. junciformis*, except for when they grew with *T. leucothrix* as a competitor (Figure 4.4), where there was no significant difference in the tuft height (Table 4.5, Figure 4.4).

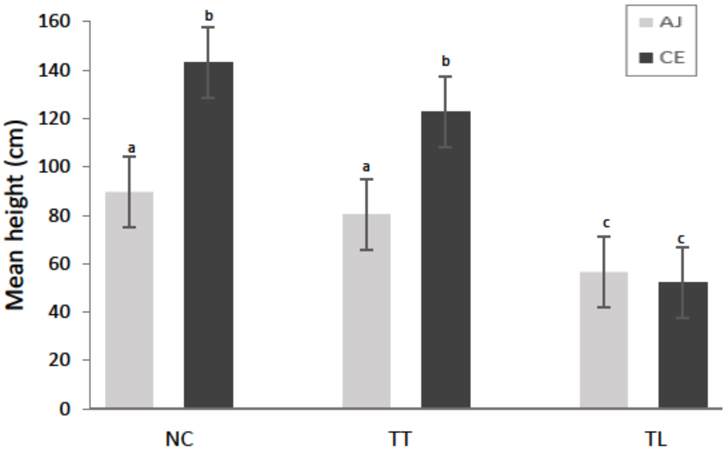

Figure 4.4: Mean (± SE) height (cm) for interactions between unpalatable phytometers *Aristida junciformis* (AJ) and *Cymbopogon excavatus* (CE) and palatable competitors *Themeda triandra* (TT), *Tristachya leucothrix* (TL) and no competition (NC).

Narrow-leaved phytometers

There was a general competitive effect from broad-leaved competitors on the biomass production, tiller numbers and tuft circumference of narrow leaved speacies (Table 4.6 & 4.7). To be precise, there was a species-specific impact of broad-leaved competitors where *Megathyrsus maximus* had a stronger competitive effect than *C. excavatus*, reducing narrow-leaved grass biomass (Figure 4.5A) and tiller numbers (Figure 4.5D). The narrow-leaved phytometers differed in terms of height, tiller number and tuft circumference with *Aristida junciformis* being significantly taller and having more tillers than *Melinis nerviglumis.*

Table 4.6: Results for generalized linear models of the main effects and interactions on mean dry biomass, for narrow leaved phytometers competing with broad leaved species, showing Wald Chi-squares (X^2), degrees of freedom (d.f), and p-value, with significant effects ($P<0.05$) in bold

Source of variation	d.f	X^2	P-value
Phytometer (P)	1	0.585	0.445
Competition (C)	2	38.183	**<0.001**
Water Level (WL)	1	0.259	0.611
P * C	2	0.958	0.619
P * WL	1	0.159	0.690
C * WL	2	0.846	0.655
P * C * WL	2	3.758	0.153

Table 4.7: Results for analysis of variance of the main effects and interactions for mean final height (cm), tiller numbers, tuft circumference (cm) and specific leaf area (SLA) on narrow leaf phytometers, showing degrees of freedom (d.f), F-value and P-value, with significant effects (p<0.05) in bold

	Height			Tiller numbers		Tuft circumference		SLA	
Source of variation	**d.f**	**F-ratio**	**P-value**	**F-ratio**	**P-value**	**F-ratio**	**P-value**	**F-ratio**	**P-value**
Phytometer (P)	1	10.256	**0.002**	6.546	**0.013**	10.255	**0.002**	1.680	0.200
Competition (C)	2	0.823	0.444	6.936	**0.002**	12.498	**<0.001**	0.246	0.783
Water Level (WL)	1	3.855	0.054	0.187	0.667	0.494	0.485	0.190	0.664
P * C	2	3.180	**0.049**	0.286	0.752	1.666	0.198	2.559	0.086
P * WL	1	2.329	0.132	2.871	0.095	0.232	0.632	0.127	0.723
C * WL	2	1.339	0.270	0.187	0.830	0.687	0.507	0.756	0.474
P * C * WL	2	0.373	0.691	0.089	0.915	0.372	0.691	0.408	0.667
Error	60								
Total	72								

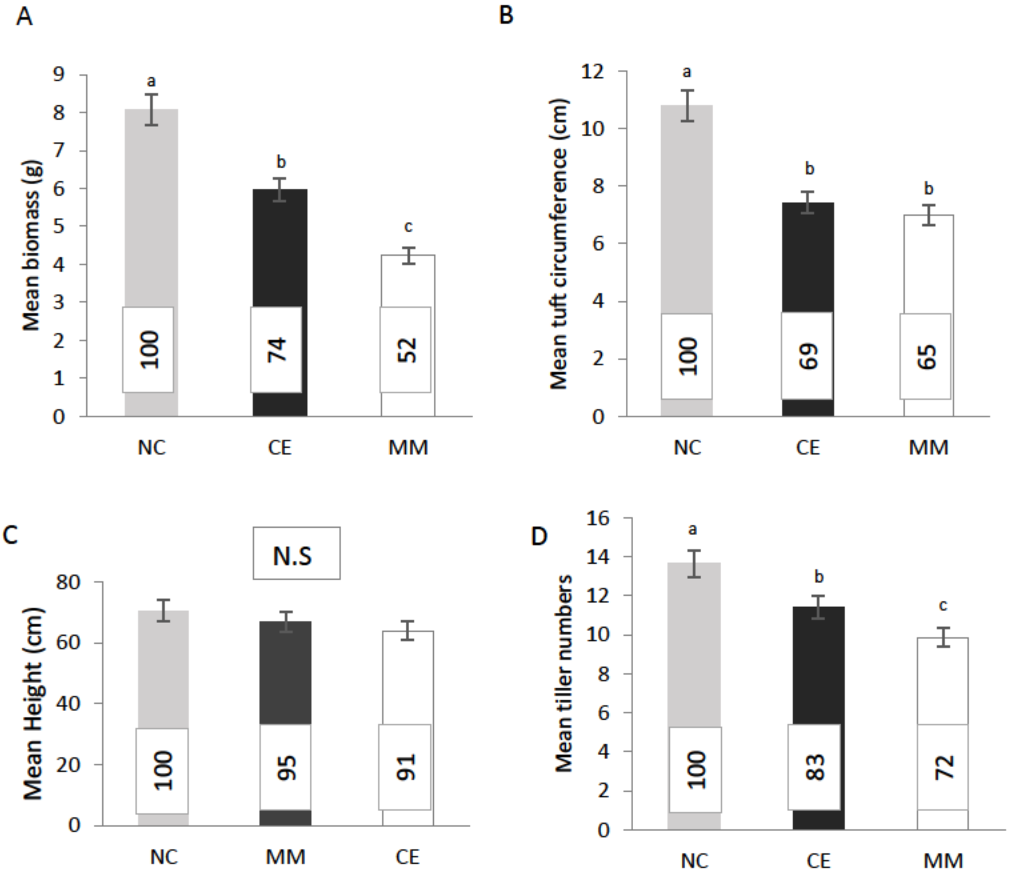

Figure 4.5: Mean (± SE) biomass (g) - A, mean tuft circumference (cm) - B, mean height (cm) - C, mean tiller numbers – D for narrow leaf phytometers *Melinis nerviglumis*, *Aristida junciformis* (combined), with broad leaf competitors and no competition – NC treatments. All bars have the percentage reduction caused by competition in each variable Treatments with letters in common are not significantly different (P > 0.05), blocks with N.S are not significant.

Broad-leaved phytometers

There was a general significant competitive effect ($P<0.05$) by narrow-leaved species on the biomass of broad-leaved species on biomass production, but there was no effect of water levels and phytometer species (Table 4.8). Plants with no competition had greater biomass than the plants experiencing competition (Figure 4.5 & 4.6). Narrow-leaved competitors did not have any significant competitive impact on the mean number of tillers, tuft circumference and height of broad-leaved species. *Melinis nerviglumis* was a fairly strong competitor as it caused a greater reduction ($P<0.05$) in broad-leaved phytometer biomass than *A. junciformis* (Figure 4.6 & 4.7). This could be due to the differences in physiological structure of the two grasses because *A. junciformis* grows more erect while *M. nerviglumis* has broader tufts leading to a greater competitive footprint. The noted difference in the pattern of response is that *M. nerviglumis* was more competitive against *M. maximus*, while *A. junciformis* was more competitive against *C. excavatus* (Figure 4.7) and this interaction was only found on tuft circumference. Both the tiller counts and tuft circumference for broad leaf species was significantly reduced by drought.

Table 4.8: Results for generalized linear models of the main effects and interactions on mean dry biomass, for broad leaved phytometers competing with narrow leaved species, showing Wald Chi-squares (X^2), degrees of freedom (d.f), and p-value, with significant effects ($P<0.05$) in bold

Source of variation	d.f	X^2	P-value
Phytometer (P)	1	0.035	0.852
Competition (C)	2	19.792	**<0.001**
Water Level (WL)	1	1.743	0.187
P * C	2	1.113	0.573
P * WL	1	0.868	0.352
C * WL	2	0.251	0.882
P * C * WL	2	2.148	0.342

Table 4.9: Results for analysis of variance of the main effects and interactions for mean final height (cm), tiller numbers, tuft circumference (cm) and specific leaf area (SLA) on broad leaf phytometers showing degrees of freedom (d.f), F-value and P-value, with significant effects ($p<0.05$) in bold

	Height			Tiller numbers		Tuft circumference		SLA	
Source of variation	d.f	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
Phyto (P)	1	0.030	0.864	1.342	0.251	0.160	0.690	0.598	0.442
Comp (C)	2	1.281	0.285	1.842	0.167	2.015	0.161	0.671	0.515
Water Level (WL)	1	3.003	0.088	7.101	**0.010**	5.875	**0.005**	3.856	0.054
P * C	2	1.331	0.272	0.305	0.738	5.666	**0.020**	0.263	0.770
P * WL	1	0.570	0.453	0.215	0.645	0.863	0.427	1.629	0.207
C * WL	2	0.491	0.615	0.144	0.866	3.052	0.055	0.715	0.493
P * C * WL	2	0.703	0.499	0.527	0.593	0.464	0.631	0.192	0.826
Error	60								

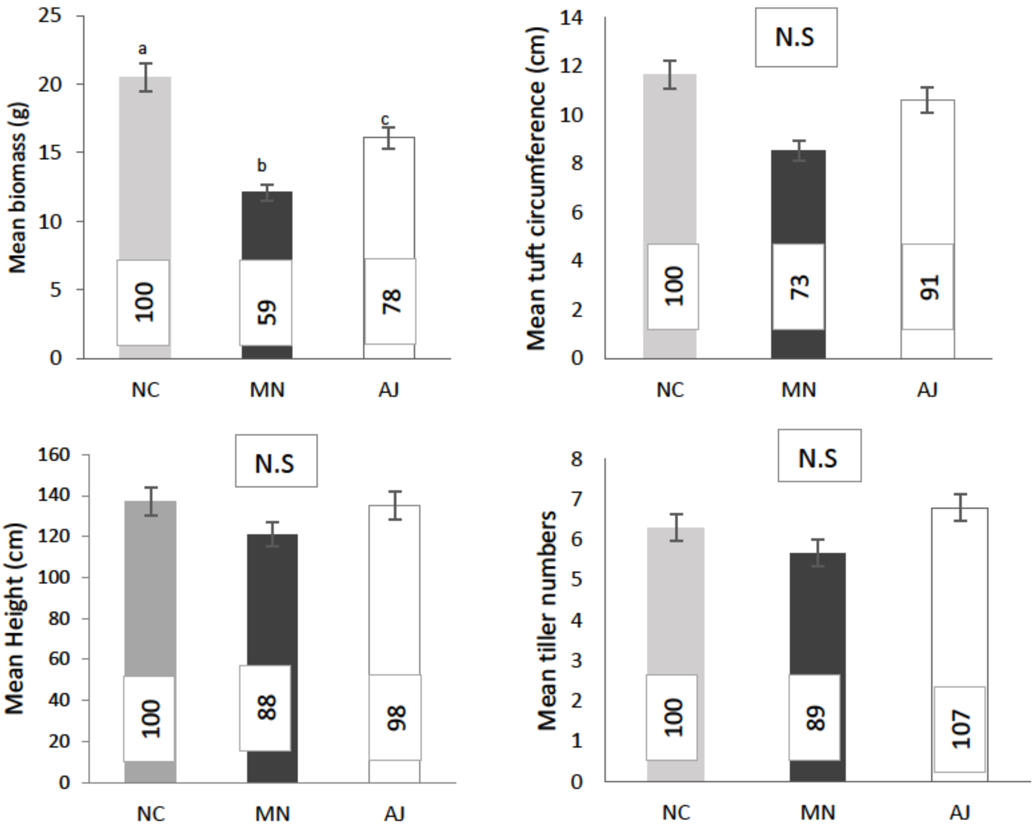

Figure 4.6: Mean (± SE) biomass (g), mean tuft circumference (cm) mean height (cm) and mean tiller numbers for broad leaf phytometers *Megathyrsus maximus* and *Cymbopogon excavatus* (combined) with narrow leaf competitors *Melinis nerviglumis* (MN), *Aristida junciformis* (AJ) and no competition (NC) treatments. Treatments with letters in common are not significantly different (P > 0.05), blocks with N.S are non-significant.

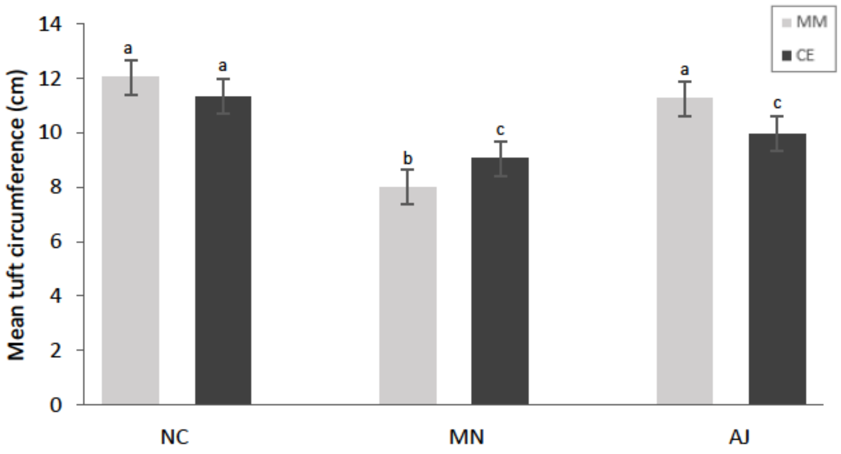

Figure 4.7: The interactions for mean (± SE) tuft circumference between broad leaf phytometers *Megathyrsus maximus* (MM) and *Cymbopogon excavatus* (CE) and narrow leaf competitors *Melinis nerviglumis* (MN), *Aristida junciformis* (AJ) and no competition (NC) plots. Treatments with letters in common are not significantly different (P > 0.05).

Both *A. junciformis* and *C. excavatus* had a lower percentage biomass when paired with *T. leucothrix* compared to *T. triandra* as a competitor. Both palatable species had a lower competitive response to *A. junciformis* compared to *C. excavatus* and that might be attributed to the differences in plant leaf size (Table 4.10). there was a strong competitive response from plants that had broad-leaf competitors rather than the ones with narrow-leaf competition (Table 4.10).

Table 4.10. Summary in reduction of biomass as a result of interaction with competitor species, expressed as percentage of a phytometer by each species relative to the biomass obtained by the phytometer grown without competition.

Phytometers	***Competitors***	
Unpalatable	**Palatable**	
	T. leucothrix	*T. triandra*
A. junciformis	34%	66%
C. excavatus	31%	45%
Palatable	**Unpalatable**	
	A. junciformis	*C. excavatus*
T. leucothrix	74%	69%
T. triandra	73%	56%
Narrow-leaf	**Broad-leaf**	
	M. maximus	*C. excavatus*
A. junciformis	46%	62%
M. nerviglumis	51%	75%
Broad-leaf	**Narrow-leaf**	
	A. junciformis	*M. nerviglumis*
C. excavatus	76%	66%
M. maximus	56%	52%

4.5. Discussion

4.5.1. Effect of competition between palatable and unpalatable species

Dry matter has been used extensively to analyse the competitive ability of grass species as it is an effective measure of resource limitation to plant growth and can also be an indicator of intraspecific or interspecific competitive effects (Fagundez and Lema, 2019). For example, by growing thinner leaves and roots, plants can produce greater leaf area and longer roots with the same amount of biomass. Consequently, reduced specific leaf area during growth will result in a reduction in plant efficiency, which impacts the competitive dynamics between plants or seedlings (Schippers and Olff, 2000). The general findings of this study show that the presence of competition significantly reduced biomass production of both palatable and unpalatable species, irrespective of the type of competing species. Similarly, Aguiar et al. (2001), found that shortgrass species experiencing competition accumulated less biomass compared to those growing with limited or no competition. For unpalatable focal plants, the negative effects of competition on aboveground biomass production were both general and species-specific. *T. triandra* and *T. leucothrix* both caused a significant reduction in the unpalatable focal plant biomass and height, while *T. leucothrix* proved to be a stronger competitor.

The significant reduction of biomass is a direct result of the negative effect of competition on other plant traits that were measured in this study, including number of tillers, tuft circumference and height. For instance, *T. leucothrix*, a strong competitor, caused a significant height reduction to unpalatable plants in general. This is unexpected as competitive success is usually correlated with size and both *A. junciformis* and *C. excavatus* are taller than *T. leucothrix*. This opposes the view that taller plants are better at capturing resources and denying those resources to other plants and in most cases smaller species often experience suppressed growth under competition (Keddy et al., 2002; Rösch et al., 1997).

Superior competitive ability in plants requires a relatively larger plant size (Goldberg, 1996; Grime, 1979). On the contrary, height is not strongly correlated with competitive ability in our study between the palatable and non-palatable species because a relatively smaller palatable grass *T. leucothrix* had a strong competitive advantage over a taller and unpalatable species such as *C. excavatus*. Competition from *T. triandra* and *T. leucothrix* significantly reduced tiller numbers and the tuft circumference of unpalatable plants and the competitive effect was species-specific whereby *T. leucothrix* was a stronger competitor again. The findings of this study indicate that palatable species may better withstand the presence of competition as the height and tiller numbers of palatable plants were not affected by competition. Given that biomass yield is positively correlated with plant height and tuft diameter, if the height of a plant is reduced by a competitor, then the biomass will ultimately also be negatively affected, disrupting the productivity of the plant, and eventually the chances of survival under drought conditions (Yan et al., 2012). The common trend between the palatable and unpalatable species as well as the narrow and broad-leaved species is that none of the treatments showed any significant effects on specific leaf area.

The stress-gradient hypothesis (SGH), which was first introduced in the early 1900s by Clements (1916), states that competitive interactions between plants are more prevalent and intense under low-stress conditions, while facilitative interactions are more frequent under high-stress conditions. However, later research provided both supporting (Lortie and Callaway, 2006; Ziffer-Berger et al., 2014) and contradictory findings that led to the refinement of the model which now states that the intensity and importance of facilitation may be highest under moderate stress and may decrease or even collapse under severe stress conditions, eventually increasing competition amongst plants under dry and harsh environmental conditions (Bu et al., 2013; Butterfield et al., 2016; Castanho et al., 2015; Maestre et al., 2005). Based on the results of this study, competition intensity was based on the physiological features of the species such as leaf size and no effect of water stress was observed. For the stress-gradient hypothesis to take effect, the intensity of the water stress may need to be changed.

4.5.2. Effect of competition between narrow and broad-leaved species

In plant communities, interactions are often measured as a ratio of performance variables, such as biomass, between plants which grow individually and plants with neighbours left intact (Armas et al., 2004). The biomass production of species with narrow leaves was significantly reduced by the presence of broad-leaf competitors. These findings align with the research that assumes that smaller plants experience size suppression from larger competitors and therefore are mostly at a competitive disadvantage (Keddy et al., 1994). Although both did show a general reduction, *Megathyrsus maximus* was a dominant competitor as it significantly reduced mean biomass production of narrow-leaved species more than *C. excavatus*. Coupled with the reduction in biomass was a reduction in the tiller counts and tuft circumference. Other studies have found similar effects of *M. maximus*, likely due to its upright, tufted, densely bunched leaves which provide extensive shading (Coêlho et al., 2021).

Narrow-leaved competitors did not have a significant effect on either height, tiller numbers or tuft circumference of the broad-leaved plants. However, narrow-leaved competitors significantly reduced the biomass of broad-leaved species, exhibiting species-specific competitive effects. However, there was an interactive effect of competition and phytometer on the tuft circumference of broad-leaved species. *Melinis nerviglumis* showed a stronger competitive effect on the tuft circumference of *M. maximus* compared to *A. junciformis*. Since the biomass production usually increases with bigger plant tuft diameter (Yan et al., 2012), tuft size is influenced by the number of tillers as well (Mganga et al., 2010; Oliveira et al., 2019). Tillers have a direct link to the tuft size of the plant, both these physiological properties having a crucial role to play in the adaptability of grasses to both biotic and abiotic stress (Laidlaw, 2005). Tuft size plays an important role in protecting topsoil and increases the likelihood of survival in drought conditions (Oliveira et al., 2019).

The findings of this study contradict the theory that limiting resources can lead to intense competition among plants (Butterfield et al., 2016; Chapin and Shaver, 1985), as limited water resources did not have a significant impact of plant competition. Again, this might be a result of low intensity in the drought applied or short duration. However, the results align with the plant interaction theory which predicts that superior competitors will eventually drive an inferior competitor to a lower abundance or in extreme cases, extinction (Grime, 1973; Tilman, 1985) because competitively superior grasses decreased physical traits of their inferiors, such as tiller numbers, height or even biomass production.

4.5.3. Effect of water levels on biomass

One of the main aims of this study was to investigate the effect of water level on biomass production of grasses. Although drought is considered to be a key environmental constraint to the productivity of plants (Farooq et al., 2009), the findings of this study showed that water levels had no significant effect on the final dry matter yield of both palatable and unpalatable grasses, as well as broad and narrow-leaved plants. This is contradictory to most findings from other ecological studies that found a significant effect of water on grass production at an experimental level (Farooq et al., 2009; February et al., 2013; Pillay and Ward, 2022; Sørensen et al., 2017; Xu et al., 2022). Water level showed a strong positive correlation with total biomass production (Xu et al., 2022), implying that water is a limiting resource for grass productivity (February et al., 2013; Pillay and Ward, 2022). The differences in our results and the cited studies may be attributed to one or more factors, such as the length of the experiment, the type of grass species planted, the quantity and frequency of water treatment and the water-use efficiency. The contradictions in the findings of these studies may also be attributed to the fact that the current study was strictly limited to grasses as competitors, while Pillay and Ward

(2022) as well as February et al. (2013), alternated woody and grass species as competitors and focal plants. Since grasses have shallower roots, they get water from the topsoil, while trees which have deeper roots use water from the subsoil (Ward et al., 2013).

Despite biomass being unaffected, tiller numbers for palatable species were significantly reduced by drought. Similarly, a decrease in tillering rate was induced by drought (Korte and Chu, 1983). It is safe to assume that if the study duration was extended, we would see a reduction in biomass resulting from the tiller reduction. Drought also significantly reduced the mean tuft circumference and number of tillers for both broad-leaf species, *C. excavatus* and *M. maximus*. Drought and competition did not affect specific leaf area of any species. Compared to traits like tuft circumference and quantity of tillers, specific leaf area is probably less plastic and may only alter after extended drought exposure.

As expected, the narrow leaved grasses were unaffected by drought in this study as narrow leaved grasses are said to be more drought tolerant compared to their broad leaved (Craine et al., 2013). What was not anticipated in this study was the broad-leaved species remaining unchanged by drought. However, the photosynthetic competitive superiority that the broad leaved grasses have (Buckley et al., 2017) might have influenced the grasses in avoiding drought impacts. Both the unpalatable and palatable species were unaffected by drought. The survival of palatable species against drought may be due to the absence of grazing because palatable species are eradicated by selective grazing during a drought while unpalatable species experience less frequent and intense herbivory, unpalatable species have a competitive advantage over other species. However, in the absence of grazing, the palatable species such *T. leucothrix* had a higher capacity for competition than unpalatable species. In general, grasses may be better able to deal with and recover from low to moderate drought, but they are more sensitive to longer and severe periods water stress compared to (Volder et al., 2010), hence if the applied drought was elongated and more intense, the results might be different.

4.6. Conclusions

The presence of a competitor significantly reduced the biomass production of all grasses, whether palatable, unpalatable, narrow-leaved or broad-leaved, but the competitor species determines the level of biomass reduction each plant will undergo. Species such as *T. leucothrix* had a strong competitive effect, reducing the focal plant's height, tiller numbers and tuft circumference as well as the biomass, despite the species small stature. This insinuates that plant size is not always a clear indication of potential competitive ability. The biomass production of grasses was, however, not affected by drought. It is evident that drought may affect different species traits in varying ways because the tuft circumference for *C. excavatus* was significantly reduced by drought. Drought also reduced the mean tiller numbers of palatable species. The effects on tiller numbers and tuft size can ultimately translate to the reduction in biomass in the long run. The species-specific responses to water shortages may be qualified by various plant characteristics such as their dehydration avoidance or tolerance to drought. Drought had an impact on the competitive effects of grasses, which ultimately affects the biomass production of plants. The species in the current study, may have morphological, molecular or physiological advantages that may assist the grasses in coping with drought and further investigation is required to reveal which of these physiological survival strategies are activated by each plant during drought.

CHAPTER 5: SPECIES COMPOSITION STRUCTURE AND BELOWGROUND RESPONSES TO DIFFERENT MOISTURE LEVELS IN A MESIC GRASSLAND

Abstract

In South Africa, the grassland biome mainly occurs on the high central plateau, inland and mountainous areas of KwaZulu-Natal, which are experiencing changing conditions due to climate change. A moisture manipulation trial was conducted to investigate the response of grassland species composition, richness and diversity, and above and belowground biomass production to varying soil moisture regimes at Ukulinga Research Farm, University of KwaZulu-Natal, Pietermaritzburg, South Africa. Three moisture levels were applied: severe drought (D), ambient rainfall (A), and above-average rainfall (R). Severe drought reduced rainfall to the 1st percentile of the 100-year rainfall record which is 53% (483mm) of the long-term mean. The water intercepted on the drought plots was diverted to the above-average rainfall plots while the ambient plots did not receive any rainfall manipulation. The trial took place over a period of 3 years and the data were collected annually during the growing season. The ambient rainfall plots had highly diverse species composition in 2019/2020 and 2020/2021, which increased in the above-average rainfall treatments in 2021/2022. Over time, the drought plots were dominated by forbs, such as *Lichtensteinia kolbeana*, *Senecio retrorsus* and *Thunbergia atriplicifolia,* while the above-average and ambient rainfall plots were dominated by palatable grasses, such as *Setaria nigrirostris,* with a lower forb abundance. *Tristachya leucothrix*, *Cymbopogon excavatus*, *Themeda triandra*, *Brachiaria serrata*, and *Heteropogon contortus* were the most common grasses across the treatments. Both diversity and richness increased in 2021/2022 while the biomass was greatest in 2020/2021. There were no changes caused by moisture manipulations in any of the aforementioned factors. Despite fluctuations in aboveground biomass, the level of photosynthetically active radiation penetrating the grass canopy was unaffected by the treatments. High moisture levels reduced root biomass and greater biomass was found in the upper soil layers, at 1-16cm below the soil surface. Annual weather patterns had a more pronounced influence on grassland biomass production, species richness and diversity than total growing season rainfall. In conclusion, the interannual climatic fluctuations have a stronger impact on grassland biomass production and species composition compared to moisture level treatments. The mesic grassland showed resilience and is well adapted to annual rainfall fluctuations, as the 53% rainfall reduction had no measurable negative impact.

Keywords: *biomass, diversity, richness, evenness, annual weather patterns*

5.1. Introduction

The importance of herbaceous grassland communities cannot be stressed enough as they provide key services including forage and habitat for livestock and wildlife, thus sustaining human livelihoods. Natural disturbances, such as drought and sporadic floods are predicted to increase in frequency and severity because of global climate change (IPCC, 2013; Wilgen et al., 2016), affecting the state of grassland communities. Drought is not only expected to increase in frequency worldwide but is also projected to change in duration and intensity (Dai, 2012; IPCC, 2013; Wilgen et al., 2016). Around the world, especially in arid and semi-arid regions, drastic changes in water availability are weakening the stability and functionality of grassland ecosystems (Song and Yu, 2015). Depending on a plant's tolerance, which can affect the plant communities within an existing vegetation type as well as the plant's capacity to withstand dry conditions, different vegetation types may have different responses to drought (Gupta et al., 2020). It is therefore beneficial for ecosystems to exhibit some resistance (presented by limited loss of function during drought) or resilience and recovery from drought.

Global climate change can have strong impacts on soil functioning and plant communities by influencing changes in species abundance and above and belowground biomass (IPCC, 2013). The growth and productivity of grasslands is regulated by temperature and soil moisture content, specifically the volume and timing of rainfall events (Hufkens et al., 2016; Huxman et al., 2004b; Knapp and Smith, 2001). Drought may have varying impacts depending on the timing, magnitude and duration (Denton et al., 2017; Zeiter et al., 2016) and is considered the most economically destructive natural hazard, leading to declines in agricultural production (Mishra and Singh, 2010). Increased drought intensity and frequency alters ecosystem function (Smith, 2011) and is likely to decrease grass growth rates, hence decreasing the general biomass production of a grassland (Cunha and Richter, 2014).

Variability in rainfall is known to have a greater impact than grazing pressure on both grass and forb cover and biomass (Fynn and O’Connor, 2000). However, compared to grasses, forbs tend to show increased establishment and dominance following environmental disturbances (Buitenwerf et al., 2011). Annual forbs are a significant component of soil seed banks, particularly under heavy grazing (Tessema et al., 2016) allowing them to quickly establish when conditions become favourable. Many annual forbs have a prostrate growth form and are characterized by an ability to retain water, which aids their survival in dry, hot conditions whilst most perennial forbs can resprout from a persistent bud bank (Coller et al., 2021). Drought exacerbates the decline of plant species diversity and cover in general (Choat et al., 2012). Species diversity, richness and evenness are indicators of functional diversity and veld condition (Bonillla-Valencia et al., 2022). Species evenness can be defined as the relative abundance of each species within a community (Bonillla-Valencia et al., 2022), while richness is the number of individual species (Wilsey and Potvin, 2000).

One of the key traits that are often used to explain and predict ecosystem functioning is the roots. Recent research has seen a shift in focus from aboveground to belowground plant functional traits for the explanation of soil and ecosystem processes in response to the environment (Bardgett et al., 2014). Additionally, it can be argued that root functional traits might be better predictors of soil and ecosystem processes compared to leaf traits. As a result of the increasing body of evidence linking root traits to measures of ecosystem functioning, it has been suggested that root traits are central to ecosystem responses to climate change (Bardgett et al., 2014; Beidler et al., 2015).

Drought can alter root traits and plant community composition (Frank et al., 2015), resulting in changes to the structure and function of the ecosystem (Bardgett et al., 2014). Indicators of ecological function, such as roots, can also be used to explain ecosystem dynamics in response to the changing environment (Bardgett et al., 2014). Root response to drought is dependent on a variety of adaptive strategies that include root physiological, and morphological plasticity giving plants their tolerance or ability to avoid drought stress (Hartmann, 2011). Drought-induced impacts on plants are closely related to the response of rooting systems to water limitations which often leads to changes in plant nutrient uptake (Chaves, 2002). It is therefore crucial to understand the responses of root systems to drought for terrestrial biosphere management applications to better predict vegetation sustainability.

Drought episodes pose a risk to grassland productivity (Knapp and Smith, 2001) and plant community structure (Tielborger et al., 2014). Healthy vegetation protects the soil against erosion by strengthening soil stability and increasing water infiltration, organic litter content and nutrient cycling (Carlier et al., 2009; SANBI, 2014). With global warming comes a predicted increase in drought occurrence, presenting challenges for grassland management and necessitating the development of management techniques that mitigate the environmental and ecological effects of drought (Vetter, 2009). Understanding the effects of disturbances, such as drought, on grassland vegetation structure and dynamics is essential for the management and conservation of mesic grasslands. Species diversity, richness, evenness and biomass production are indicators of plant community health, and all indicators can be affected by drought. It is important to study the relationship between the indicators of plant community health and drought. From a species composition perspective, changes from a climax to a pioneer state or vice versa, and changes from a sward dominated by palatable species towards an unpalatable sward or vice versa can have important implications for agriculture and conservation land uses. Research into the effects of varying soil water levels on herbaceous community dynamics allows for better predictions of grassland ecosystem responses to future climatic changes under different land-use regimes, potentially improving the management and conservation of these ecosystems (Riginos et al., 2018; Vetter, 2009).

5.1.1. General objective

To determine the effects of long-term, severe drought on grass sward dynamics and morphology.

5.1.2. Specific objectives

- To investigate the impacts of induced severe drought, ambient and above-average rainfall addition on species composition dynamics such as diversity, richness and evenness.
- To assess the effects of different moisture levels on biomass production and light availability.
- To determine the impacts of different moisture levels on below-ground root biomass and length.

5.2. Materials and methods

5.2.1. Study site

The Drought-Net experiment is located at Ukulinga Research Farm, University of KwaZulu-Natal, (29°24’ E, 30°24’ S), Pietermaritzburg, South Africa. This experiment was situated at an altitude of 840 m above sea level. The area receives an average annual rainfall of 838 mm (Ward et al., 2017). It is categorized as subtropical, with recorded extreme temperatures ranging from 0.2°C to 41.9°C (Allan, 1996). The summers are warm (mean monthly maximum 26.4°C) and winters are mild (mean monthly minimum 8.8°C) with occasional frost. The soil is derived from shales under the Westleigh classification, and the soils are generally considered to be infertile and acidic. The Westleigh soil form comprises an orthic A horizon over a soft plinthic B horizon (Soil Classification Working Group 1991). The field trial was located within a closed canopy, forb-rich grassland biome, classified as Hinterland Thornveld (Mucina and Rutherford, 2006).

5.2.2. Experimental layout and design

To determine vegetation sensitivity to drought, a rainfall manipulation trial was erected comprising of three treatments namely; severe drought (D), ambient rainfall (A) and above-average rainfall (R). The severe drought treatment reduced rainfall to the 1st percentile of the 100-year rainfall record which is 53% (483mm) of the long-term mean, using passive shelters with transparent plastic roof sheets. The water collected from the drought plots was diverted to the above-average rainfall plots through a gutter and PVC pipes. In the ambient rainfall plot, the sward was allowed to grow naturally with no manipulation, as a control. The trial was arranged in a randomized block design, with three replicates, to account for any site-level effects. Each plot was 3m x 5m and contained six 1m^2 subplots separated by a 0.5m buffer. A plastic sheet, 250 micrometres thick, was buried vertically to a depth of 0.5m around each plot for hydrological isolation. This experiment was established in 2019 and the first species composition sampling was done during the 2019/2020 growing season. In each plot two 1m^2 quadrats were used for sampling, one for quantifying species composition and aboveground biomass and the other for destructive sampling for root length and biomass data collection. All plots were mowed at the beginning of winter to remove moribund biomass and prevent woody encroachment. No grazing took place in the field trials. Daily rainfall and temperature data were recorded for the duration of the trial using the existing weather station at the site.

5.3. Vegetation assessment

5.3.1. Species composition and biomass

Species composition was surveyed during the early growing season (December) and the late season (March). The aerial cover of each species rooted within each 1m^2 quadrat was recorded by visually estimating the aboveground cover to the nearest percent. At the end of the growing season, biomass samples were clipped from each 1m^2 quadrat in two 1m x 1m long strips. The biomass was divided into functional groups namely, grasses, forbs and legumes, then oven dried at 60°C for 48 hours and weighed to determine the final dry biomass. For the determination of photosynthetically active radiation (PAR), light readings were taken during the late growing season using a LP-80 LAI Ceptometer. The readings were recorded on a cloudless, sunny day between 10h00 am and 14h00 pm, with no obstructions above the light meter beside the drought treatment which had plastic roof sheets. One reading was taken above the canopy, while 2 readings were taken at ground level below the vegetation canopy at diagonal angles across the 1m^2 plot.

5.3.2. Belowground biomass

For the measurement of root biomass and length, a hole of about 50cm in depth and 30cm wide (to allow for the diagonal placing of mesh bags) was dug in the destructive sampling plot. Rectangular plastic mesh bags (10cm in breadth and 50cm in length) were used as ingrowth cores. The mesh had openings of 5 mm^2, which allowed roots and soil microorganisms to enter. The mesh bags were filled with soil that was initially dug from the hole after all roots, including dead roots and decomposing organic matter, were carefully removed by handpicking and rinsing, similar to the method used by Osawa and Aizawa (2012). The filled mesh bags were then placed at a slightly diagonal angle back into the soil, to help properly fit into the hole, up to a depth of 0.5m and the hole was completely covered with soil. The mesh bags remained *in* situ for 6 months through the growing season for the accumulation of roots. Following that, the mesh bags were removed and the accumulated roots were separated from the soil through sieving. The roots were then washed and roots from the bottom, middle and top positions in the mesh bag were separated for data stratification. Since the mesh bags were placed 50cm into the soil stratum, the top portion represented the first 0 - 16 cm, the middle portion, 17-33cm and the bottom portion, 34-50cm in depth. The roots were then oven-dried at 80°C for 48 hours (Osawa and Aizawa, 2012) and weighed to determine biomass. From each position in the mesh bag (low, middle and top), determined by the soil depth, all roots at different positions or soil depth were measured to determine the average root length from each soil depth.

5.4. Statistical analysis

To examine the relationships between the plant community composition of grasses and forbs with the environmental factors and year of sampling, a redundancy analysis (RDA) was performed using PAST software (version 4.10). Species with an occurrence of <5% were excluded from the ordination plot for the reduction of clutter. The effects of annual weather patterns represented by years and moisture level (drought, ambient and above-average rainfall), as well as their interactions on species composition were analysed with permutational multivariate analysis of variance (PERMANOVA) using the Euclidean distance method due to continuous data, with 9999 permutations. Then an analysis of similarities (ANOSIM) was performed using Bray Curtis dissimilarity, followed by a pairwise comparison once significant differences were observed. For the generation of average dissimilarity, percentage contribution (%), cumulative percentage (%), and species means, a similarity percentages routine (SIMPER) analysis for species abundance between the years 2020, 2021 and 2022 using a Bray-Curtis dissimilarities test was performed using PAST software (version 4.10). This statistical analysis was performed using R Studio (version 4.0.2) with the following packages: vegan, tidyverse, and pairwiseAdonis. All variables were checked for normality prior to analysis. Species richness (S) and Pielou’s evenness (J’) were used as metrics to measure functional diversity (Bonillla-Valencia et al., 2022; Keylock, 2005) as well as the Shannon–Wiener diversity index (H’) (Keylock, 2005; Mendes et al., 2008). An Analysis of Variance (ANOVA) was used to investigate the effects of drought treatments and year of sampling on species richness (S), diversity (H’) calculated using the Shannon–Wiener diversity index (H’), and Pielou’s evenness (J) and a Tukey’s test was used to separate means, where the ANOVA revealed significant differences.

The Shannon–Wiener diversity index (H’) was calculated using the following equation:

$H' = SUMpi \log(b)pi$

where, pi = proportional abundance of species, and b = base of logarithm (Keylock, 2005)

Pielou’s evenness (J′) was calculated using the following equation:

$J' = H'/\ln(S)$

where, H′ = Shannon–Wiener diversity index and S = total number of species in a sample, across all samples (Mendes et al. 2008).

Furthermore, an analysis of variance to examine the main effects and interactions of rainfall variation and year on mean aboveground dry biomass was performed. An analysis of variance was also performed to investigate the impact of soil depth layer and rainfall variation on root biomass and length. These analyses were performed using SPSS 26.0 (IBM Systat Software), using SigmaPlot 12.5 (Systat Software) for visual representation of results.

5.5. Results

5.5.1. Rainfall and temperature

Mesic grasslands receive an annual rainfall of >600mm per year (Palmer and Ainslie, 2005). Generally, the annual rainfall between 2010 and 2020 was within the normal range for a mesic grassland, except for 2014 and 2015, which both fell below 600mm and are recognized as drought years (Figure 5.1). Figure 5.2 represents monthly rainfall for 2019/2020, 2020/2021 and 2021/2022. The graphs are focused on the growing season (September to May). The 2019/2020 cycle has a gradual curve which shows a normal increase in rainfall around the growing season. However, this cycle has lower total growing season precipitation than 2020/2021 and 2021/2022. There was a sharp increase in rainfall in 2021/2022 resulting in this period having the highest total growing season rainfall of the three study years. Figure 5.3 shows a gradual increase in the average monthly minimum and maximum temperature (°C) for all three growing seasons. For both the minimum and maximum temperature, the 2019/2020 season had a higher temperature for most months, while 2021/2022 had the lowest maximum temperatures compared to other growing seasons.

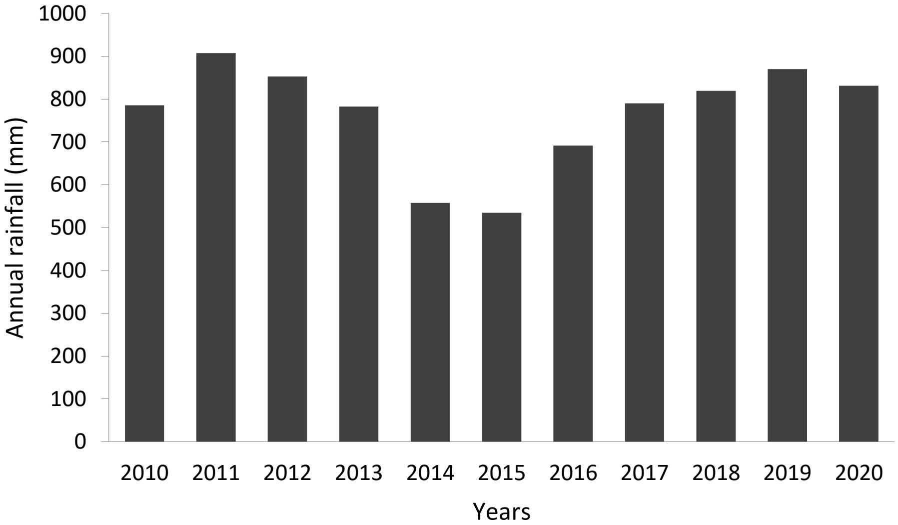

Figure 5.1: Average annual rainfall over a period of 10 years, from 2010 to 2020 at Ukulinga Research Farm, Pietermaritzburg, South Africa.

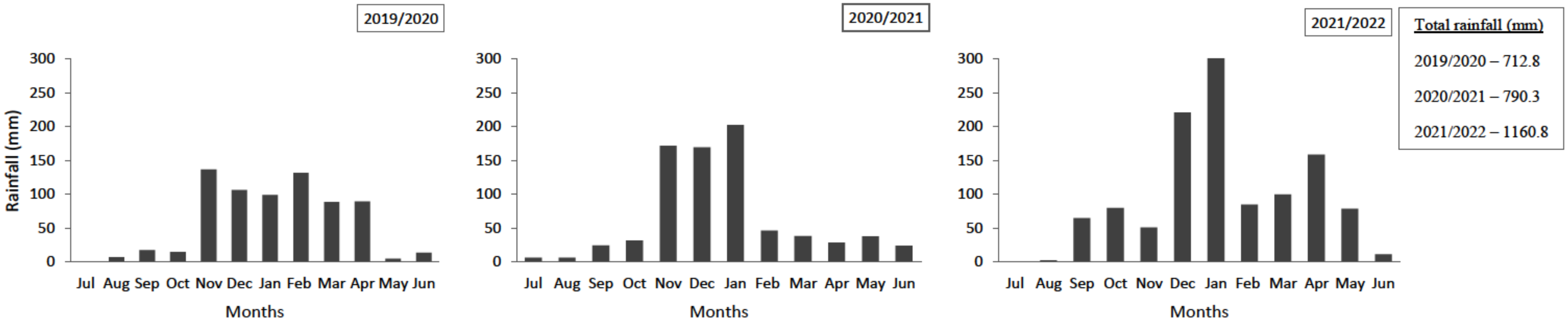

Figure 5.2: The monthly rainfall (mm) for 2019/2020, 2020/2021 and 2021/2022 at Ukulinga Research Farm, Pietermaritzburg, South Africa.

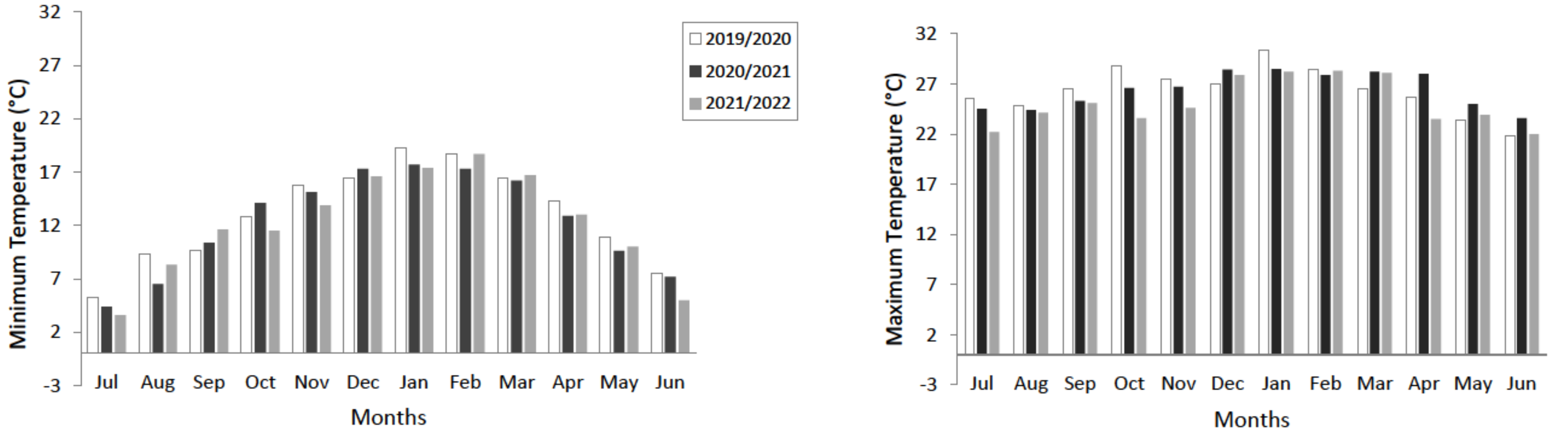

Figure 5.3: The monthly average minimum and maximum temperature (°C) for 2019/2020, 2020/2021 and 2021/2022 at Ukulinga Research Farm, Pietermaritzburg, South Africa.

5.5.2. Species composition

In 2019/2020 and 2020/2021 the ambient rainfall plots showed the greater biodiversity of species, suggesting a greater species diversity (Figure 5.4A & 5.4B) while in 2021/2022 the above-average rainfall treatments had the greatest variability (Figure 5.4C). In all three years the drought plots had limited biodiversity and a greater concentration of forbs, dominated by species such as *Senecio coronatus*, *Cephalaria pungens* and *T. atriplicifolia* with a comparatively number of grass species. On the other hand, both the above-average and ambient rainfall plots were dominated by grasses such as *H. contortus* or *S. nigrirostris* (Figure 5.4C).

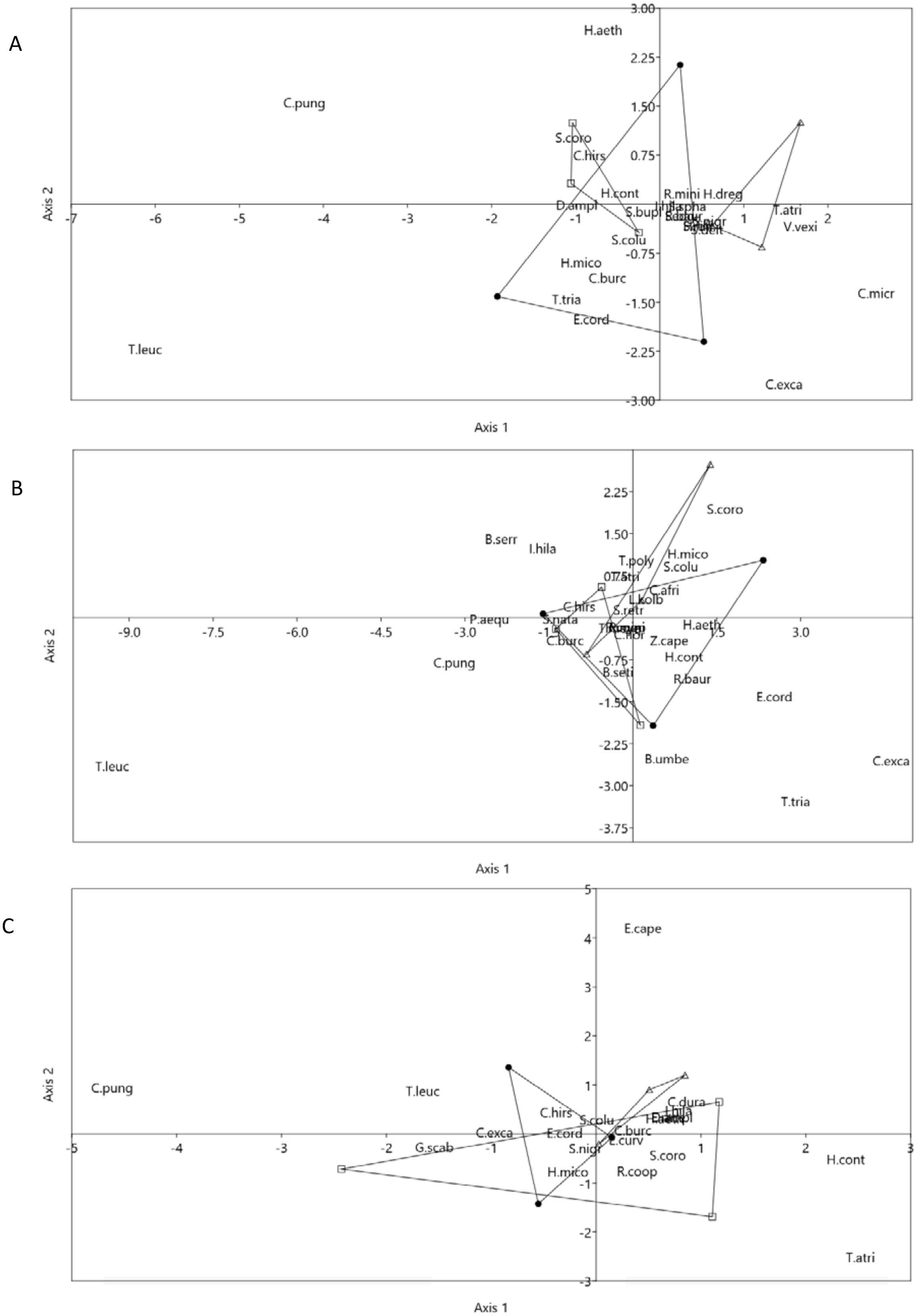

Figure 5.4: Redundancy analysis of the grass and forb species community composition under drought (triangle), ambient (dot) and above average rainfall (square) from Ukulinga Research Farm, Pietermaritzburg, South Africa in A) 2019/2020, B) 2020/2021 and C) 2021/2022. Species with an occurrence of <5% were excluded from the ordination plot for the reduction of clutter. Full names are included in Appendix 5B.

The permutational multivariate analysis of variance revealed that only the year, indicating annual weather patterns, had a statistically significant impact on the grassland plant community composition ($F_{2, 18} = 1.7988$, p = 0.0322). Contrary to expectations the moisture level, as well as the interaction between the year and moisture level did not affect the grassland community composition (Table 5.1). The SIMPER analysis for species abundance showed the distribution and relative abundance of species in terms of their means, cumulative percentage and percentage contribution towards the dissimilarity between the years 2019/2020, 2020/2021 and 2021/2022 (Table 5.2).

Table 5.1: Two-way permutational multivariate analysis of variance (PERMANOVA) for all years (2019/2020, 2020/2021, 2021/2022) between the drought, ambient and above-average rainfall moisture levels, showing *P*-values with significant effects ($P < 0.05$) in bold at Ukulinga Research Farm, Pietermaritzburg, South Africa

Source	df	Mean square	F-value	P-value
Year	2	8546	1.7988	**0.0322**
Moisture level	2	7273	1.5308	0.0788
Y*ML	4	4729	0.4976	0.9992
Residual	18	42760		
Total	26	63309		

In terms of grasses, *T. leucothrix*, *Cymbopogon excavatus*, *T. triandra*, *Brachiaria serrata*, and *H. contortus* contributed to 52.13% of the cumulative dissimilarity in grass species composition. The cover of *T. leucothrix*, *C. excavatus* and *T. triandra* increased from 2019/2020 to 2020/2021 but noticeably decreased in 2021/2022, while *B. serrata* and *H. contortus* cover remained the same in 2019/2020 and 2020/2021, followed by a slight increase in 2021/2022. *Eragrostis capensis, Eragrostis racemosa* and *Eragrostis curvula* were not recorded in the first two years of the study but then appeared in 2021/2022. The increased rainfall by 2021/2022 may have driven the germination of species from the soil seedbank. By contrast, some grass species such as *Setaria sphacelata,* and *Hyparrhenia dregeana*, and some forbs such as *Vigna vexillata*, were present at the beginning of the study, but had disappeared by the final year. *S. nigrirostris* and *Diheteropogon amplectens* are the only species that are present, but in low abundance, in 2019/2020, then disappear in 2020/2021 and reappear in the 2021/2022 growing season (Table 5.2). This could be an indication that species may stay dormant until they receive adequate moisture resources and resprout (Klupczyńska and Pawłowski, 2021).

Table 5.2: SIMPER analysis for species abundance between the years 2019/2020, 2020/2021 and 2021/2022 using Bray-Curtis dissimilarities test showing descending order of average dissimilarity, percentage contribution (%), cumulative percentage (%), and mean abundance

Species	**Av. dissimilar**	**Contribution %**	**Cumulative %**	**Mean 2019/2020**	**Mean 2020/2021**	**Mean 2021/2022**
Grasses						
T. leucothrix	10.16	15.6	15.6	27.7	55	33.9
C. excavatus	4.11	7.537	23.13	13.7	17.8	3.22
T. triandra	4.32	6.629	36.67	17	20.9	19.7
B. serrata	3.703	5.682	42.35	6.44	6.44	7.78
H. contortus	2.766	4.244	52.13	1.11	1.11	11.2
E. capensis	2.312	3.548	59.9			10.6
S. nigrirostris	1.291	1.981	79.73	0.889		5.33
P. aequinerve	0.6317	0.9695	90.26		3.89	
D. amplectens	0.5506	0.8451	92.02	2.22		0.556
E. racemosa	0,5217	0.8006	92.82			2.22
S. sphacelata	0.1325	0.2033	98.95	0.556		
E. curvula	0.1321	0.2028	99.15			0.556
Forbs						
C. pungens	4.501	6.907	30.04	14.4	12.2	9.78
T. atriplicifolia	3.608	5.537	47.89	5.89	10.9	8.89
E. cordatum	2.745	4.213	56.35	4	11.1	2.44
S. coronatus	2.23	3.423	63.32	3.44	8.11	1.89
I. hilaris	1.971	3.024	66.34	3.11	6.11	0.556
C. burchellii	1.633	2.506	68.85	5.33	4.78	1.11
C. hirsute	1.549	2.378	71.23	2.33	2.78	3.67
C. macrocarpa	1.538	2.361	73.59	6.56		
A. bakerianus	1.364	2.093	75.68	1.11	5.67	0.556
H. aethiopicus	1.351	2.073	77.75	4.78	1.67	0.556
H. miconiifolium	1.086	1.667	81.4	1.11	4	1.44
V. vexillata	1.079	1.656	83.06	4		
H. dregeana	0.9858	1.513	84.57	4.44		
G. scabra	0.8496	1.304	85.87			3.89
R. baurii	0.8451	1.297	87.17	0.556	3.67	
T. polystachya	0.7337	1.126	88.3		2.78	
C. dura	0.6512	0.9993	89.3			2.78
B. setifera	0.5899	0.9053	91.17		0.889	2.22
B. umbellata	0.4256	0.6531	93.47		2.22	
S. columbaria	0.4148	0.6366	94.11	0.556	1.11	0.556
S. bupleuroides	0.3694	0.567	94.67	1.67		
R. minima	0.3431	0.5266	95.2	0.889	0.778	
B. species	0.3176	0.4875	95.69	1.67		
R. cooperi	0.3123	0.4793	96.17		0.889	0.556
L. kolbeana	0.2935	0.4504	96.62		1.11	
S. natalensis	0.2707	0.4155	97.03		1.67	
S. retrorsus	0.262	0.402	97.43	0.667	0.556	
S. deltoides	0.2463	0.378	97.81	0.889		
A. angustata	0.2413	0.3703	98.18		1.11	
H. filiformis	0.1847	0.2835	98.47	0.667		
T. meyeriana	0.1805	0.277	98.74		1.11	
Sedge	0.126	0.1934	99.34	0.556		
C. africana	0.1096	0.1681	99.51		0.556	
Z. capensis	0.1064	0.1633	99.67		0.556	
A. chlorophyllum	0.1064	0.1633	99.84		0.556	
C. floribunda	0.1064	0.1633	100		0.556	

Table 5.3: Analysis of variance for diversity, richness and evenness between the years 2019/2020, 2020/2021 and 2021/2022 and moisture levels (ambient, drought and above-average rainfall) showing total sum of squares, *F*-value and *P*-value with significant effects ($P < 0.05$) in bold

			Diversity		**Richness**		**Evenness**	
Source	**ss**	**df**	**F-value**	**P-value**	**F-value**	**P-value**	**F-value**	**P-value**
Year	0.972	2	9.001	**0.002**	24.796	**<0.001**	0.054	0.947
Moisture level	0.077	2	0.710	0.505	0.242	0.787	4.122	**0.034**
Y * ML	0.150	4	0.696	0.605	1.154	0.364	0.516	0.725
Error	0.971	18						
Total	136.665	27						

Like species composition, species diversity ($F_{2, 18} = 9.001$, p = 0.002) and richness ($F_{2, 18} = 24.796$, p = <0.001) were significantly affected by year indicating sensitivity to interannual climatic fluctuations, while both were unaffected by moisture manipulations. Both species diversity and richness were significantly higher in the 2021/2022 growing season (Figure 5.5). However, the contrary was observed when it came to species evenness, which was significantly affected by the moisture levels ($F_{2, 18} = 4.122$, p = 0.034) but was not by annual weather patterns (Table 5.3). The above-average rainfall area had the greatest evenness, followed by the drought site (Figure 5.6).

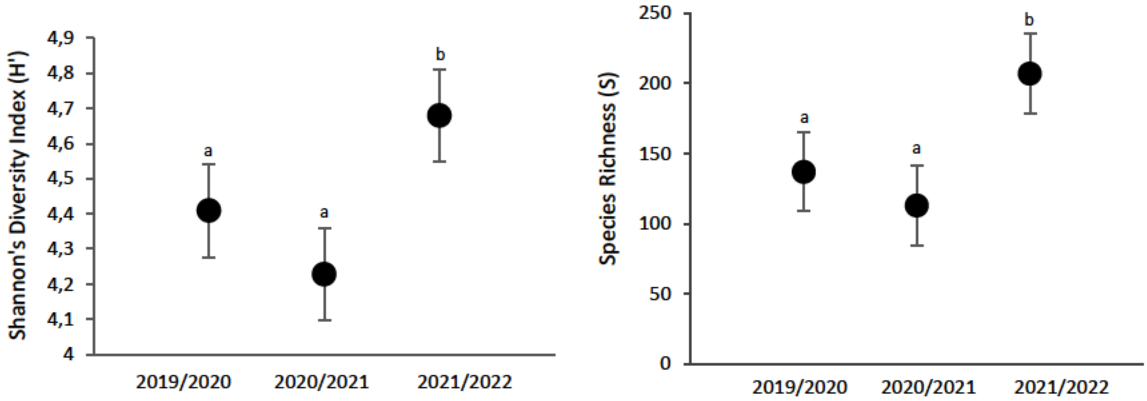

Figure 5.5: Shannon's diversity (H') and species richness (S) for 2019/2020, 2020/2021 and 2021/2022 at Ukulinga Research Farm, Pietermaritzburg, South Africa. Years with different letters are significantly different ($P < 0.05$).

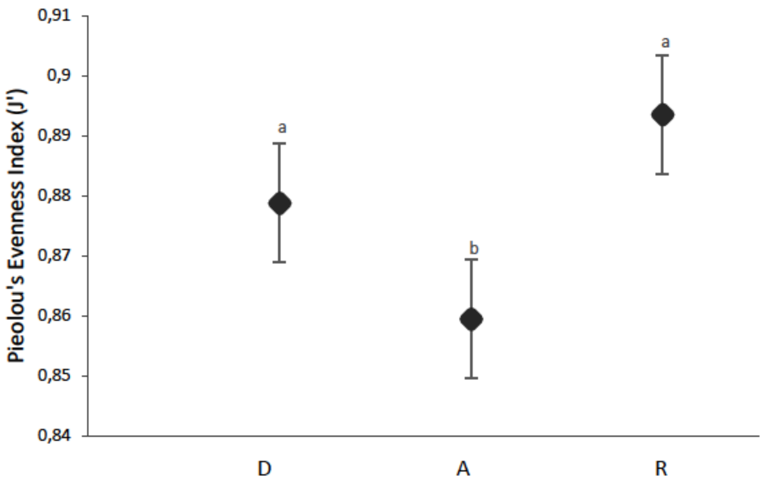

Figure 5.6: Pielou's evenness index (J') between the drought (D), ambient rainfall (A), and above-average rainfall (R) plots at Ukulinga Research Farm, Pietermaritzburg, South Africa. Treatments with different letters are significantly different ($P < 0.05$).

5.5.3. Biomass results

Annual weather patterns (year) significantly affected the biomass production of the grassland community, while the moisture level, as well as the interaction between moisture level and year, did not affect sward biomass (Table 5.4). In 2020/2021 the sward biomass was significantly greater than in 2019/2020 or 2021/2022 (Figure 5.7). Despite these fluctuations in biomass, the level of photosynthetically active radiation penetrating the grass canopy was unaffected, possibly because the canopy was not sparse enough to allow excessive photosynthetically active radiation penetration even when biomass production fluctuated (Table 5.4).

Table 5.4: Results for analysis of variance for main effects and interactions of ambient rainfall, above average rainfall and drought between the years 2019/2020, 2020/2021 and 2021/2022 on mean aboveground dry biomass, as well as photosynthetically active radiation, showing degrees of freedom (df), *F*-ratio and *P*-value with significant effects ($P < 0.05$) in bold

		Biomass		**PAR**	
Source	**df**	***F*-ratio**	***P*-value**	**F-value**	**P-value**
Year	2	6.182	**0.009**	2.958	0.078
Moisture level	2	1.281	0.302	0.633	0.543
Y * ML	4	0.866	0.503	2.852	0.054
Error	18				
Total	27				

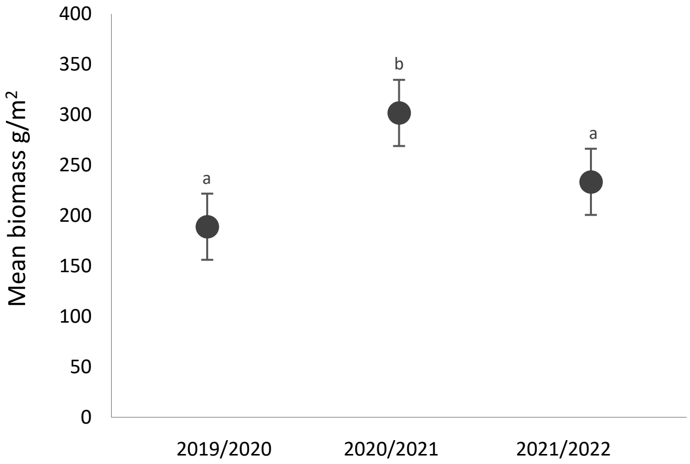

Figure 5.7: The mean dry biomass (g/m^2) main effect of interannual climatic variability between the years 2019/2020, 2020/2021 and 2021/2022 at Ukulinga Research Farm, Pietermaritzburg, South Africa. Superscripts showing different letters are significantly different ($P < 0.05$).

5.5.4. Root traits

Soil depth and moisture level significantly affected the biomass and average length of roots. However, the interaction between soil depth and moisture levels did not affect the root biomass and length (Table 5.6). Root biomass and root length decreased with increasing rainfall, but there was no significant difference between ambient rainfall and drought ($P < 0.05$) (Figure 5.8). Root biomass and length were significantly greater in the initial 1-16cm below the soil surface and decreased into deeper soil layers (Figure 5.9).

Table 6: Analysis of variance for root biomass and average length in different soil depths (top, middle, bottom) and treatments (drought, ambient, above average rainfall) showing total *F*-value and *P*-value with significant effects ($P < 0.05$) in bold

		Biomass		**Length**	
Source	**df**	**F-value**	**P-value**	**F-value**	**P-value**
Depth	2	12.577	**<0.001**	19.809	**<0.001**
Moisture level	2	8.059	**0.003**	10.152	**0.001**
D * ML	4	0.798	0.542	1.473	0.252
Error	18				
Total	27				

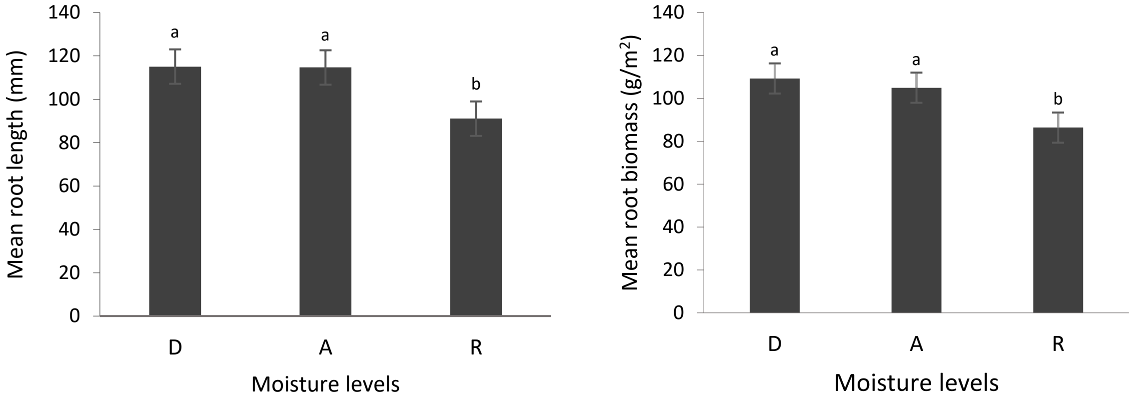

Figure 5.8: The effect of moisture levels (drought -D, ambient -A, above average rainfall – R) on mean root length (mm) and mean root biomass (g) at Ukulinga Research Farm, Pietermaritzburg, South Africa. Superscripts with the different letter are significantly different ($P < 0.05$).

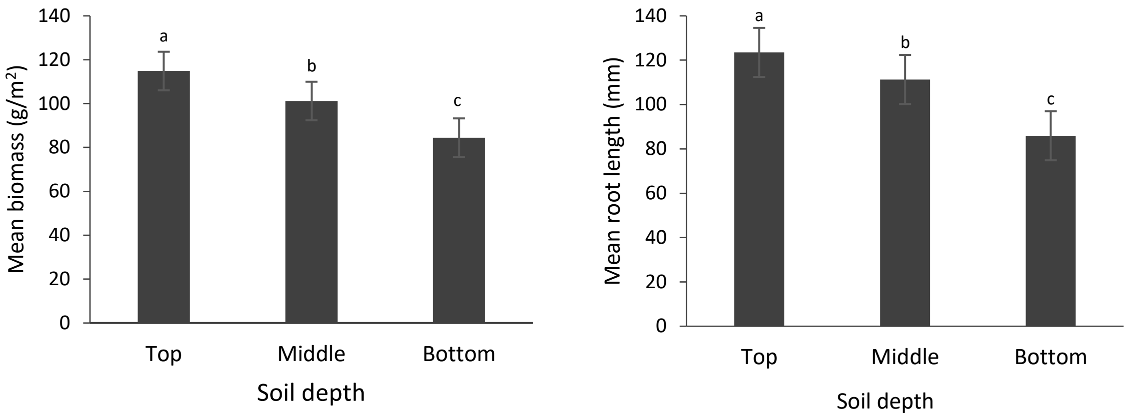

Figure 5.9: The effect of soil depth (top: first 0- 16 cm of the top soil; middle: 17-33cm and bottom: 34-50cm deep) on mean biomass (g/m^2) and mean root length (mm) at Ukulinga Research Farm, Pietermaritzburg, South Africa. Superscripts with the different letter are significantly different ($P < 0.05$).

5.6. Discussion

5.6.1. Species composition

Annual weather patterns had a significant influence on the grassland species composition, diversity and richness. The resulting fluctuations in species diversity, richness and aboveground biomass production can be associated with the increased rainfall and lower maximum temperatures of the growing season of 2021/2022. There was an increase in the abundance of reasonably palatable species *T. leucothrix* and *T. triandra*, from 2019/2020 to 2020/2021, with a slight decrease in 2021/2022. Cover of moderately palatable species such as *H. contortus* remained the same in 2019/2020 and 2020/2021, increasing in abundance in 2021/2022. Unpalatable grasses such as *E. capensis, E. racemosa* and *E. curvula* were not recorded in the first two years of the study but then appeared in 2021/2022. The palatable *S. nigrirostris* and *D. amplectens* were present in low abundance in the 2019/2020 growing season, disappearing in 2020/2021 and reappearing in the 2021/2022 growing season. This could be caused by these species may remaining dormant until they receive suitable water resources and resprout (Klupczyńska and Pawłowski, 2021). Additionally, the aforementioned species were suppressed in the year with the highest biomass production but showed up in the other 2 years when biomass was lower. This may be related to the inverse relationship found between species diversity and biomass (Barnes et al., 2016; Grime, 1998), which was also found in this study.

The 2021/2022 growing season had a comparatively higher rainfall and this may have influenced the germination of species from the soil seedbank. This suggests that species composition may be able to withstand drought without any major changes but may fluctuate with rainfall variation from year to year. Drought had little impact on the composition of grass species in a study conducted in the Kruger National Park as all of the grass species recorded prior to the occurrence of drought were also present during the peak- and post-drought periods and no key grass species were lost as a result of moisture decline (Kennedy et al., 2003; Wilcox et al., 2020). These results revealed the same trend, where the moisture treatments had no effect, yet the years significantly affected the grassland composition, with higher rainfall years 2020/2021 and 2021/2022.

The relative abundance of most species fluctuated annually. For instance, the abundance of the most dominant forb, *C. pungens*, gradually decreased from 2019/2020 to 2021/2022 while the relative abundance of the most dominant grass species, *T. leucothrix, C. excavatus* and *T. triandra*, peaked briefly in 2020/2021 and slightly declined during the 2021/2022 growing season. Perennial species dominated the grassland throughout the study and this could have contributed to the lack of impact that moisture levels had on the species composition as the abundance of perennial grasses can be an effective mitigation strategy to reduce the impacts of climate fluctuations. Perennial grasses have evolved adaptive features in response to heat stress and soil moisture shortages. The two main characteristics that have been demonstrated to boost perennial grass plants' tolerance to heat and drought are their deep roots and dormancy under unfavourable conditions (Nie and Norton, 2009). Additionally, c the amounts of water-soluble carbohydrates in tiller bases may increase drought resistance (Volaire and Lelièvre, 1997). Species abundance is influenced by by rainfall variability due to soil moisture’s direct influence on seedling recruitment, basal cover and plant mortality rate (O'Connor, 1994).

New grass species such as *E. racemosa* and *E. capensis* were recorded in 2021/2022, while *H. dregeana* and *S. sphacelata* disappeared from the grassland population after the third treatment year. Grasses are able to sacrifice their aboveground tissues and remain dormant belowground, relying on dehydration-tolerant roots (Volaire et al., 2020). The disappearance of the tall, densely tufted grasses may have a negative impact on the grassland because tall plants with a large canopy that lowers microclimatic temperatures modify the soil temperatures (He et al., 2022). Annual weather patterns significantly affected both species diversity and richness while moisture level had no significant impact. The final year of the experiment had the greatest species richness and diversity. The fact that the grassland was not affected by the soil moisture regime may be attributed to the grassland community having a relatively high species richness and diversity. The Shannon-Weiner diversity index value normally ranges from 1.5 to 3.5 and on rare occasions exceeds 4.5 (Spellerberg and Fedor, 2003). In this study, the species diversity was consistently well over 4, indicating a species-rich or healthy species diversity throughout the experiment.

Multi-species communities have a greater probability of having species which are well adapted to global warming and drought, hence a highly diverse plant community has a better chance of survival against drought (Naeem and Li, 1997). Biomass production, energy transmission, biogeochemical processes, and ecological services are among the functions of an ecosystem that are enhanced by increased species richness (Naeem and Li, 1997). Hence, species-rich communities are likely to retain ecosystem function under environmental stress as species loss is unlikely to translate into functional group loss. Ecological communities with high species richness, are believed to benefit from functional redundancy since the loss of any one species would not negatively impact the communities' ability to function over time (Naeem, 1998).

These ecological communities with more functionally redundant species are more likely to show increased resilience after environmental disturbance compared to communities with fewer functionally redundant species (Biggs et al., 2020). Biomass production, energy transmission, biogeochemical processes, and ecological services are among the ecosystem functions that are enhanced by increased species richness (Naeem and Li, 1997).

Species-rich and diverse grassland communities are generally able to capture more sunlight and ultimately photosynthesize more, resulting in greater productivity. Additionally, high species richness creates a dense canopy, correlated with more extensive basal cover (Middelboe and Binzer, 2004) which protects the soil from erosion. Denser canopy cover also decreases wind speed and limits transpiration rates (Larcher, 2003). High species richness can also be a positive indicator that the plant community can withstand or resist drought because the water-use efficiency of plant communities rises as species richness increases (Boeck et al., 2006; Naeem and Li, 1997; Rodriguez-Ramirez et al., 2017). Hence, high species richness can promote the drought resistance of grassland communities by increasing complementarity among species and increasing species evenness (Wang et al., 2021). A highly variable plant community can be an advantage as it increases the frequency of biotic interactions and competition which can mediate the effects of climate change experienced by individual species (Srivastava et al., 2021). High soil moisture levels promote a highly variable or diverse plant community. In this study, species evenness was significantly increased by above-average moisture levels, with the ambient rainfall treatment having the lowest species evenness. High species richness and evenness are key indicators of the community's ability to resist dry environmental conditions (Wang et al., 2021).

5.6.2. Biomass production

Aboveground biomass production was sensitive to annual climatic changes even though there was no distinctive trend as biomass production was only significantly greater in 2020/2021. These findings concur with those of Hsu et al. (2012) who reported that annual precipitation and temperature variability are the main drivers of annual biomass productivity. These results correspond with those of Wigley-Coetsee and Staver (2020), who found that grass biomass increased with increasing annual rainfall. Ma et al. (2018), identified a strong correlation between productivity and annual climatic factors, thus we must take other climatic factors into consideration in our study, for example, the average minimum and maximum monthly temperature which was higher in 2019/2020 but decreased in 2021/2022. The grassland biomass is strongly related to intra-seasonal distribution of rainfall. Higher environmental temperatures are predicted to result higher biomass allocation towards roots (Scheiter and Higgins, 2009).

Our results suggest that biomass production in mesic southern African grasslands is resilient to drought because biomass is reduced during periods of water limitation and quickly recovers as soon as water resources increase. Similarly, a rapid post-drought recovery of grass biomass in other mesic southern African savanna has been observed (Abbas et al., 2019; Wigley-Coetsee and Staver, 2020). In fact, in several studies, drought appears to have improved grazing conditions for herbivores immediately post-drought, compared to the pre-drought conditions. Of course, this is subject to change as droughts increase in frequency and recovery time is significantly reduced. In an adaptive strategy to combat drought, the production of biomass and plant growth is temporarily reduced (Staniak and Kocon, 2015), to survive the dry season and regrow during rehydration when metabolic processes are reactivated (Staniak and Kocon, 2015). The level at which each species resists dehydration and regrows indicates species specific adaptive capabilities. Some species are more tolerant of dry conditions while others can regrow rapidly with rehydration (Staniak and Kocon, 2015). *Tristachya leucothrix, C. excavatus* and *T. triandra* retained a relatively high abundance throughout the experiment, with small fluctuations which could be a sign of tolerance or resilience to shifts in annual weather patterns. Annual weather patterns and moisture level treatments did not have any effect on photosynthetically active radiation reaching the soil surface. This means that, even when biomass production fluctuated annually, the canopy remained dense and closed. When the plant canopy cover remains dense, it shields the grassland from exposed bare ground, hence surface water flow is reduced and soil erosion is prevented (Yucesan et al., 2019).

5.6.3. Root biomass and soil depth

Both soil depth and moisture level significantly impacted root biomass and length, with reductions in both properties with increasing soil depth and under above-average rainfall. Greater root dry matter content has been linked to drought resistance (Wahl and Ryser, 2002) but in our study, the root biomass and length under drought and ambient rainfall conditions remained the same. After three years of drought application, the belowground system did not show a drought response to the 53% rainfall reduction treatment that was applied. As rainfall increases, plants do not need to maintain high root production as moisture is easily accessible from the topsoil. A similar result was observed by Kou et al. (2022), where moisture from high rainfall was easily accessible in the topsoil.

This study found greater root biomass in the topsoil and since the root biomass is directly proportional to root length, increased root length was also found at the topsoil. These findings concur with Mori and Inagaki (2012), who stated that a 4 month drought had no effect on root length (Mori & Inagaki, 2012). However, the root biomass findings oppose Lozano et al. (2020), who showed that forbs and grasses under drought-induced conditions showed an increase in root diameter, consequently increasing root biomass. The findings of a meta-analysis based on 128 published research studies in grasslands show that drought significantly increases root diameter, which also increases the biomass of roots (Zhou et al., 2018). Since the level of water reduction in our study did not invoke a drought response from the grassland, the results suggest that our grassland may need more severe water reductions to display a drought response.

The unchanged root biomass and length may be an indication of drought avoidance. In this study, plants utilized drought avoidance strategies by maintaining root biomass during dry conditions to survive the induced drought, making the root system of the grassland resistant to drought. The results of the current study are in contrast with those of de-Vries et al. (2016) who found that root biomass was significantly reduced by a two-week severe drought. We must, however, consider the time difference and drought intensity of the current study and that of de-Vries et al. (2016), who ran the experiment for 2 weeks in a greenhouse at 30% water reduction. Some species have developed the ability to withstand dry conditions using drought avoidance and drought tolerance strategies through a combination of various plant functional traits. Drought avoidance strategies integrate high water-use efficiency with low stomatal conductance while investing in a high root-to-shoot ratio. As a result, drought survival through avoidance, allows plants to use available resources they as minimally as possible for the maintenance, while production is reduced or sustained at the same level and further production is avoided. Unlike drought avoidance, drought tolerance strategies allow increased plant production (Roumet et al., 2008). Each strategy is related to certain plant functional traits where there is a trade-off between fast growth or acquiring more resources and slow growth or conserving and using available resources. Slow growth confers stress resistance by reducing the demand for growth and production, thereby allowing for greater investment in defence traits (Chapin, 1993). Plants with fast growth characteristics facilitate a quick resource intake for growth but are usually adapted to resource-rich environments (Reich, 2014).

Drought tolerance is sometimes associated with a longer or deeper root system, making it possible to exploit resources in lower levels of the soil (Fuentealba et al., 2015; Ma et al., 2018;

Volaire et al., 1998). Additionally, drought-tolerant species often have a greater investment in root biomass, whereas drought-avoiding species may promote longer roots (Chaves, 2002; Lavinsky et al., 2015). The effects of drought on root traits and responses examined in previous individual studies are highly contradictory (Chapman et al., 2012; Chaves, 2002). For instance, drought may decrease (Guha et al., 2010), increase (Manoharan et al., 2010), and have no effect on root length (Mori and Inagaki, 2012). These highly diverse results may be due to the difference in the intensity and duration of drought (Comas et al., 2013) and differences in plant functional types (Kashiwagi et al., 2005). Despite the length of the dry period, a severe drought generally invokes a decrease in root biomass (de-Vries et al., 2016; Lozano et al., 2020).

5.7. Conclusions

Most of the aboveground variables such as species composition, diversity, richness and biomass production were not affected by drought but instead were significantly affected by annual weather patterns. Throughout the experiment, the grassland community maintained healthy levels of species richness and diversity. Annual climatic fluctuations with the greatest impact on aboveground variables occurred in the growing seasons of 2020/2021 and 2021/2022. Both species diversity and richness were significantly higher in the growing season of 2021/2022, while species evenness was significantly affected by the moisture levels where the above-average rainfall area had the highest species evenness, followed by drought. These effects could be due to the greater rainfall in the 2021/2022 growing season combined with reduced evaporation due to the low average monthly temperatures. The main conclusion is that annual weather patterns have a stronger influence on grassland biomass production, species richness and diversity than the moisture level treatments. The belowground responses were, however, different as both root biomass and length were significantly affected by moisture level. Root traits were consistent under drought and ambient rainfall and reduced under above-average rainfall. The understanding of root system responses to drought is important for better projections of vegetation dynamics and adaptations to climate change (Fisher et al., 2014). In conclusion, the mesic grassland biomass production showed resilience by recovering when there were high moisture levels during the 2021/2022 growing season. This signifies that grassland productivity may be resilient to drought by using strategies such as decreasing biomass production. Communities with high species richness also benefit from functional and ecological communities with more functionally redundant species are more likely to show increased resilience after environmental disturbance (Biggs et al., 2020) and since the loss of certain species is unlikely to result in the loss of a functional group, species-rich communities are therefore likely to maintain ecosystem function under environmental stress. The fairly high species diversity throughout the grassland is a positive trait as multi-species communities have a greater chance of having species which are well adapted to global warming and drought.

6. CHAPTER 6: THE INFLUENCE OF NUTRIENT ADDITION (NITROGEN, PHOSPHORUS, POTASSIUM) ON VEGETATION DYNAMICS UNDER DIFFERENT MOISTURE LEVELS IN A MESIC GRASSLAND

Abstract

Grasslands provide vital resources for human well-being and grazing for livestock. The importance of plant community productivity, diversity and species richness in grassland ecosystems is well known, and both drought and nutrient inputs have a significant impact on these attributes. Uncertainty still exists over the impact that climate change may have on these factors. A rainfall manipulation experiment was conducted over three years (2020–2022) to assess species composition and biomass production sensitivity to varying moisture levels and nutrient addition at Ukulinga Research Farm, University of KwaZulu-Natal, in Pietermaritzburg, South Africa. Three moisture levels were used: drought (D), ambient rainfall (A), and above-average rainfall (R). Drought reduced rainfall to the 1st percentile of the 100-year rainfall record, which was 53% (483mm) of the long-term mean, while the intercepted water was diverted to the above-average rainfall plots. One subplot within each moisture level was allocated for the annual nutrient addition of nitrogen, phosphorus, and potassium. Species composition was surveyed and biomass was harvested for the estimation of aboveground biomass production, annually. In the first year, there were no nutrient addition and moisture effects but there was a main effect of moisture level in the 2nd year and finally a nutrient effect on species composition in the 3rd year of the experiment. Annual weather patterns significantly increased species richness and diversity in 2021/2022, regardless of moisture level. An interaction between nutrient addition and years showed a significant difference in response to annual weather patterns across nutrient levels ($P < 0.05$), where unfertilized plots were unaffected and fertilized plots showed significantly greater diversity in 2021/2022, the growing season with the greatest rainfall. Species evenness fluctuated annually in the high and ambient rainfall treatments, while it remained unchanged in the drought treatment. Several grasses were abundant in both unfertilized and fertilized sites but most species increased in abundance under nutrient enrichment. The increase in rainfall significantly increased plant biomass production ($355.169 g/m^2 \pm 111,3568$), and drought had the opposite effect. Both nutrient addition and annual weather patterns significantly affected species composition and biomass production but the effective use of additional nutrients was dependent on the amount of moisture available. The observed alterations in the fertilized community were stimulated by the increased usage of available nutrients due to the increased soil moisture, therefore, moisture is a factor that limits the impact of nutrient addition. The grassland may be resistant to the imposed 50% rainfall reduction and need longer periods (up to 3 years) of nutrient addition before any notable changes take place because it typically experiences erratic temperatures and rainfall.

Keywords: *rainfall, nutrient addition, biomass, species composition*

6.1. Introduction

The current atmospheric warming and consequent climate change can potentially affect the productivity of agricultural lands and grasslands as changes in rainfall amount and distribution may result in increased drought (Anwar et al., 2013). Thurow and Taylor (1999) classify droughts into four categories: meteorological, agricultural, hydrological, and socioeconomic. Meteorological drought occurs when there is a significant reduction in precipitation relative to the climatologically expected mean, which varies depending on the location. A hydrological drought occurs when there is not enough water in the river flows or lakes, reservoirs, and groundwater (Tallaksen and Van Lanen, 2004) and a socioeconomic drought occurs when the water supply from local water resources cannot meet the water demand of that given society (Shi and Chen, 2018). Agricultural drought takes place when soil moisture falls below the climatically appropriate moisture availability, thereby adversely affecting plant yields and increasing plant stress and mortality. Drought not only reduces plant productivity through increased mortality rates, but also limits the distribution and diversity of species while accelerating grassland degradation (Craine et al., 2013). Extreme droughts have the potential to cause significant and long-term ecological damage (Knapp et al., 2015a). In general, drought stress can depress the growth of plants and their reproductive abilities by limiting nutrient uptake, transport, and partitioning (Gessler et al., 2017). In comparison with many arable croplands, grasslands can offer a buffer against climate unpredictability because of their diversity of plants, which respond differently to extreme environmental conditions (Pecl et al., 2017).

A lack of water has the potential to decrease plant productivity (Guo et al., 2017), while excessive rainfall can cause soil nitrogen leaching, reducing the available nitrogen in the soil (Shi and Chen, 2018). Plants usually adapt to given environmental conditions by adjusting their patterns of growth and resource allocation. For instance, the plant's ability to obtain resources increases when water supply is sufficient (Li et al., 2009). Since plant internal nutrient cycling is an important biological process and ecosystem function, it can influence plant growth and productivity (Wright et al., 2002). Currently, grassland ecosystems have undergone extreme changes, such as declines in species biodiversity, richness or evenness, due to climate change (Su et al., 2021). Species evenness is the relative abundance or relative number of individuals of each species within a community (Maureaud et al., 2019) and is a function of species diversity.

Plant productivity, diversity and richness are important indicators of ecosystem health and functionality (Tilman et al., 2006; Wu et al., 2020). The extent to which these aforementioned characteristics might alter as a result of climate change is still unknown. The most significant limiting elements in varied grasslands are nitrogen (N) and phosphorus (P), both of which are essential for plant development (Fay et al., 2015). Identifying plant responses to nutrient addition combined with severe drought is important to better understand the major physiological mechanisms determining ecosystem processes under drought conditions. The knowledge gained from this research allows for improved predictions of vegetation responses to climatic variations, influenced by global warming. The key focus of this chapter is to investigate to what extent moisture variability and nutrient addition affect the plant community of mesic grassland.

6.2. General objective

To determine the effects of multi-year, severe drought and nutrient addition on grass sward dynamics and plant productivity.

6.2.1. Specific objectives

- To investigate the impacts of the addition of Nitrogen, Phosphorus, Potassium (NPK) and micronutrients on species composition dynamics such as diversity, richness and evenness as well as biomass production and Photosynthetic Active Radiation (*PAR*).
- To assess the effects of different water levels on species composition, biomass production and Photosynthetic Active Radiation (*PAR*).

6.3. Materials and methods

6.3.1. Study site

The Drought-Net experiment is located at Ukulinga Research Farm, University of KwaZulu-Natal, (29°24’ E, 30°24’ S), in Pietermaritzburg, South Africa. This experiment was established in 2019 and is part of the International Drought Network, which is a series of globally distributed experiments conducting research to understand drought sensitivity patterns across various biomes and climatic gradients. Ukulinga Research Farm is situated at an average altitude of 840m above sea level. The area receives an average annual rainfall of 838 mm and is categorized as subtropical (Ward et al., 2017). The summers are warm (mean monthly maximum 26.4°C) and winters are mild (mean monthly minimum 8.8°C) with occasional frost. The soil is generally considered to be infertile and acidic, derived from shales under the Westleigh classification, (Soil Classification Working Group 1991) with classification as acrisols , overlaying shales of the Karoo (Fynn et al., 2005). The field trial was located within a closed canopy, forb-rich grassland biome, classified as KwaZulu-Natal Hinterland Thornveld (Mucina and Rutherford, 2006).

6.3.2. Experimental layout and design

To determine species composition sensitivity to drought and nutrient addition, a rainfall manipulation experiment was initiated comprising of three moisture levels; drought (D), ambient rainfall (A) and above-average rainfall (R). The drought treatment reduced rainfall to the 1st percentile of the 100-year rainfall record which is 53% (483 mm) of the long-term mean, using passive shelters with transparent plastic roof sheets for the diversion of rain. The water collected from the drought plots was diverted to the above-average rainfall plots through a gutter and PVC pipes. The experiment was arranged in a randomized block design to account for any site effects. There were 9 plots in total, with each plot measuring 3 m x 5 m, with six 1 m^2 subplots separated by a 0.5 m buffer. A 250-micrometre thick plastic sheet, was buried 0.5 m deep around each plot for hydrological isolation. One 1 m^2 subplot was used for the addition of nitrogen, phosphorus, and potassium (NPK), and quantifying species composition and biomass production. All plots were mowed at the beginning of winter to prevent woody encroachment and remove moribund biomass, as no grazing takes place in these experiments. Daily rainfall and temperature data were recorded for the duration of the experiment using the existing weather station at the site. Fertilizers containing nitrogen, phosphorus and potassium were broadcast annually in the early growing season in November. For each subplot, the fertilizers were applied as follows: 23.3 g Nitrogen (time release urea), 50g Phosphorus (triple super phosphate), 22.3 g for Potassium sulphate and 100 g for micronutrients. The micronutrient mix contained boron, manganese, copper, molybdenum, iron, zinc and sulphur pentoxide and was only applied once in the first treatment season. The selected nutrients and application amounts were adapted from the experimental protocol of the global Nutreint Network experiments (adapted from http://nutnet.scence.oregonstate.edu/exp_protocol).

6.3.3. Species composition and biomass

Species composition was surveyed annually during the early (December) and the late (March) growing season. The aerial cover of each species rooted within each 1m^2 subplot was recorded by visually estimating the aerial cover for each species to the nearest percent. Annually, at the end of the growing season, biomass samples were clipped from each 1m^2 subplot in two 1m x 0.1 m long strips. The plant biomass was divided into functional groups namely, grasses, forbs and legumes, then oven dried at 60°C for 48 hours and weighed. Before analysis, the final data set was developed using the maximum abundance selected from either the early or late season for each species. Photosynthetically active radiation (PAR), readings were taken during the late growing season using a LP-80 LAI Ceptometer. The readings were recorded on a cloudless, sunny day between 10h00 and 14h00, with no obstructions above the light meter beside the drought treatment plastic roof sheets. One reading was taken above the canopy, while 2 readings were taken below the vegetation canopy at diagonal angles across the 1m^2 subplot.

6.4. Statistical analysis

The relationships between the plant community composition under different moisture levels and nutrient addition, allowing for annual weather patterns were examined with a Canonical Correspondence Analysis (CCA), using R Studio (version 4.0.2), with the analysis package “vegan” and “ggplot2” for plotting the ordination graphs (RStudio team, 2020). Permutation tests were used to determine the significance of the CCA models and significant effects were graphed. Species with an occurrence of <5% were excluded from the ordination plot for the reduction of clutter. All variables were checked for normality. The data for species evenness and biomass were not normally distributed, hence generalized linear models were applied for statistical analysis, only for these variables. The effects of nutrient addition and moisture levels as well as their interactions on species composition were analysed with permutational multivariate analysis of variance (PERMANOVA) using a Euclidean distance method due to continuous data, with 9999 permutations. Where significant effects were observed a pairwise comparison was performed. The Shannon–Wiener diversity index (H’) was calculated using the following equation (Keylock, 2005)

$H' = SUMpi \log(b)pi$

where, pi = proportional abundance of species, b = base of logarithm.

Pielou’s evenness (J′) was calculated using the following equation (Mendes et al., 2008):

$J' = H'/\ln(S)$

where, H′ = Shannon–Wiener diversity index and S = total number of species in a sample, across all samples.

An analysis of variance was used to investigate the effects of drought treatments and year on species richness (S) and diversity, calculated using the Shannon–Wiener diversity index (H’). When the analysis revealed significant effects the Least Significant Difference post hoc test was used to separate means. The statistical analyses were performed using R Studio (version 4.0.2) and using the following packages: “vegan”, “tidyverse”, and “pairwiseAdonis”. Furthermore, generalized linear models using Poisson distributions? were used to examine the main effects and interactions of ambient rainfall, above-average rainfall and drought between 2020, 2021 and 2022 on mean aboveground dry biomass.

6.5. Results

The annual rainfall graph shows the total rainfall for growing seasons from 2005 to 2022, over 17 years in the mesic grassland at Ukulinga Research Farm, Pietermaritzburg, South Africa (Figure 6.1). Mesic grasslands generally receive high annual rainfall of >600 mm per year (Palmer and Ainslie, 2005). For most years, the annual rainfall fell within the average range for a mesic grassland, except for two years 2014 and 2015, a regional drought, where both years fell below 600 mm. From 2019, the drought treatments induced a 53% rainfall reduction in the natural rainfall of the grassland. Figure 6.1 draws attention to the fact that grassland naturally experiences high and low rainfall cycles ranging from 500 mm to approximately 1100 mm. The graph shows the growing seasons from September to May in a single bar, from 2005 to 2022 (Figure 6.1). The monthly rainfall for the study period is shown in Figure 6.2. The 2021/2022 growing season had the greatest total rainfall at 1160.8 mm (Figure 6.2). Figure 6.3 shows both the monthly average minimum (A) and maximum temperatures ºC (B) for 2019/2020, 2020/2021 and 2021/2022 at Ukulinga Research Farm. Generally, the 2019/2020 cycle had the highest average minimum temperatures (ºC) and the same was observed for maximum temperatures (ºC) from July to February, except December (Figure 6.3).

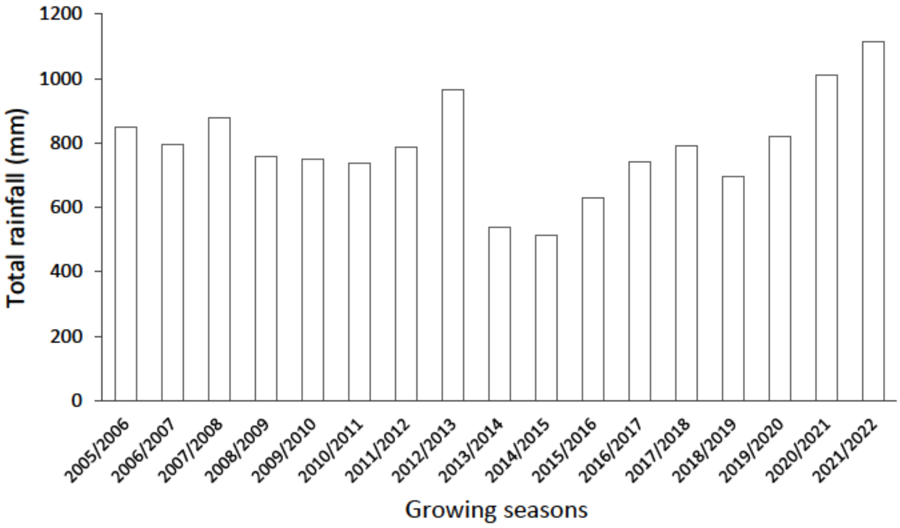

Figure 6.1: Growing season rainfall from 2005 to 2022 at Ukulinga Research farm, Pietermaritzburg, South Africa.

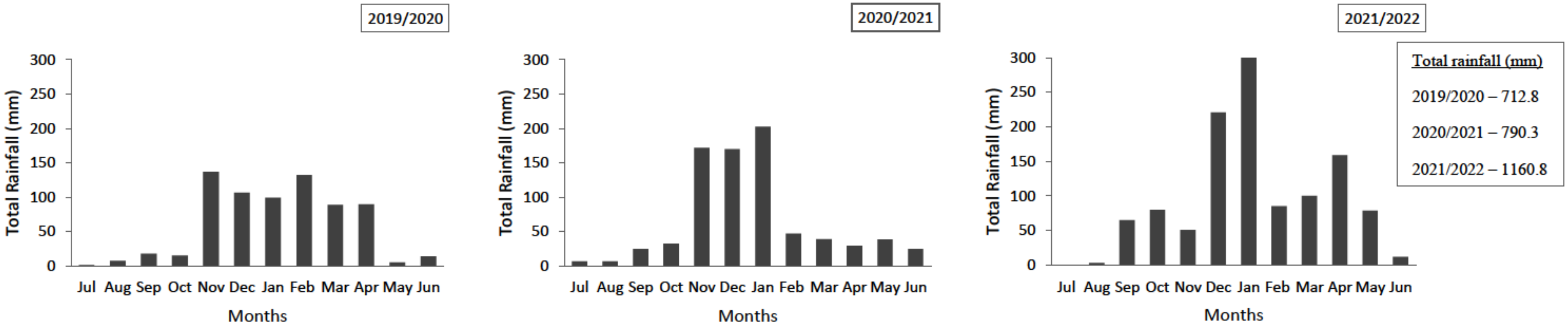

Figure 6.2: The total monthly rainfall (mm) for the 2019/2020, 2020/2021 and 2021/2022 seasons at Ukulinga Research Farm, Pietermaritzburg, South Africa.

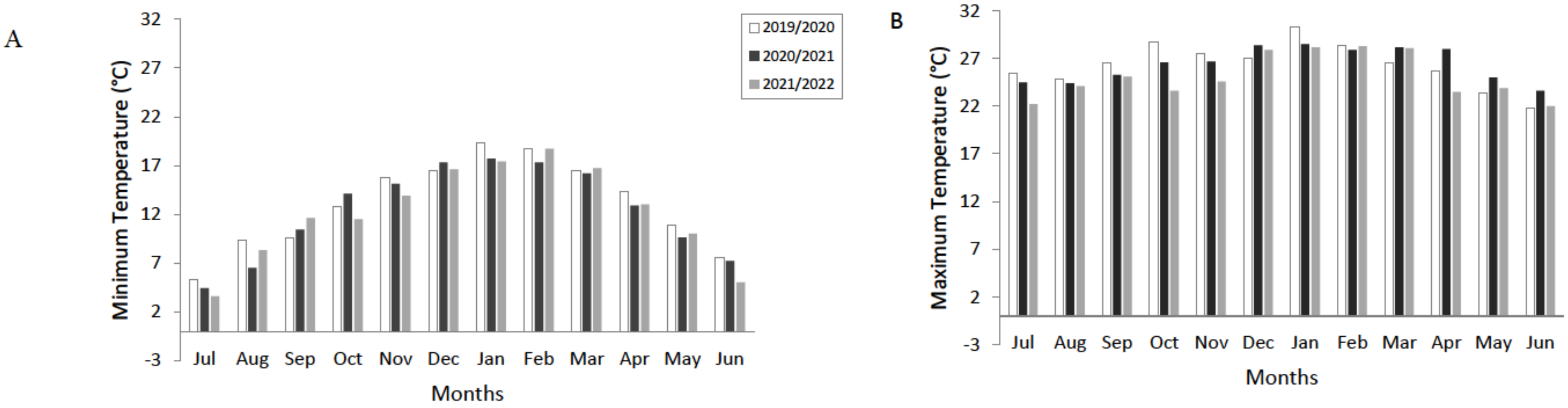

Figure 6.3: The monthly average minimum temperature °C (A) and maximum temperature °C (B) for 2019/2020, 2020/2021 and 2021/2022 at Ukulinga Research Farm, Pietermaritzburg, South Africa.

6.5.1. Species composition

The ambient rainfall treatment retained a higher species abundance in the unfertilized areas of 2019/2020 and 2020/2021 (Figure 6.4A, 6.5A), containing species such as *Eriosema cordataum*, *Cymbopogon excavatus*, and palatable grasses: *Tristachya leucothrix*, and *Bracharria serrata*. Meanwhile fertilization reduced general species abundance in the same treatment and growing seasons (Figure 6.4B, 6.5B). There was a notable shift in the final growing season where the nutrient addition treatment showed higher species abundance and variation in the ambient rainfall treatment (Figure 6.6B). The 2020/2021 fertilized drought treatment had a high forb abundance of *Thunbergia atriplicifolia*, *Rhynchosia totta*, and *Indigofera hilaris* (Figure 6.5B). but species abundance decreased in 2021/2022 (Figure 6.6B).

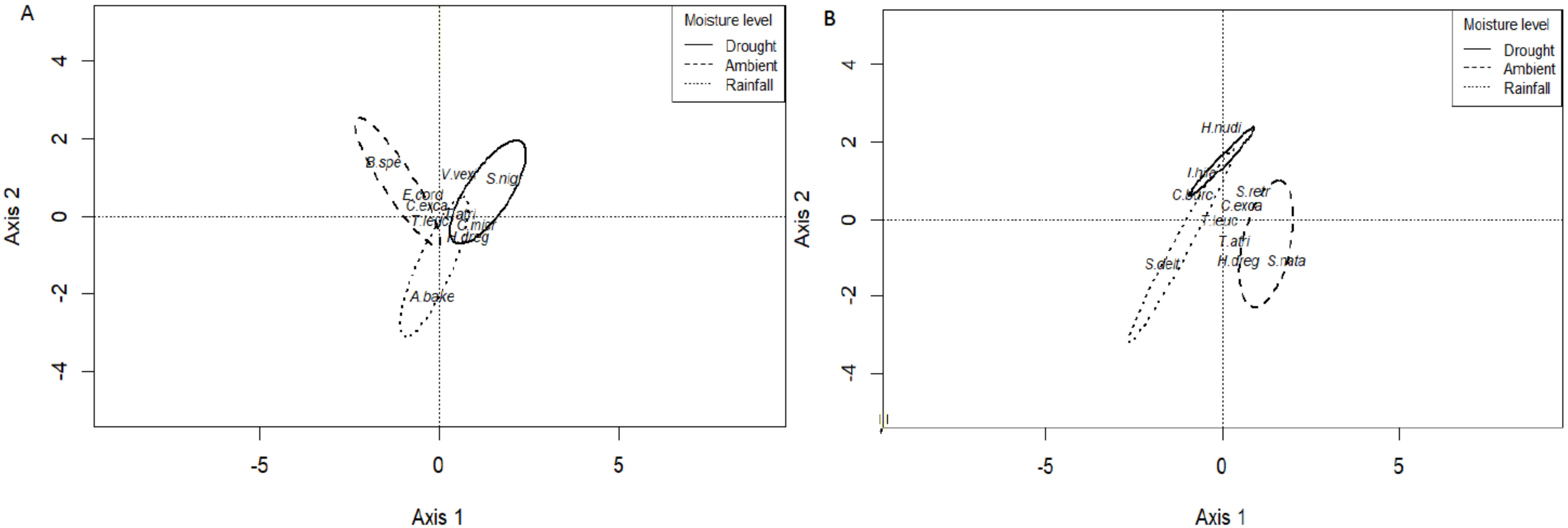

Figure 6.4: Canonical correspondence analysis of the grass and forb species community composition in unfertilized (A) and fertilized (B) plots under drought, ambient and above average rainfall from Ukulinga Research Farm, Pietermaritzburg, South Africa for 2019/2020. Species with an occurrence of <5% were excluded from the ordination plot for the reduction of clutter. Full names of the species are included in Appendix 6A.

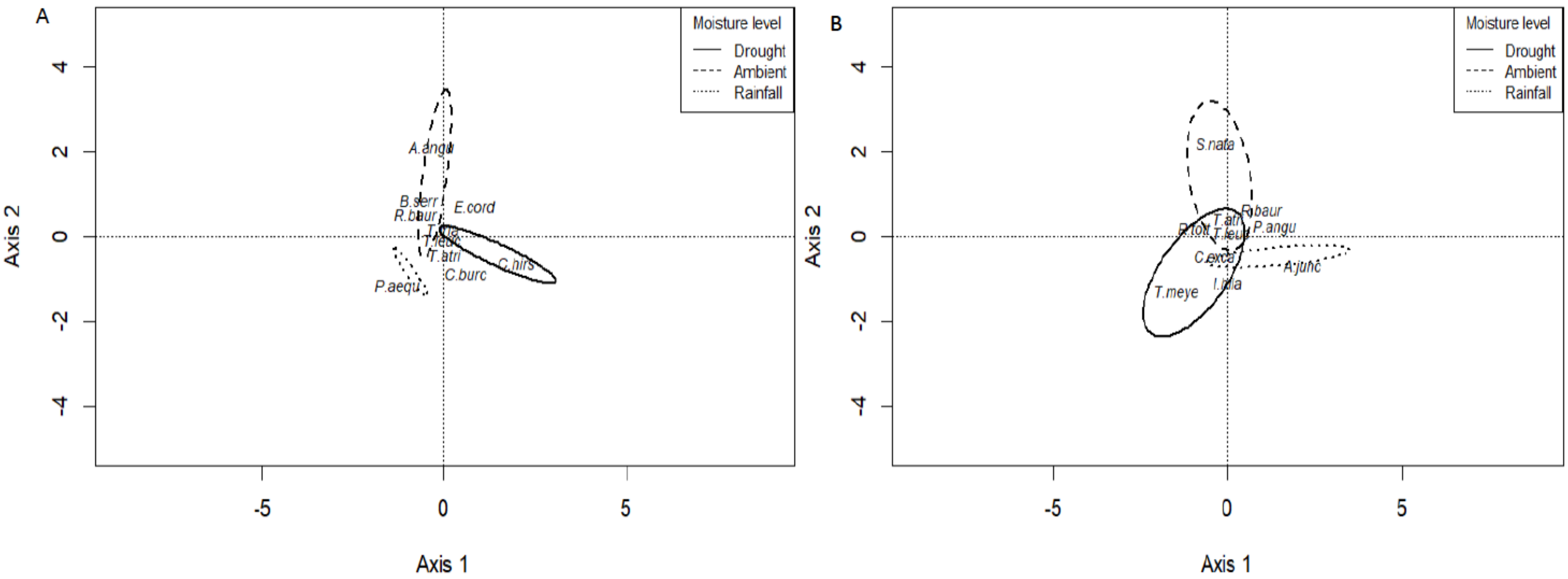

Figure 6.5: Canonical correspondence analysis of the grass and forb species community composition in unfertilized (A) and fertilized (B) plots under drought, ambient and above average rainfall from Ukulinga Research Farm, Pietermaritzburg, South Africa for 2020/2021. Species with an occurrence of <5% were excluded from the ordination plot for the reduction of clutter. Full names of the species are included in Appendix 6A.

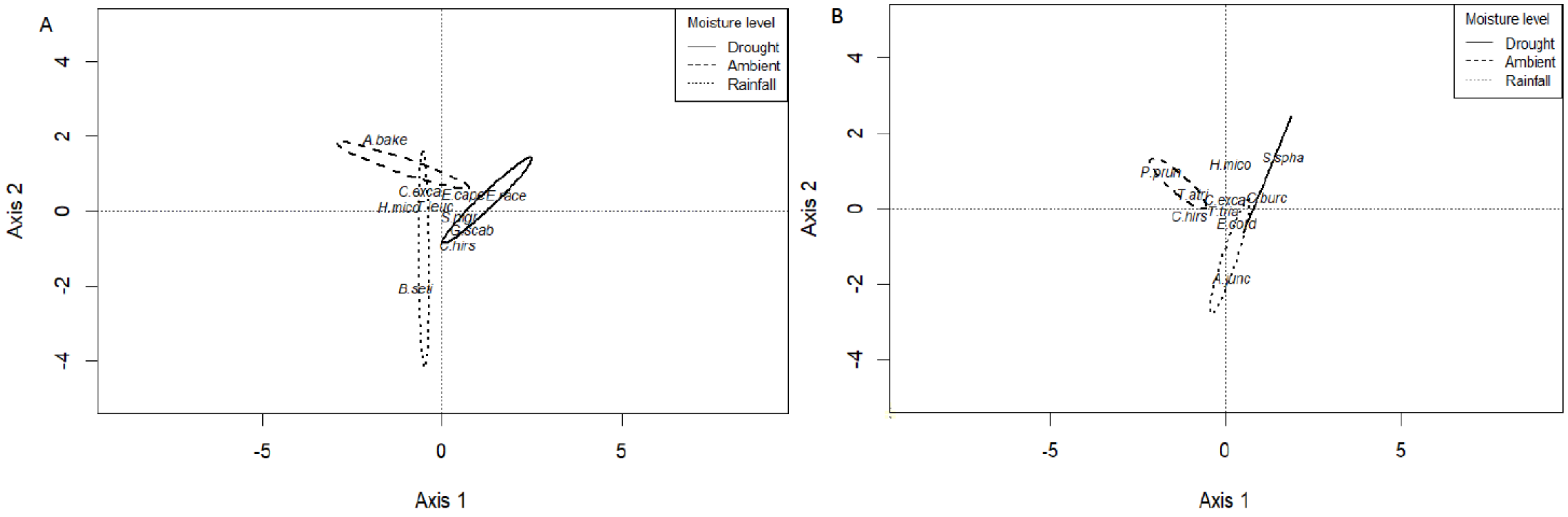

Figure 6.6: Canonical correspondence analysis of the grass and forb species community composition in unfertilized (A) and fertilized (B) plots under drought, ambient and above average rainfall from Ukulinga Research Farm, Pietermaritzburg, South Africa for 2021/2022. Species with an occurrence of <5% were excluded from the ordination plot for the reduction of clutter. Full names of the species are included in Appendix 6A.

The permutational multivariate analysis of variance suggests that it took three years for the nutrient addition to exhibit an effect on species composition (Table 6.1). There was a significant effect of moisture level only in 2020/2021, while there were no observed effects of interactions (Table 6.1). This was the second year with rainfall below 800 mm, the long-term average, and in the following year, the rainfall was abnormally high which is likely the cause of the observed effects. The species composition in the drought treatments was significantly different to the above-average rainfall treatment, while the ambient rainfall had no significant difference to the rest of the moisture levels. *T. leucothrix*, *T. triandra* and *C. excavatus* were the most abundant grasses in both unfertilized and fertilized plots (Table 6.2), however, *T. leucothrix* showed a reduction in abundance in the fertilized area in 2021/2022.

Table 6.1: Two-way permutational multivariate analysis of variance (PERMANOVA) between fertilization and the drought (D), ambient rainfall (A) and above average rainfall (R) moisture levels at permutation 9999, using Bray-Curtis for the year 2019/2020, 2020/2021 and 2021/2022, showing *P*-values with significant effects ($P < 0.05$) in bold at Ukulinga Research Farm, Pietermaritzburg, South Africa

		2019/2020		**2020/2021**		**2021/2022**	
Source	**Df**	**F-value**	**P-value**	**F-value**	**P-value**	**F-value**	**P-value**
Fertilization	1	1.2628	0.2585	1.3306	0.1951	2.0831	**0.0237**
Moisture level	2	0.70763	0.8405	1.8896	**0.0297**	0.98705	0.4908
Interaction	2	0.57219	0.9383	0.95875	0.4941	0.83272	0.6857
Residual	12						
Total	17						

Table 6.2: Average species (forbs and grasses) abundance contributing to the dissimilarity between unfertilized (NF) and fertilized (F) plots between in three moisture levels (drought, ambient and above average rainfall) throughout the years 2019/2020, 2020/2021 and 2021/2022 resulting from SIMPER analysis plots using Bray-Curtis dissimilarities test. Species in shaded blocks contribute more than 5% and species in bold more than 15% to the dissimilarity between unfertilized (NF) and fertilized (F) sites

	2019/2020		2020/2021		2021/2022	
Species	**NF**	**F**	**NF**	**F**	**NF**	**F**
			Grasses			
Tristachya leucothrix	**27.7**	**43.9**	**55**	**64.4**	**33.9**	**18.6**
Themeda triandra	**17**	**18.4**	**20.9**	**24.1**	**19.7**	**28**
Cymbopogon excavatus	13.7	10	**17.8**	**22.8**	3.22	5.67
Setaria nigrirostris	0.889	6.11		1.67	5.33	**20.6**
Brachiaria serrata	6.44	0.556	6.44		7.78	1.11
Setaria sphacelata	0.556	1.22		**24.4**		4.22
Hyparrhenia dregeana	4.44	2.78	1.67	2.22		
Aristida junciformis		1.33		4.44		5
Eragrostis capensis			**20.9**	**24.1**	10.6	1.67
Diheteropogon amplectens	2.22	1.67		3.89	0.556	
Heteropogon contortus	1.11		1.11		11.2	0.667
Panicum aequinerve			3.89	1.67		
			Forbs			
Cephalaria pungens	14.4	8.67	12.2	16.2	9.78	5.89
Carissa macrocarpa	6.56	7.33	6.11	11.1		
Thunbergia atriplicifolia	5.89	11.7	10.9	**17.4**	8.89	11.9
Chaetacanthus burchellii	5.33	3.56	4.78	8.11	1.11	2.22
Eriosema cordatum	4	3.89	3.67	2.78	2.44	4
Senecio bupleuroides	1.67	6.67		2		2.78
Senecio coronatus	3.44	4.44	8.11	8.33	1.89	4
Hibiscus aethiopicus	4.78	2.44	1.67	5.11	0.556	0.556
Crabbea hirsute	2.33		2.78	4.56	3.67	2.11
Indigofera hilaris	3.11	3.11	6.11	11.1	0.556	
Spermacoce natalensis		2	4			1.67
Crotalaria dura			10.9	**17.4**	2.78	8.89
Acalypha angustata		2.22		4.67		
Aster bakeriana	1.11	1.33	5.67	4.44	0.556	
Vigna vexillata	4	0.556		1.67		
Commelina africana				3.89		1.11
Helichrysum miconiifolium	1.11		4		1.44	2.33
Ruellia baurii	0.556		3.67	2.78		

Species diversity was significantly greater in 2021/2022, compared to 2019/2020 and 2020/2021 (Figure 6.7 & 6.9). Only diversity was affected by nutrient addition, where the average species diversity was lower in the unfertilized plots compared to the fertilized plots (Table 6.3). The interaction between nutrient addition and year shows a significant response to annual weather patterns across fertilizer levels, where unfertilized plots were unaffected by climate variability and fertilized plots showed significantly greater diversity in 2021/2022 when greater total rainfall was recorded (Figure 6.8). Additionally, species richness was significantly higher in 2021/2022, than 2019/2020 and 2020/2021 (Figure 6.9).

Table 6.3: Results for analysis of variance for diversity, richness and interactions between the years 2019/2020, 2020/2021 and 2021/2022 and treatments (ambient, drought and above average rainfall) as well as drought and fertilization plots showing total the *F*-value and *P*-value with significant effects ($P < 0.05$) in bold

		Diversity		**Richness**	
Source	**Df**	**F-value**	**P-value**	**F-value**	**P-value**
Year	2	4.223	**0.023**	29.328	**<0.001**
Fertilization	1	4.862	**0.034**	0.380	0.542
Moisture level	2	0.449	0.642	1.234	0.303
Y * F	2	3.565	**0.039**	2.746	0.078
Y*ML	4	1.981	0.118	0.836	0.511
F * ML	2	0.751	0.479	2.083	0.139
Y * F * ML	4	1.074	0.384	0.935	0.455
Error	36				
Total	54				

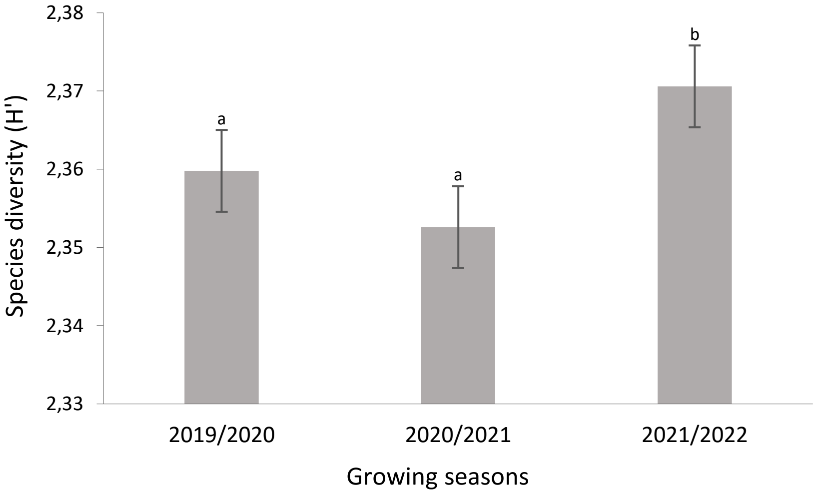

Figure 6.7: Mean species diversity calculated based on Shannon's diversity index (H') and between the growing seasons from the years 2019/2020, 2020/2021 and 2021/2022 at Ukulinga Research Farm. Year with different letters are significantly different ($P < 0.05$).

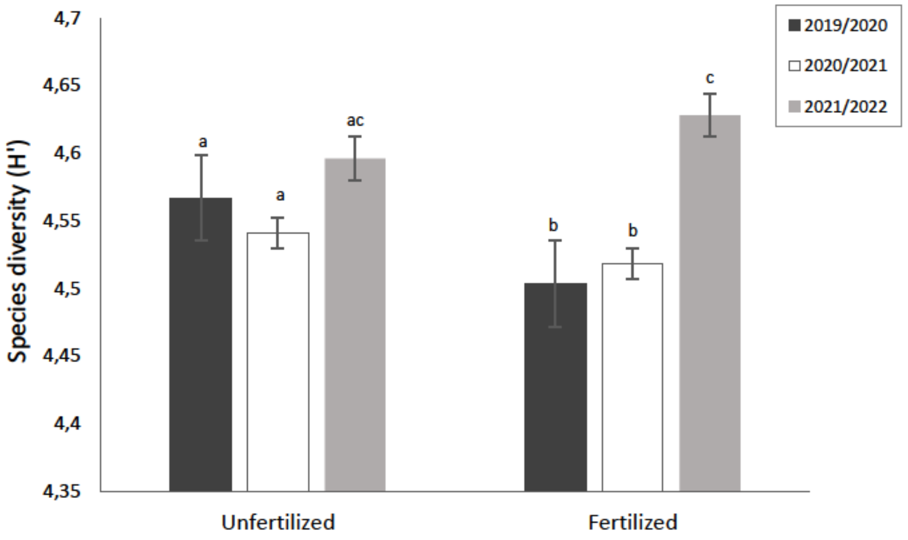

Figure 6.8: The interactions of mean species diversity calculated using Shannon's diversity Index(H') between the growing seasons 2019/2020, 2020/2021 and 2021/2022 across the fertilized and unfertilized plots at Ukulinga Research Farm. Superscripts showing different letters are significantly different to one another ($P < 0.05$).

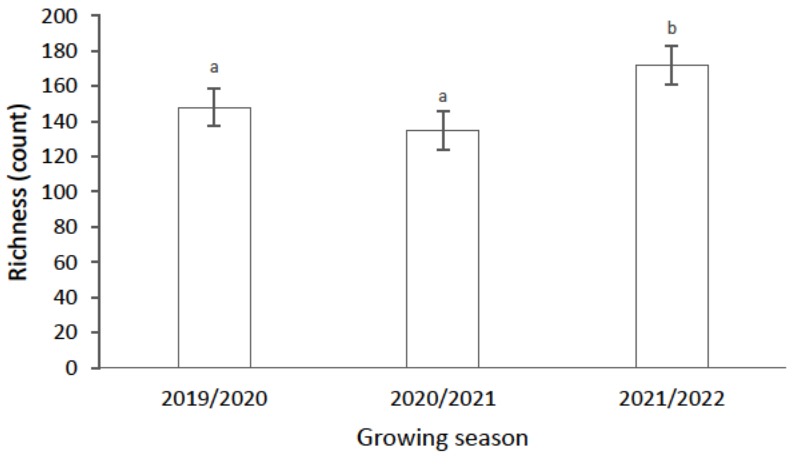

Figure 6.9: Mean species richness (count) between years 2020, 2021 and 2022 at Ukulinga Research Farm. Years with the different letters are significantly different ($P < 0.05$).

Mean species evenness was significantly increased by fertilization. Unlike both diversity and richness, species evenness was significantly reduced by 2021/2022 (Figure 6.10A). Species evenness gradually decreased from 2019/2020 to 2021/2022 in the sites with nutrient addition while there was no significant effect of annual weather patterns in the unfertilized sites (Figure 6.10A). Species evenness was unaffected by annual weather fluctuations under the drought treatment while under ambient rainfall it was significantly decreased from 2019/2020 to 2021/2022. For the above average rainfall plot, annual weather patterns only affected species evenness in 2020/2021 and no effect was observed both in 2019/2020 and 2021/2022 (Figure

10B). Nutrient addition significantly increased species evenness in the ambient rainfall plot compared to the unfertilized area (Figure 6.10C).

Table 6.4: General linear models for the effects of interannual climatic variability (year), moisture level (ambient, drought and above average rainfall) and fertilization on species evenness showing total the Chi-square value (X^2), *F*-value and *P*-value with significant effects ($P < 0.05$) in bold

Source	X^2	Df	P-value
Year	8.240	2	**0.016**
Fertilization	6.905	1	**0.009**
Moisture level	0.263	2	0.877
Y * F	6.404	2	**0.041**
Y * ML	15.905	4	**0.003**
F * ML	10.253	2	**0.006**
Y * F * ML	8.783	4	0.067

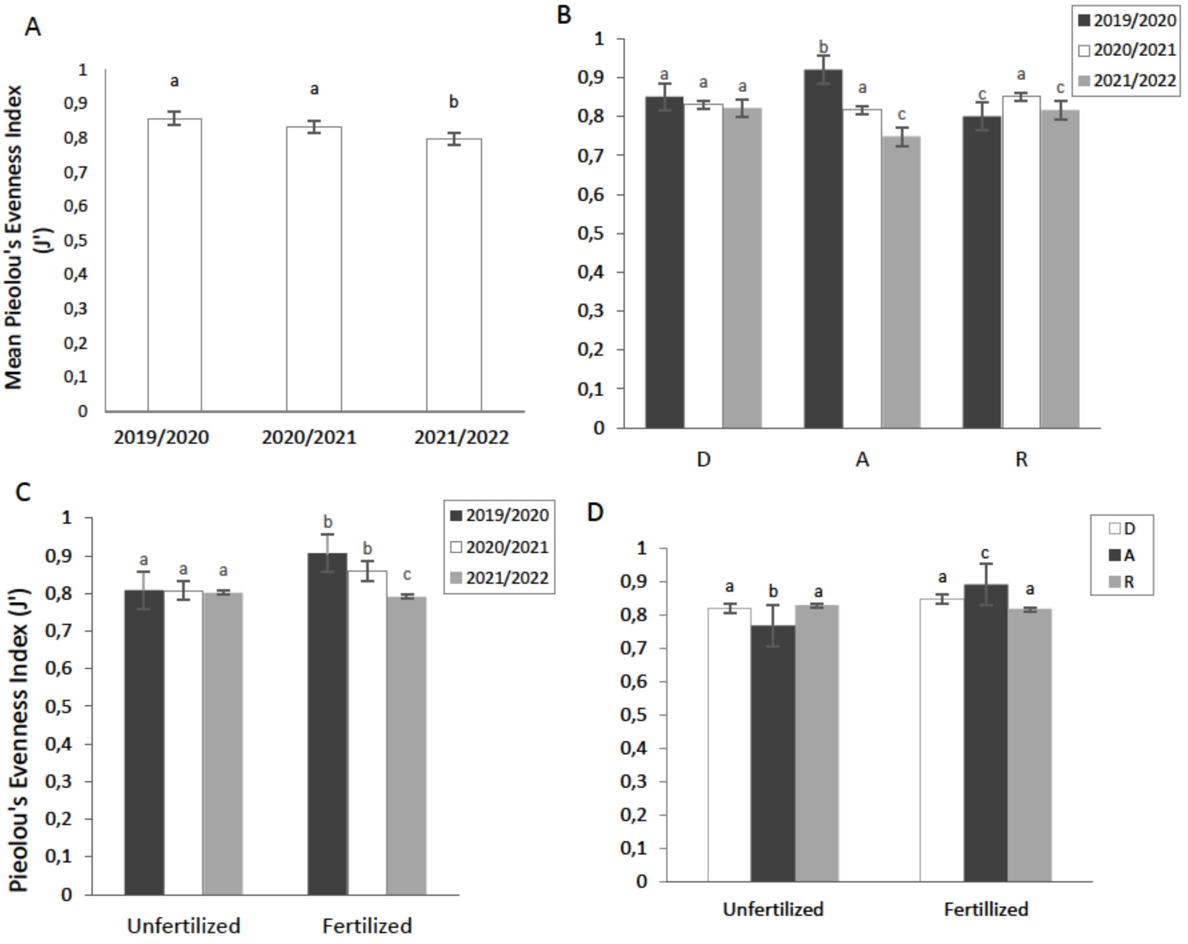

Figure 6.10: The mean species evenness (J') between growing seasons (A) and interactions for the effects of interannual climatic variability between 2019/2020, 2020/2021, and 2021/2022 on drought (D), ambient (A) and above average rainfall (R) (B). C represents the interactions for the effects of interannual climatic variability between 2019/2020, 2020/2021, and 2021/2022 on mean species evenness of fertilized and unfertilized plots while D shows interactions for the effects of treatments drought (D), ambient (A) and above average rainfall on mean species evenness of fertilized and unfertilized plots at Ukulinga Research Farm, Pietermaritzburg, South Africa. Superscripts showing different letters are significantly different ($P < 0.05$).

6.5.2. Biomass production

The results from the general linear models analysis showed a significant main effect ($P < 0.05$) of nutrient addition, rainfall, annual weather patterns, as well as the interaction of nutrient addition and rainfall on biomass production of the grassland community (Table 6.5). The year 2020/2021 had significantly greater biomass production (310.765g/m^2 ± 116,9456) compared to all the other years, while 2019/2020 was not significantly different to 2021/2022 (Figure 6.11A). Drought significantly reduced sward biomass (*256.381*g/m^2 ± 98,4792) compared to ambient and above-average rainfall which promoted greater biomass production (Figure 6.11B). The sward biomass production was directly proportional to rainfall level (Figure 6.11B). Nutrient addition was ineffective in the drought and ambient rainfall areas until there was an increase in moisture resource availability which then resulted in increased sward biomass production (Figure 6.11C). In the unfertilized area, drought significantly reduced sward biomass production while there was no significant difference between biomass production in the ambient and above-average rainfall plots (Figure 6.11C). The amount of photosynthetically active radiation that penetrated the grass canopy was unaltered despite these variations in sward biomass (Table 6.6).

Table 6.5: Results for general linear models for main effects and interactions of moisture levels between the years 2019/2020, 2020/2021 and 2021/2022 on mean aboveground dry biomass, showing Wald Chi-square (X^2), value, degrees of freedom (df) and *P*-value with significant effects ($P < 0.05$) in bold

Source	X^2	**df**	**P-value**
Fertilization	11.738	1	**<0.001**
Moisture level	6.514	2	**0.038**
Year	52.630	2	**<0.001**
F * ML	10.587	2	**0.005**
F * Y	5.284	2	0.071
ML * Y	1.077	4	0.898
F * ML * Y	8.802	4	0.066

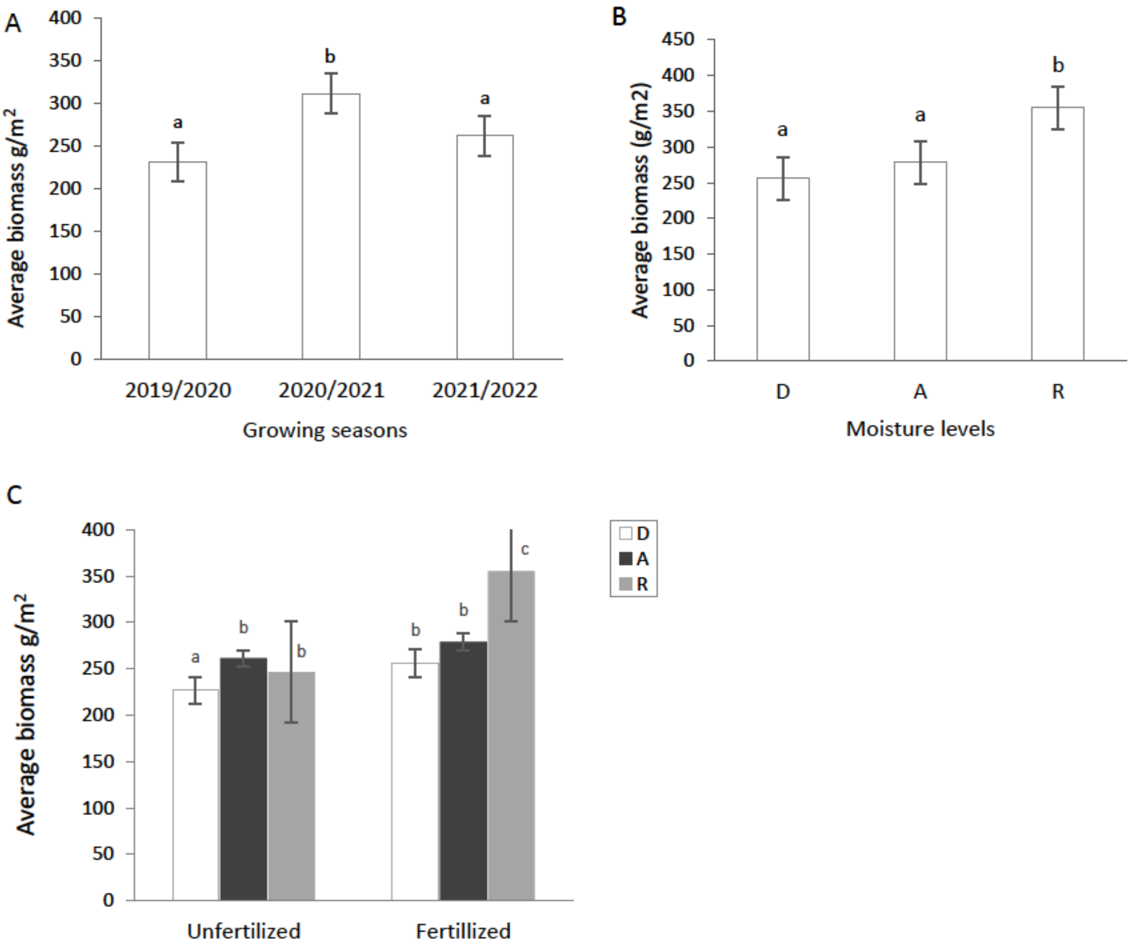

Figure 6.11: The average sward biomass (g/m^2) for the main effects of interannual climatic variability between years (2020, 2021, 2022), moisture levels: drought (D), ambient (A) and above average rainfall (R) and interactions between fertilization and moisture levels at Ukulinga Research Farm, Pietermaritzburg, South Africa. Superscripts showing different letters are significantly different ($P < 0.05$).

Table 6.6: Results of analysis of variance for Photosynthetic Active Radiation (*PAR*) and interactions between the years 2019/2020, 2020/2021 and 2021/2022 and treatments (ambient, drought and rainfall) showing total sum of squares, *F*-value and *P*-value with significant effects ($P < 0.05$)

Source	Sum of squares	df	F-value	P-value
Year	44.362	3	0.272	0.845
Moisture level	16.869	2	0.155	0.857
Y * ML	161.739	6	0.495	0.806
Error	1306.494	24		
Total	4540.825	36		

6.6. Discussion

6.6.1. Species composition

The composition of plant communities is influenced by climate change, specifically drought (Knapp et al., 2015a). The influence of nutrient availability on drought-stricken grasslands remains unclear as limited work has focussed on this (Stampfli et al., 2018). There is a dearth of literature on drought impacts on fertilized grasslands, however, our results reveal a general significant effect of fertilization and, to a more limited extent, moisture level. However, there were no treatment effects in the first year but there was a main effect of moisture level on the second year and then a main effect of fertilizer was only observed in year three.

The fertilized 2020/2021 drought treatment showed an increase in the abundance of a few grasses such as *Setaria nigrirostris*, *Setaria sphacelata* and the introduction of *Eragrostis plana*. The highly variable species composition suggests that nutrient addition promoted a higher species diversity. Species diversity is subject to change at consistently low fertilizer inputs because species diversity initially increases at low resource supply levels but declines over time as the environment becomes species-rich (Tilman, 1982). Several studies have also found a decreasing effect of specifically nitrogen or phosphorus addition on species diversity and richness (Humbert et al., 2015; Midolo et al., 2019; Yue et al., 2020). The majority of these research studies focused on the addition of NPK as separate nutrients without taking water levels into consideration, as was the case in the current study. Given that higher species diversity and richness are typically linked to higher soil porosity and infiltration capacity (Fischer-Bedtke et al., 2015), which in turn improve soil aggregation in response to organic matter accumulation and increased microbial activity (Gould et al., 2016), it is assumed that these increases in diversity and richness may be advantageous to mesic grasslands.

Greater species diversity and richness reduce surface runoff, subsequently reducing soil erosion, resulting in improved ecosystem stability (Berendse et al., 2015). Regardless of species identity, the increased species diversity and richness observed in the 3rd year of the experiment in response to nutrient addition can help the grassland to withstand soil erosion during times of rainfall unpredictability. Therefore, areas with nutrient-rich soils may be likely to cope with extreme weather events better than areas with nutrient poor soils. This may not be the case in extremely nutrient-poor areas, as loss of soil fertility often leads to a further decline of plant species diversity, hence a weaker resistance to erosion (Grime, 2001). Species evenness was affected by both annual weather patterns and nutrient addition, with evenness remaining higher in fertilized areas compared to the unfertilized. Short-term research has the potential to under represent long-term processes by not detecting certain environmental thresholds, such as species adaptation (Kidd et al., 2017)). Therefore, it is important to consider that the results from this study over the 3-year period of nutrient addition may not persist in the long term. Long-term nutrient enrichment studies show an increase in above-ground net primary productivity with N and P enrichment (Vitousek et al., 2010) and a decrease in plant species diversity and richness (Cleland and Harpole, 2010; Harpole et al., 2016; Muehleisen et al., 2023).

Numerous functional diversity indicators such as richness or evenness, can identify the complementing effects or facilitation implications that coexisting species with different trait values have on ecological processes (Lavorel and Grigulis, 2012). These functional diversity components are used to evaluate the variety of functional traits within a plant community. Richness and evenness are believed to be critical drivers of ecosystem processes, such as productivity (Wilsey, 2000). However, much less attention has been paid to how much evenness is affected by climatic changes. As opposed to richness and diversity, evenness, regardless of treatment, was significantly reduced from 2019/2020 (0.921) to 2021/2022 (0.796). this may have been caused by the disappearance, as well as the increase in abundance of the different species. Species evenness can account for more variance in plant community diversity than species richness ((Maureaud et al., 2019; Wilsey et al., 2005).

Nutrient addition gradually increased the abundance of many palatable grass species, such as *T. triandra* and *S. nigrirostris,* but also increased the abundance of unpalatable grass species, such as *C. excavatus, Aristida congesta* and forbs. Based on these results, as much as nutrient addition promoted a higher species diversity and richness in the grassland, this does not necessarily mean that fertilization is good for the grassland as it has been noted that this is a short-term effect and long term effects are negative based on much experimental work. Nutrient addition did not only increase the abundance of palatable species, but also increased diversity and richness of other unpalatable species and some forbs that might not be considered to be good for the veld condition. Fertilization caused the disappearance of *B. serrata* in 2020/2021, and a decrease in the abundance of the species in 2021/2022. Again, *Heteropogon contortus* completely disappeared with fertilization in 2019/2020 and 2020/2021. Nutrient effects vary along a vegetation and climatic gradients, with grasslands showing that nutrient addition drastically decreased plant diversity (Boch et al., 2021; Hautier et al., 2009; Jacquemyn et al., 2003; Kölliker et al., 2002). A commonly documented reaction to low-level nitrogen application is the disappearance of certain species from plant communities, resulting in a decrease in abundance and local extinction (Midolo et al., 2019; Simkin et al., 2016; Wilkins et al., 2016). For instance, legumes generally decline, as increasing soil N availability is detrimental to N fixation (Craine et al., 2002). Nutrient enrichment studies are often inclusive of mowing and grazing interactions. Therefore, other management practices such as mowing or grazing may be worth considering given that the other studies have noted a decrease in species richness and diversity with nutrient addition (Boch et al., 2021; Hautier et al., 2009; Kölliker et al., 2002).

The effect of nutrient addition on increasing species richness was only recorded in the 3rd year of treatment application, 2021/2022. Coincidentally, this directly followed the growing season with the highest rainfall of the study period, which suggests that the fertilizer requires some time and available moisture resources to be effective. This also means that available moisture is a key factor that determines the efficient utilization of available nutrients required by mesic grassland species. The increase in soil moisture promotes use of available nutrients, enough to stimulate the observed changes in the nutrient enriched grassland. The significance of annual weather patterns may also suggest that the grasslands require time to respond to variations in ecosystem drivers, hence the observed changes were mostly in 2021/2022 since there was a significant effect of moisture level only in 2020/2021. Moderate soil fertility has also been reported to yield the greatest plant species richness (Zechmeister et al., 2003). One study in particular also showed that nutrient addition caused an increase in grassland species richness (Boch et al., 2021). Nutrient addition also changes the composition of the plant community by favouring fast-growing, resource-acquisitive plants and species with higher biomass productivity and moisture efficiency. However, Boch et al. (2021), also found that maximum yield can be achieved with a lower addition of nutrients, suggesting moderate soil fertility can be important for high plant diversity in mountain grasslands

There is evidence supporting a positive relationship between plant diversity and above-ground plant biomass production in natural ecosystems (Tylianakis et al., 2008). The positive relationship between biomass production and species diversity was recorded in this study whereby both increased species diversity and biomass were observed in 2021/2022, compared to the first two years of the study. These could be short-term trends given that the majority of nutrient enrichment studies show reduced richness, diversity with increase in nutrient levels and biomass (Cleland and Harpole, 2010; Fay et al., 2015; Harpole et al., 2016; Muehleisen et al., 2023). However, a positive relationship was also observed between species richness and biomass (Naeem et al., 1996) which is agreement with the findings of this study. This can protect the grassland from the impacts of drought as greater biomass production increases canopy cover, protecting the soil from erosion (Srivastava et al., 2020). It is believed that an ecosystem will be more tolerant to disturbances if its diversity is greater leading to more stable ecosystems (Tilman, 1994).

6.6.2. Biomass production

Nutrient addition, moisture level, annual weather patterns, as well as the interaction of nutrient addition and moisture level significantly affected biomass production of the grassland community. In 2020/2021, the overall above-ground biomass production was significantly greater than the rest of the experimental years. This may be attributed to the changes in the rainfall patterns which fluctuated from year to year as well as within each year's growing season. This average sward biomass also increased with increasing moisture level. It is difficult to solely base the results of increased biomass on rainfall, as temperature and humidity may be contributing factors to biomass production. Since moisture level is the most influential abiotic factor for grassland plant productivity (Huxman et al., 2004a; Lieth and Whittaker, 1975; Sala et al., 1988), specifically driven by growing season precipitation (Fay et al., 2003) it may be concluded that moisture level is a contributing factor to the effects we observed on biomass production. Our results concur with those of other studies (Hossain and Beierkuhnlein, 2018; Kahmen et al., 2005; Pierre et al., 2016) who found increased aboveground biomass production with increased precipitation. In mesic grasslands, this confirms that drought reduces sward biomass production while additional moisture resources promote biomass production.

When interacting with nutrient addition, drought significantly reduced sward biomass production in the unfertilized area. Similarly, low aboveground biomass was recorded in the drought treatment in general. Contrary to our results, Van Sundert et al. (2023), recorded a reduction in biomass under nutrient-enriched treatments of both nitrogen combined with phosphorus, as well as nitrogen, phosphorus and potassium addition. In our case, nutrient addition promoted an overall higher average biomass compared to the unfertilized plots. These findings coincide with other studies that found fertilizing herbaceous plant communities increased above-ground biomass (Clark and Tilman, 2008; Gross et al., 2005; Suding et al., 2005). Higher nutrient availability can mitigate the impacts of drought but only under moderate droughts and not extreme water limitations (Shi et al., 2018) through increased water-use efficiency (Guo et al., 2017), or by promoting a deeper rooting depth (Gessler et al., 2017).

The ability of plants to maintain metabolism and the production of biomass under limited water availability is called drought tolerance which means the plants have a high water-use efficiency (Jones et al., 2017). In this case, the grassland biomass production was negatively correlated to drought, despite nutrient addition, hence it was not drought tolerant. However, the grassland is resilient to drought because as soon as moisture availability increased, then the biomass production also increased. The fact that biomass was reduced during drought in a nutrient enriched grassland means that the plants were not able to sufficiently utilize the available nutrients in the absence of adequate moisture, therefore water is the most limiting resource in the productivity of mesic grasslands. Grasslands are known to be resilient ecosystems, with a high capacity for recovery after environmental distress (Stuart-Haëntjens et al., 2018). For instance, the annual biomass production of a mesic grassland recovered to pre-drought levels after one year (Wilcox et al., 2020). In this study, the increase in rainfall for the 2021/2022 growing season, coupled with the addition of nitrogen, potassium and phosphorus stimulated an increase in biomass production that was initially lost through drought, showing the resilience of mesic grasslands, and that these areas are able to recover upon the elimination of resource limitation.

Drought impacts on grassland productivity strongly depend on the plant community composition, because plant functional groups may differ considerably in their drought response. For instance, grasses generally have a higher phenotypic plasticity in traits that are related to the impact of drought than forbs, which allows them to better withstand drought (Wellstein et al., 2017). In this study, throughout all moisture level plots, grasses were the functional group that dominated the sward biomass, compared to forbs and legumes. Grass responses to drought are anticipated to be less reliant on nutrient addition regimes than forbs which are drought sensitive under high land-use intensity with frequent fertilizer application (Stuart-Haëntjens et al., 2018). A general loss of forb and legume richness has also been shown under nutrient addition (Vargova et al., 2020).

There was no significant effect of either nutrient addition, moisture level, or annual weather patterns on light penetrating the sward canopy. This implies that biomass production maintained sufficient basal and aerial cover, thereby reducing the potential for erosion to occur. With the grassland having sufficient vegetation coverage, the plant community is protected from soil erosion because vegetation cover directly protects the soil surface from erosive winds, reducing runoff (Webb et al., 2021). This level of biomass coverage could be a positive contributing factor to the mesic grassland's resilience to climatic variations as the biomass remains sufficient to protect the soil from erosion throughout climatic variations.

6.7. Conclusions

Annual weather patterns significantly affected both species richness and diversity, where species diversity was significantly greater in 2021/2022, compared to 2019/2020 and 2020/2021. However, only species diversity was increased by nutrient enrichment in the 3rd year of the experiment. The changes in species diversity occurred after a higher rainfall cycle, which implies that there needs to be sufficient moisture in order for the grassland to utilize the available nutrients. Taking moisture into consideration, nutrient addition also increased the abundance of many species, but this is not necessarily good for the grassland because the abundance of unpalatable species may be increased. As much as the unfertilized plant community remained less productive and diverse compared to the nutrient enriched areas, greater moisture levels are necessary to bring the grassland to a highly productive state and also for rapid recovery of the plant community after a drought. The fact that the changes caused by nutrient addition were in the final year of this experiment signifies that not only is moisture a limiting factor but the species composition requires some time for visible changes to occur. It is also important to note that the observed increase in species diversity may not persist after additional years of nutrient addition because many long-term studies state that prolonged nutrient enrichment decreases both the diversity and richness of species (Boch et al., 2021; Cleland and Harpole, 2010; Harpole et al., 2016; Muehleisen et al., 2023). The species composition of the grassland was not affected by drought, however, higher moisture levels significantly increased sward biomass production. The high aboveground biomass in the grassland produced with higher rainfall contributed to the photosynthetically active radiation that remained unchanged as higher biomass provides a dense canopy cover. This also means that a dense grass canopy was maintained which helps avoid excessive runoff and erosion under extreme climatic events. Since the grassland generally receives fluctuating rainfall and temperatures, it may be resilient to the applied 50% rainfall reduction and require longer periods of nutrient addition for any significant changes to occur. For future research, it is recommended that the consideration or use of specific grass species in a controlled environment to understand which species make use of the available nutrients and moisture the most efficiently and which species contribute to the quick recovery of a grassland following a drought. The results can identify the key species that help the natural grassland to withstand drought.

CHAPTER 7: GENERAL DISCUSSION AND CONCLUSIONS

7.1. Introduction

With climate change, more intense and frequent episodes of heat stress and water deficiency have become more prevalent, significantly limiting productivity (IPCC, 2013) and affecting grassland ecosystem function globally (Knapp et al., 2015b). There is a lack of research into the responses of grassland systems to drought and to how the grassland plant community will react to the fast-changing environment. Additionally, it is well recognized that the nutrients nitrogen, phosphorus, and potassium can alter the plant community and limit terrestrial productivity (Fay et al., 2015). Drought stress affects the absorption, transport, and partitioning of nutrients, including nitrogen, phosphorus and potassium, and other essential minerals, which can impede plant growth and reproduction (Gessler et al., 2017; Hu and Schmidhalter, 2005). However, little is currently known about the effects of nutrient addition combined with drought stress as well as prescribed fire combined with drought in grassland systems. This study attempted to address these challenges, given that forecasting the biotic impacts of climate change is essential to developing mitigation measures. This chapter summarizes the main conclusions from the experimental chapters, highlighting key findings that advance our understanding of fire, nutrient addition, drought, and grass-grass interactions in a mesic South African grassland. It discusses findings, logical implications, and recommendations for further research.

7.2. Summary of research findings

One of the main aims of this study was to investigate the response of a mesic grassland plant community to a short-term drought event and prescribed burning. The thesis is composed of different experimental approaches for the various chapters which include a 20-week long drought in pot trials (Chapter 4), a short-term drought on grassland managed with prescribed fire (Chapter 3), a 3-year drought manipulation experiment (Chapter 5) plus a nutrient addition trial (Chapter 6). The key findings showed that a short-term drought did not result in significant changes to the grassland plant community under different burning frequencies. Instead, there were significant changes caused by fire to the species composition in terms of diversity and richness. The different burning frequencies resulted in clear and distinct patterns in the grassland species composition. For instance, annual burning resulted in significantly higher species diversity and richness, promoting the abundance of palatable grasses such as *Tristachya leucothrix* and *Themeda triandra*. This suggests that fire is an important management tool for the survival and maintenance of a highly diverse and species-rich grassland community, even under fluctuating moisture levels. By promoting the dominance of palatable grass species, frequent fire can create good veld condition suitable for grazing livestock. Long-term fire exclusion resulted in a dominance of woody species such as *Vachellia nilotica*, forbs and tall, low palatability grasses including *Cymbopogon validus* and *Eragrostis curvula,* while under triennial burning there was a co-dominance of grasses and forbs (Chapter 3). These findings align with other research from South African mesic grasslands indicating that the exclusion of fire leads to changes in the forb composition (Fynn et al., 2005; Fynn et al., 2004; Uys et al., 2004) and a decline in the abundance of palatable grass species (Everson et al., 1985b; Fynn et al., 2011). As a result, using fire to control the distribution and local concentration of woody species in grasslands can be a financially advantageous management strategy.

Annual burning had a high grass abundance, while the triennially burned site was dominated by forbs. This means frequent fire is important for the survival and maintenance of a grassland community. Given that grasses are dominant in some of the driest habitats, they are often quite resistant to drought. Since fire promotes the dominance of grasses, regular burning can contribute to the shielding of the grassland from drought impacts. Furthermore, they exhibit some competitive traits, such as quick regrowth and rapid nitrogen uptake (Stampfli et al., 2018). The grass community adapts to changing conditions by maximizing water consumption through individual strategies and competitive interactions among the current species (Kardol et al., 2010). Additionally, Walter et al. (2011) highlighted that grasses can be more resistant to recurring droughts, depending on the severity and length of drought. C_4 plants have efficient delivery of CO_2 which improves photosynthetic efficiency at high temperatures (Osmond et al., 1982) that are associated with climate change. Thus, C_4 photosynthesis has a significant impact on leaf water-use efficiency, and net CO_2absorption per unit of water loss (Osmond et al., 1982). Warm-season C4 plants can withstand dry conditions by controlling transpiration through stomatal closure during photoperiods, which reduces water loss (Kazemi et al., 2023). All the grasses found in this study were mostly perennial and C4 species, which could have contributed to the grassland being unaffected by drought.

Most forbs can withstand drought conditions by having deeper rooting profiles, while grasses are generally better suited to withstand drought, particularly if there is a higher percentage of C4 photosynthetic species (Nippert and Knapp, 2007) and as previously mentioned, the annually burned area had a higher grass abundance. Even though the fire treatments resulted in different forb-grass distributions, ultimately all sites had a combination of forbs and grasses in varying abundance, thus, both have the aforementioned physical characteristics to withstand drought. This may help to explain why the grassland in this study did not respond to the drought. The short-term duration and intensity of the drought may also contribute to the fact that drought did not have a significant impact on the grassland species composition. It is also crucial to note that the data collection occurred in 2020 and the 2014–2015 drought was followed by a number of years of normal to high rainfall. This is indicative of one of two things, either the grassland was not affected by the drought or recovered quickly during the subsequent normal rainfall years. The time between the drought and the assessment allowed for any short-term fluctuations in species composition to stabilise, allowing for a realistic assessment of the impact of drought on species composition. The long-term fire treatments created three distinct grassland communities (the annually burned area dominated by grasses, triennial had both grasses and forbs while no burn site was bush encroached) and none of these communities were negatively affected by the drought (Chapter 3).

Results similar to Chapter 3 were observed in Chapter 5 whereby the 3-year drought application had no effect on the majority of aboveground variables, including species composition, diversity, richness, and biomass production; instead, annual weather patterns had a major impact. Similarly, the main conclusions in chapter 3 demonstrated that, under varying fire frequency conditions, drought did not alter the grassland plant community. Chapter 5 focused on how the grass sward composition, biomass production as well as root biomass and length were influenced by drought (50% rainfall reduction). The 3-year drought application did not have any significant effects on the species diversity and richness of the plant community. Instead, the species composition, which was assessed using aerial cover, was affected by annual weather patterns where both the species diversity and richness were significantly higher in the growing season of 2021/2022. Given that biomass fluctuated from year to year, this can be linked to changes in aerial cover. The fact that the grassland remained unaffected by drought implies its’ resilience to the impacts of climate change, as the plant community remained with fairly high species diversity, which is associated with resilience to environmental changes and is an indication of the plant community’s ability to self-regulate (Irob et al., 2023).

The grassland’s lack of response to drought can also be attributed to greater rainfall and reduced evaporation due to the low average monthly temperatures in the 2021/2022 growing season. The belowground biomass production was significantly lowered by above-average rainfall while it was greater under drought and ambient rainfall. This could be attributed to a shift towards greater aboveground biomass production with increased rainfall, as drought can generally cause a shift in biomass allocation from stems and leaves to roots (Eziz et al., 2017).

However, different climate adaption techniques across biogeographical regions may affect the allocation of biomass in response to drought. For example, even though roots require a larger initial investment, under moderately dry conditions, deeper roots are required to ensure access to water (Guswa, 2010). Interannual climate variability increased the aboveground biomass production in 2020/2021, even though 2020/2021 growing season did not have the highest rainfall, this is when the total annual rainfall showed a significant increase from 2019/2020 (Chapter 5). During this growing season, the biomass production of the mesic grassland demonstrated resilience by rebounding after high moisture levels. This suggests that plants reallocate resources and reduce biomass production during drier conditions, promoting the grassland's drought resistance. The grassland community sustained healthy levels of species richness and biodiversity during the trial. According to Spellerberg and Fedor (2003), the Shannon-Weiner diversity index value typically falls between 1.5 and 3.5, with rare exceptions where it surpasses 4.5. Throughout the experiment, the species diversity in this study was continuously well over 4, indicating a species-rich or healthy species diversity. The relatively high species diversity in the grassland is advantageous because it increases the likelihood that species which are well-adapted to drought would exist in multispecies communities.

Chapter 4 assessed the influence of drought on grass-on-grass interactions in pot trials. The results presented similar findings to those of Chapter 5 whereby there was minimal impact of applied drought on the species composition. However, the competitive effect had a stronger significant impact on grass species interactions. In an attempt to explore the competitive effects of broad vs narrow-leaved grasses as well as palatable vs unpalatable grasses on plant traits under drought conditions, it was discovered that competition, irrespective of the kind of species exerting the competition, decreased the biomass production of plants. Additionally, the level at which biomass production and other morphological traits were affected, was also dependent on the type of competition present. For instance, broad-leaved species had a stronger competitive effect compared to narrow-leaved species as they reduced the number of tillers and tuft size, hence ultimately reducing the biomass of the narrow-leaved species. Fine grass leaves intercept more water than broad leaves, from the small rain events typical in semiarid grasslands (Lauenroth et al., 2014) meaning that smaller-leaved species have a better chance of survival against long-term drought. In this study, both the broad and narrow-leaved species were unaffected by drought. The palatable species had a dominant competitive effect over unpalatable species without exposure to defoliation. This was directly reflected by the results from Chapter 5 where palatable species, such as *T. leucothrix* and *T. triandra*, were dominant throughout the field trial. In the absence of defoliation such as grazing, the mesic grassland can be resilient enough to maintain a competitive palatable plant community despite drought.

Chapter 4 is a more in-depth look at the responses observed in Chapter 5 to see if shifts in competitive interactions drive the changes in species composition reflected in the field. Again, the drought did not have any significant impact on the final biomass of plants in the 20-week drought application in the greenhouse (Chapter 4) as opposed to the reduction in biomass in the three-year field drought observed in Chapters 5 and 6 which assessed the influence of nutrient addition and 3-year drought on species composition and biomass production. The drought application in the pot trials may have been less severe and shorter than in the drought manipulation in the field trial. To emphasize, all grasses, whether palatable, unpalatable, narrow-leaved, or broad-leaved, saw a significant decrease in biomass output when a rival species was present; however, the competitor species controlled the degree of biomass reduction that each plant experienced. Despite their relatively smaller stature, palatable species like *T. leucothrix* exhibited a substantial competitive effect, lowering the height, tiller numbers, tuft circumference, and biomass of the focus plant. This suggests that prospective competitive ability may not always be directly correlated with plant size, but palatability as well.

Chapter 6 investigated the influence of annual weather patterns and nutrient addition (nitrogen, phosphorus and potassium) on species composition and biomass production under different moisture levels. Interannual climate variability significantly increased species richness and diversity by 2021/2022. Additionally, the results showed that in the first year, there were no treatment effects but there was a main effect of moisture level fluctuation in the 2nd year and finally a fertilizer effect in the 3rd year of the experiment. Similar to Chapter 5, the unfertilized plots remained unchanged by moisture levels while fertilization promoted significantly greater diversity. *T. leucothrix, T. triandra* and *C. excavatus* remained highly abundant in both unfertilized and fertilized sites but most forb species increased in abundance in the fertilized areas. It took three years for the annual addition of NPK fertilization to cause visible changes to the species composition, however, species composition was unaffected by moisture availability. This might be attributed to one of two factors, the fact that the final growing season of the experiment had a higher-than-average rainfall or the plant community response to the given amount of fertilization required up to three years to show significant changes.

A study that assessed plant community changes caused by experimental fertilization with nitrogen, phosphorus and potassium found that species richness changes were observed after a period of 8 to 10 years across 30 grasslands (Muehleisen et al., 2023). Not only may the grassland need more time to reflect changes caused by nutrient addition, the observed increase in species diversity may also change over time as prolonged nutrient enrichment is said to decrease species richness and diversity (Cleland and Harpole, 2010; Harpole et al., 2016). The possible reasons for the contradiction of this study vs the long-term nutrient enrichment could be the comparatively shorter length of nutrient addition in our study and the fact that moisture was a limiting factor. The addition of nutrients in grasslands typically results in the competitive exclusion of some species, leading to a decreased species biodiversity (Boch et al., 2021; Crawley et al., 2005; Harpole et al., 2016; Jacquemyn et al., 2003). Fertilization increases competition for light, hence it promotes the dominance of taller plants and the shading out of smaller species (Hautier et al., 2009). The ecological implications of the dominance by taller plants is the expectation of an increase in biomass with fertilization (Humbert et al., 2016). In conclusion. for the grassland to effectively use the available nutrients and maximize sward biomass production, species diversity, and richness, there must be adequate moisture resources. Nutrient addition, influenced by moisture availability, also makes many species more abundant; however, this may not be beneficial for the grassland as it may lead to an increase in the population of undesirable species and ultimately cause a diversity reduction over time (Harpole et al., 2016; Muehleisen et al., 2023). Even though the unfertilized plant community was less diversified and prolific than the nutrient-enriched regions, higher moisture levels are required to transform a grassland into an extremely productive area and to hasten the plant community's recovery following an environmental event such as drought.

7.3. Concluding remarks

The similarities in both the field trials and pot trial chapters that showed minimal or no effect of drought could potentially suggest that the length of the drought is important in causing significant changes to the plant community. In the case of the greenhouse experiment (Chapter 4), the drought period of 20 weeks might have been too short to cause significant changes to the biomass production of plants. Likewise, in the long-term fire experiment (Chapter 3), the short-term drought event could have been comparatively short to cause significant changes in the grassland species composition by 2019-2022, or the vegetation had recovered by this time. However, even in the rainfall manipulation trial (Chapters 5 and 6), the three-year drought period had no significant effects on the species composition of the grassland. The fact that the grassland often experiences fluctuation in both average annual rainfall and temperature may be an indication that it may take a very long drought at extreme intensities to cause a significant impact on the species composition of the mesic grassland. The aboveground biomass that was reduced by drought over three years quickly recovered once the rainfall increased. This may suggest that our grasslands can remain resilient in the event of drought and be able to recover once moisture resources are reintroduced. Drought only caused a significant decrease in the biomass production of plants but caused no significant changes in species diversity or richness. However, both nutrient addition and interannual climate variability caused significant changes to species composition and biomass production but the effective use of the nutrients was dependent on the amount of moisture available. The observed changes to the fertilized plant community suggest that in the presence of nutrient enrichment, moisture availability is the limiting resource. This study has attempted to address the lack of knowledge on the effects of nutrient addition combined with drought stress as well as fire and drought in mesic grassland systems. The innovative approach of examining the effects of drought in field trials as well as potted plant trials and rainfall manipulation trials has contributed to an understanding of the impact of drought on the sustainable management of these grasslands.

7.4. Management and research recommendations for South African mesic grasslands

In general, the drought did not have a significant impact on the grassland. However, nutrient addition increased diversity and richness in this study, while extended fertilization had limiting and reductive effects on species diversity in other studies (Fay et al., 2015; Harpole et al., 2016; Muehleisen et al., 2023). It should be considered that this increase was caused by forb colonisation, so the high diversity was not necessarily indicative of good veld condition. The recommendation would be that nutrient enrichment, especially over long periods should be avoided in natural rangelands. The changes in the plant community were only visible after three years of nutrient addition, indicating that fertilization needs time to cause community changes. Nutrient addition is also moisture-dependant as the fertilized grassland only showed changes after increased rainfall, therefore rainfall is a limiting factor. Finally, frequent fire, specifically annual burning, is beneficial to the maintenance of high species biodiversity and richness with an increased abundance of palatable grasses. Fire should be a consistent management regime for mesic grassland and a cost-effective means of eliminating woody species encroachment. Short and intermediate-term drought do not cause significant, permanent changes to the grassland, and this can be caused by the regular fluctuation of minimum and maximum temperatures and rainfall in the mesic grassland. For distinct plant community changes, the drought needs to be quite severe or extended. These mesic grasslands showed resilience to the impacts of climatic change.

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APPENDIX 5A

GRASS SPECIES LIST

Brachiaria serrata	*B. serr*
Cymbopogon excavatus	*C. exca*
Eragrostis capensis	*E. cape*
Eragrostis curvula	*E. curv*
Heteropogon contortus	*H. cont*
Setaria nigrirostris	*S. nigr*
Setaria sphacelata	*S. spha*
Themeda triandra	*T. tria*
Tristachya leucothrix	*T. leuc*

FORB SPECIES LIST

Carissa macrocarpa	*C. macr*
Cephalaria pungens	*C. pung*
Chaetacanthus burchellii	*C. burc*
Conyza floribunda	*C. flori*
Crabbea hirsuta	*C. hirs*
Eriosema cordatum	*E. cord*
Helichrysum miconiifolium	*H. mico*
Hibiscus aethiopicus	*H. aeth*
Hyparrhenia dregeana	*H. dreg*
Indigofera hilaris	*I. hila*
Lichtensteinia kolbeana	*L. kolb*
Rhynchosia cooperi	*R. coop*
Rhynchosia minima	*R. mini*
Scabiosa columbaria	*S. colu*
Senecio bupleuroides	*S. bupl*
Senecio coronatus	*S. coro*
Senecio deltoides	*S. delt*
Senecio retrorsus	*S. retro*
Tephrosia polystachya	*T. poly*
Thunbergia atriplicifolia	*T. atri*
Vigna vexillata	*V. vexi*

APPENDIX 6A

GRASS SPECIES LIST

Aristida junciformis	*A. junc*
Brachiaria serrata	*B. serr*
Cymbopogon excavatus	*C. exca*
Diheteropogon amplectens	*D. ampl*
Eragrostis capensis	*E. cape*
Eragrostis racemosa	*E. race*
Heteropogon contortus	*H. cont*
Panicum aequinerve	*P. aequ*
Setaria nigrirostris	*S. nigr*
Setaria sphacelata	*S. spha*
Themeda triandra	*T. tria*
Tristachya leucothrix	*T. leuc*

FORB SPECIES LIST

Acalypha angustata	*A.angu*
Aster bakerianus	*A. bake*
Berkheya setifera	*B.seti*
Bulbostylis speies	*B. spec*
Cephalaria pungens	*C. pung*
Chaetacanthus burchellii	*C. burc*
Crabbea hirsuta	*C. hirs*
Eriosema cordatum	*E. cord*
Graderia scabra	*G. scab*
Helichrysum miconiifolium	*H. mico*
Helichrysum nudifolium	*H.nudi*
Hibiscus aethiopicus	*H. aeth*
Hyparrhenia dregeana	*H. dreg*
Indigofera hilaris	*I. hila*
Pentanisia angustifolia	*P. angu*
Rhynchosia totta	*R. tott*
Ruellia baurii	*R. baur*
Senecio bupleuroides	*S. bupl*
Senecio deltoides	*S. delt*
Senecio retrorsus	*S. retro*
Spermacoce natalensis	*S. nata*
Thunbergia atriplicifolia	*T. atri*
Tragia meyeriana	*T.meye*
Vigna vexillata	*V.vexi*

TABLE 5.2. SPECIES

Grasses

Brachiaria serrata	*B. serrata*
Conyza floribunda	*C. flori*
Cymbopogon excavatus	*C. excavates*
Diheteropogon amplectens	*D. amplectens*
Eragrostis capensis	*E. capensis*
Eragrostis curvula	*E. curvula*
Eragrostis racemosa	*E. racemosa*
Heteropogon contortus	*H. contortus*
Panicum aequinerve	*P. aequinerve*
Setaria nigrirostris	*S. nigrirostris*
Setaria sphacelata	*S. sphacelata*
Themeda triandra	*T. triandra*
Tristachya leucothrix	*T. leucothrix*

FORBS

Acalypha angustata	*A.angustata*
Agathisanthemum chlorophyllum	*A. chlorophyllum*
Aster bakerianus	*A. bakerianus*
Berkheya setifera	*B. setifera*
Berkheya umbellata	*B. umbellata*
Bulbostylis species	*B. species*
Carissa macrocarpa	*C. macrocarpa*
Cephalaria pungens	*C. pungens*
Chaetacanthus burchellii	*C. burchellii*
Commelina africana	*C. africana*
Conyza floribunda	*C. floribunda*
Crabbea hirsuta	*C. hirsute*

Crotalaria dura	*C. dura*
Eriosema cordatum	*E. cordatum*
Graderia scabra	*G. scabra*
Helichrysum miconiifolium	*H. miconiifolium*
Hibiscus aethiopicus	*H. aethiopicus*
Hyparrhenia dregeana	*H. dregeana*
Hypoxis filiformis	*H. filiformis*
Indigofera hilaris	*I. hilaris*
Lichtensteinia kolbeana	*L. kolbeana*
Rhynchosia cooperi	*R. cooperi*
Rhynchosia minima	*R. minima*
Ruellia baurii	*R. baurii*
Scabiosa columbaria	*S. columbaria*
Senecio bupleuroides	*S. bupleuroides*
Senecio coronatus	*S. coronatus*
Senecio deltoides	*S. deltoides*
Senecio retrorsus	*S. retrorsus*
Spermacoce natalensis	*S. natalensis*
Tephrosia polystachya	*T. polystachya*
Thunbergia atriplicifolia	*T. atriplicifolia*
Tragia meyeriana	*T. meyeriana*
Vigna vexillata	*V. vexillata*
Zonia capensis	*Z. capensis*

Appendix A: Species abundance across moisture treatments (Drought, Ambient and Rainfall) in each year.

Year	**Species**	**Drought**	**Ambient**	**Rainfall**
2021/2022	*Tristachya leucothrix*	35	50	28
2021/2022	*Thunbergia atriplicifolia*	20	20	0
2021/2022	*Themeda triandra*	15	22	40
2021/2022	*Setaria nigrirostris*	8	5	5
2021/2022	*Senecio coronatus*	4	3	5
2021/2022	*Senecio bupleuroides*	4	4	3
2021/2022	*Scabiosa columbaria*	5	4	3
2021/2022	*Ruellia baurii*	0	0	2
2021/2022	*Rhynchosia cooperi*	0	0	5
2021/2022	*Indigofera hilaris*	0	0	5
2021/2022	*Hibiscus aethiopicus*	5	0	2
2021/2022	*Heteropogon contortus*	8	0	45
2021/2022	*Helichrysum minoniifolium*	0	6	5
2021/2022	*Graderia scabra*	5	0	0
2021/2022	*Eriosema cordatum*	6	6	0
2021/2022	*Eragrostis racemosa*	8	0	0
2021/2022	*Eragrostis capensis*	12	30	7
2021/2022	*Diheteropogon amplectens*	0	0	4
2021/2022	*Crabbea hirsute*	6	0	6
2021/2022	*Cephalaria pungens*	5	20	30
2021/2022	*Crotalaria dura*	25	0	0
2021/2022	*Brachiaria serrata*	0	32	0
2021/2022	*Berkheya setifera*	0	0	20
2021/2022	*Afroaster hispida (Aster bakerianus)*	3	5	3
2021/2022	*Cymbopogon excavatus*	0	6	8
2021/2022	*Dyschoriste burchelli (Chaetacanthus burchellii)*	5	0	3

Year	**Species**	**Drought**	**Ambient**	**Rainfall**
2020/2021	*Tristachya leucothrix*	80	70	65
2020/2021	*Thunbergia atriplicifolia*	12	15	22
2020/2021	*Tragia meyeriana*	0	0	10
2020/2021	*Themeda triandra*	30	25	20
2020/2021	*Tephrosia polystachya*	25	0	0
2020/2021	*Spermacoce natalensis*	0	0	15
2020/2021	*Setaria nigrirostris*	0	0	0
2020/2021	*Senecio coronatus*	15	20	10
2020/2021	*Senecio bupleuroides*	0	0	0
2020/2021	*Scabiosa columbaria*	10	3	0
2020/2021	*Ruellia baurii*	0	10	5
2020/2021	*Rhynchosia minima*	0	0	7
2020/2021	*Panicum aequinerve*	0	0	25
2020/2021	*Indigofera hilaris*	25	0	25
2020/2021	*Hibiscus aethiopicus*	0	8	5
2020/2021	*Heteropogon contortus*	0	10	0
2020/2021	*Helichrysum nudifolium var pilosellum (helichrysum pilosellum)*	0	6	4
2020/2021	*Helichrysum minoniifolium*	15	0	7
2020/2021	*Graderia scabra*	3	0	0
2020/2021	*Eriosema cordatum*	15	50	0
2020/2021	*Eragrostis capensis*	0	0	0
2020/2021	*Diheteropogon amplectens*	0	0	0
2020/2021	*Crabbea hirsute*	12	0	0
2020/2021	*Cephalaria pungens*	5	50	15
2020/2021	*Brachiaria serrata*	0	50	8
2020/2021	*Berkheya setifera*	0	0	15
2020/2021	*Afroaster hispida (Aster bakerianus)*	12	5	10
2020/2021	*Cymbopogon excavatus*	10	12	25
2020/2021	*Dyschoriste burchelli (Chaetacanthus burchellii)*	8	3	10
2020/2021	*Acalypha angustata*	0	5	0
2020/2021	*Zonia capensis*	0	5	0
2020/2021	*Lichtensteinia kolbeana*	10	0	0

Year	**Species**	**Drought**	**Ambient**	**Rainfall**
2019/2020	*Tristachya leucothrix*	55	40	38
2019/2020	*Thunbergia atriplicifolia*	10	5	26
2019/2020	*Themeda triandra*	25	20	15
2019/2020	*Spermacoce natalensis*	0	0	2
2019/2020	*Setaria nigrirostris*	4	0	0
2019/2020	*Senecio coronatus*	15	6	0
2019/2020	*Senecio bupleuroides*	8	4	4
2019/2020	*Scabiosa columbaria*	1	5	0
2019/2020	*Ruellia baurii*	0	0	5
2019/2020	*Rhynchosia minima*	0	0	5
2019/2020	*Rhynchosia cooperi*	3	0	0
2019/2020	*Indigofera hilaris*	8	3	10
2019/2020	*Hibiscus aethiopicus*	4	18	4
2019/2020	*Heteropogon contortus*	5	0	0
2019/2020	*Helichrysum nudifolium var pilosellum*	0	10	2
2019/2020	*Helichrysum minoniifolium*	0	3	3
2019/2020	*Hyparrhenia hirta*	3	0	20
2019/2020	*Graderia scabra*	0	0	3
2019/2020	*Eriosema cordatum*	4	10	8
2019/2020	*Diheteropogon amplectens*	10	5	3
2019/2020	*Crabbea hirsute*	15	0	0
2019/2020	*Cephalaria pungens*	22	20	25
2019/2020	*Crotalaria dura*	50	0	0
2019/2020	*Brachiaria serrata*	0	50	8
2019/2020	*Berkheya setifera*	0	0	3
2019/2020	*Afroaster hispida (Aster bakerianus)*	1	1	5
2019/2020	*Commelina africana*	3	0	1
2019/2020	*Cymbopogon excavatus*	10	45	15
2019/2020	*Dyschoriste burchelli (Chaetacanthus burchellii)*	8	10	10
2019/2020	*Acalypha angustata*	0	3	0
2019/2020	*Vigna vexillata*	2	6	0