

**Understanding the Effects of Changing Climate and Land Use on  
Woody Plant Encroachment in South African Grasslands and Savannas**

**by**

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## PREFACE

The research contained in this thesis was completed by the candidate while based in the School of Life Sciences, of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa, under the supervision of Professor David Ward. The research was financially supported by the National Research Foundation.

This thesis, submitted for the degree of Doctor of Philosophy (PhD), is the product of the candidate's original work in substance, with due acknowledgment and credit given and cited in appropriate sections of the text and not having been submitted in whole or in part to be examined for any degree, nor is it being submitted in candidate for any other degree.

The thesis was written as papers and follows the format of the journal where each paper has been/will be submitted for publication, except the Introduction (Chapter 1) and Conclusions (Chapter 6). As a result the thesis does not follow a consistent format and tables and figures are at the end of each paper as they were/will be submitted to the respective journal.



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## DECLARATION 1: PLAGIARISM

I, Tiffany Prileeni Pillay, 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;
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- (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;
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## DECLARATION 2: PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis. At the time of submission, this thesis contains four papers submitted to peer-reviewed journals.

*Author contributions:*

**Publication 1:** Pillay TP and Ward D. Drought suppresses grass productivity and warming promotes tree performance. Formatted for *Global Change Biology*. TP conceived the study presented, collected and analysed the data and the paper. DW contributed to experimental design, data analysis and manuscript preparation.

**Publication 2:** Pillay TP and Ward D. Interactive effects of fire, fertilizer and grazing on an encroaching woody species: the importance of grass competition in limiting sapling growth and performance. Submitted to *Plant Ecology*. TP conceived the study presented, collected and analysed the data and the paper. DW contributed to experimental design, data analysis and manuscript preparation.

**Publication 3:** Pillay TP, Mureva A, Ward D and Cramer MD. Soil, litter and plant dynamics across a precipitation gradient: the effects of fertilization and woody plant encroachment. Formatted for *Ecosystems*. TP conceived the study presented, collected and analysed the data and the paper. DW, AM and MC contributed to experimental design, data analysis and manuscript preparation.

**Publication 4:** Pillay TP, Ncgobo S and Ward D. Fertilizer and fire influences biological nitrogen fixation and soil respiration in *Acacia sieberiana*. Submitted to *Plant and Soil*. TP conceived the study presented, collected and analysed the data and the paper. SN assisted with the lab component of the analysis. DW contributed substantially to experimental design, data analysis and manuscript preparation.

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## ABSTRACT

The increase in dominance of woody species, observed in many grasslands and savannas worldwide, highlights the sensitivity of woody herbaceous biomass ratios to changed environmental conditions. While the major factors driving the tree–grass continuum have been identified as precipitation, nutrients, increased CO<sub>2</sub> concentrations, fire and herbivory, the interaction of these driving forces determines the tree:grass ratio, and ultimately the occurrence/ absence of woody plant encroachment. Furthermore, with forecasted alterations to the global climate (such as an increase in the frequency and magnitude of climate extremes), and increased nutrient availability (either through extensive use of fertilizer or increased nitrogen deposition), more knowledge is required on the factors which promote woody plant performance.

We conducted a series of greenhouse experiments to manipulate these factors, and a two-year field trial to disentangle the effects of varying climate and land use on woody plant encroachment. These studies are summarized below:

(1) We investigated the effects of manipulated water availability and passive warming on the productivity of the commonly occurring grass species *Eragrostis curvula*, and the emergence and performance of seedlings of the woody encroacher, *Acacia sieberiana*. We simulated a typical savanna microhabitat by planting tree species within a grass matrix. Watering treatments altered to represent drought (minus 40% of the average), normal (same amount as the long-term average of the area), and excess (addition of 40% of the long term average) precipitation. Passive warming was achieved using open-top chambers. Tree seedling emergence was monitored in the first two weeks. Thereafter, weekly tree growth rate

measurements were recorded. The final above-ground grass biomass, tree root:shoot ratio and specific leaf area were quantified. We found that water availability was the main limiting factor for grass productivity, with low grass biomass attained under the drought treatments. Warming had no significant effect on grass productivity, but had a large positive effect on tree growth and performance when coupled with excess or normal water treatments. Tree seedling root:shoot ratios were highest in the drought treatment, possibly as an adaptation to low water supply. Higher specific leaf area was recorded with warming, further elucidating the positive effect of increased temperature for tree growth.

(2) Using a completely randomized experimental design in the greenhouse, we investigated the effects of fertilizer, fire and grazing on the survival, growth and biomass of *Acacia sieberiana*. We simulated a typical savanna microhabitat by planting a common savanna grass species, *Eragrostis curvula*, together with saplings of *A. sieberiana*. Treatments of fertilizer, simulated grazing and fire were applied. Weekly measurements of relative growth rate ( $RGR_{\text{height}}$  and  $RGR_{\text{diameter}}$ ) were recorded. At the end of 24 weeks, survival, total above- and below-ground biomass, and key functional traits (specific leaf area and average thorn length) were recorded to assess differences in tree performance. We found that fertilization was beneficial to grasses only, increasing their total biomass. Consequently, we observed increased tree sapling mortality with high grass biomass. Fire increased the saplings' investment in shoot growth and stem diameter. However, we found no evidence of differential post-fire allocation to roots or shoots when grass competition was absent. Grass biomass was also found to decrease the specific leaf area of tree saplings.

(3) We conducted a two-year field fertilizer trial, using pairs of open grassland and encroached plots at four sites across a precipitation gradient, ranging from 300–1500 mm

mean annual precipitation (MAP). We assessed the effects of encroachment, N, P and N+P addition on soil N stocks and soil P, litter % N and C:N ratio (index of decomposition), plant productivity and species richness. We found that soil N stocks were on average 15 times higher in the higher precipitation sites regardless of nutrient addition or encroachment. Larger variation in soil P was noted at the high precipitation sites, with some evidence of P-limitation in the encroached area with the highest precipitation. We found significant positive effects of encroachment on litter % N (quality), C:N ratios and forb cover across the precipitation gradient. Fertilization increased grass biomass and reduced species richness in the high-precipitation sites only. The purportedly beneficial effects of encroachment on litter and plant species richness was more pronounced at the high-precipitation sites. Overall, increased nutrient availability was found to be of importance in high precipitation sites only, possibly due to water scarcity at the low-precipitation sites.

(4) To elucidate the effects of fertilizer addition, simulated fire and grazing on the rate of nitrogen (N) fixation and soil respiration we conducted a greenhouse experiment using a woody encroaching species, *Acacia sieberiana*, and the commonly occurring grass species *Eragrostis curvula*. Treatments of fertilizer, simulated grazing and fire were applied.

Thereafter the Acetylene Reduction Assay was used to determine the rate of biological Nitrogen fixation (BNF). We found a significant decrease in BNF with fertilizer addition, and increases in BNF after fire application. Soil respiration increased with fertilizer addition and decreased after fire application. Grazing had no independent effect on any of the response variables. However, decreased grass biomass resulted in increased BNF across all treatments. Furthermore we found that larger saplings achieved a higher rate of BNF, with a positive correlation between the rate of BNF and both the number and weight of root nodules. The implications of these studies are:

(1) Future climate-change predictions of increased drought may constrain grass biomass, thereby promoting woody plant success. Predicted warming is likely to further enhance woody plant performance.

(2) Increased nutrient availability, whether as fertilizer addition or increased nitrogen deposition, may promote the competitive ability of the grass component, thereby limiting woody plant invasion. This is based on the assumption that grass productivity is not limited by frequent fire or intensive grazing.

(3) Encroachment of leguminous woody plants is purportedly beneficial in higher precipitation areas, due to increased soil nitrogen, higher grass foliar quality under trees, greater forb cover and increased decomposition. Hence, encroachment control should be prioritized in low precipitation sites, particularly in rangelands where woody plants are undesirable.

(4) Low intensity or infrequent fire is ineffective at controlling woody plant proliferation, particularly if the invader is able to resprout and is capable of biological nitrogen fixation.



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## CHAPTER 1: Introduction

### 1.1 Rationale for the research

Woody plant encroachment (also known as shrub or bush encroachment) is the increasing abundance of woody vegetation, which over time leads to closed-canopy environments (Archer *et al.* 2017; Bond *et al.* 2017). In southern Africa, tree and shrub densities have increased by 30-50 % (Hoffman & Ashwell 2001; Hudak & Wessman 2001; Bond 2008; Ward *et al.* 2014). Increased density of woody plants reduces the grazing capacity of grasslands and savanna rangelands, resulting in substantial economic and biodiversity losses (Archer *et al.* 1995; Wiegand *et al.* 2006). Grasslands and savannas are important for ecotourism, which serves as a major source of income for several African countries (Gray & Bond 2013). Woody plant encroachment negatively affects tourist perceptions through reduced game-viewing opportunities (Gray & Bond 2013). However, a meta-analysis by Eldridge *et al.* (2011) has highlighted that the commonly mentioned link between woody plant encroachment and degradation is not universal, with one of the benefits of encroachment including increased resource stocks (e.g. below-ground carbon (C) and nitrogen (N) or above-ground net primary productivity). A major issue regarding the effects of woody plant encroachment is that current poor management practices such as heavy stocking can lead to landscape degradation many years after their implementation. Therefore, an in-depth understanding of the factors promoting woody plant encroachment, as well as the relative effects of encroachment under different land-uses is needed. This thesis aimed to disentangle the effects of climate (precipitation and warming) and land use (nutrients, fire and grazing) on woody plant encroachment across a precipitation gradient in South Africa.

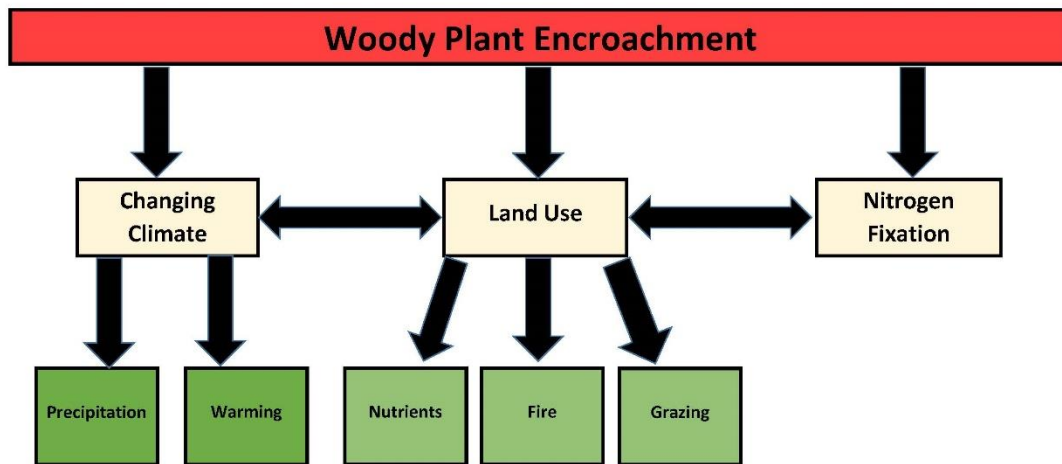


Fig. 1.1 The effects of changing climate (precipitation and warming) and land use (nutrients, fire and grazing) on woody plant encroachment.

Savannas are dynamic on both temporal and spatial scales, and vary with changes in climate (primarily precipitation), soil nutrient content, fire regime, herbivory and physical disturbance (Wiegand *et al.* 2005; Calabrese *et al.* 2010; Bond *et al.* 2017). These ecosystems are driven by resource- and disturbance-mediated patterns, with competitive interactions playing a vital role (Bond 2008; Riginos & Grace 2008; Pillay & Ward 2014). Although coexistence of species may occur through resource partitioning, one life form may often dominate another, as is the case with woody plant encroachment. The availability of resources (water and nutrients) and disturbances (fire and large mammalian herbivory) have been identified as interactive mechanisms that regulate savanna structure (Higgins *et al.* 2000; Jeltsch *et al.* 2000; Wiegand *et al.* 2006; Meyer *et al.* 2008; Accatino *et al.* 2010, 2017; Archer *et al.* 2017; Devine *et al.* 2017). Primary areas of research in savanna ecology are the mechanisms of coexistence of two contrasting life forms (grass and trees), and the factors that influence the relative abundance of each life form (Accatino *et al.* 2010).

Further alterations to the grass:tree ratio exist due to the effects of climate change, increased carbon dioxide concentrations, altered precipitation patterns and temperature changes (Hibbard *et al.* 2001; Bond & Midgley 2012; Carlyle *et al.* 2014). Anthropogenic activities, mainly the combustion of fossil fuels for energy, transportation and industrial activities, have increased global emissions of carbon dioxide, methane and nitrogen dioxide (Wenig *et al.* 2003). The Intergovernmental Panel on Climate Change (IPCC 2014) has predicted a reduction in precipitation over the south-western parts of South Africa, and an increased warming trend by the end of the 21st century (IPCC 2014). Altered precipitation, climate warming, increased nitrogen deposition and changes in land use all contribute the uncertainties of vegetation dynamics in ecosystems where woody plant encroachment has occurred, and also where it may potentially occur.

## **1.2 Woody plant encroachment and future climate change**

### ***Precipitation and warming***

Theoretical models of tree–grass interactions are well explained in the literature (Higgins *et al.* 2000; Jeltsch *et al.* 2000; Wiegand *et al.* 2006; Accatino *et al.* 2010, 2017; Archer *et al.* 2017; Devine *et al.* 2017). However, empirical studies investigating future climate-change scenarios in southern African savannas are lacking (Bond *et al.* 2010; Kgope *et al.* 2010; Leakey *et al.* 2012; Parr *et al.* 2014). Indeed, savanna tree–grass interactions may be particularly sensitive to climate change, and of great importance considering the vast expansion of woody plant density in grasslands and savannas globally (Volder *et al.* 2013). Determining the relative responses of trees and grasses to changing climates may also help predict shifts in vegetation structure, or plasticity in traits resulting from future climate changes (Alpert & Simms 2002; Violle & Jiang 2009).

Water availability, being the most limiting factor in most ecosystems (Ludwig *et al.* 2004), is likely to have its greatest impact on plant communities in arid environments (Weltzin *et al.* 2003; Kraaij & Ward 2006; Throop *et al.* 2012). While changes in precipitation-event size and distribution may have larger impacts on plant performance and productivity than the effects of elevated CO<sub>2</sub> in grassland and savanna ecosystems, responses are highly variable across experiments (White *et al.* 2012; Carlyle *et al.* 2014). In grasslands and savannas, precipitation events are usually patchy, both on spatial and temporal scales (Wiegand *et al.* 2006). Sparse, and often low, amounts of precipitation are only sufficient to infiltrate the uppermost soil layers, which are frequently only available to the shallow root profiles of grasses (Ward *et al.* 2013). However, when grasses are heavily grazed, the competitive effect of grasses is greatly reduced, and soil moisture usually intercepted by the grass layer, is now able to seep down to the subsoil layers, which is then used by trees (Walter 1971). This may allow for the proliferation of woody plant biomass (Knoop & Walker 1985). However, rooting-niche separation cannot be an exclusive explanation for the initiation of woody plant encroachment because young trees use the same subsurface soil layer as grasses in the sensitive early stages of growth (Ward *et al.* 2013). For example, Kraaij & Ward (2006) found that germination of the heavily encroaching species *Acacia mellifera* is much more sensitive to precipitation supplementation than to grazing.

A meta-analysis of African savanna field sites showed a “broken stick” relationship between woody plant cover and mean annual precipitation (MAP) (Sankaran *et al.* 2005). Below 650 mm MAP, herbivory, fire, and soil properties can only reduce woody cover below its maximum, which depends linearly on MAP (Meyer *et al.* 2007). Above this threshold, there is an asymptote where savannas are unstable and disturbances such as fire or herbivory are necessary to prevent woody plant encroachment or canopy closure (Sankaran *et al.* 2005). Water stress during the dry season of savannas has a major impact on the survival of woody

seedlings (Gignoux *et al.* 2009). The length and severity of the dry season is also very important in determining tree establishment (Kraaij & Ward 2006). Although tree seedlings rapidly develop an extensive root system to gain better access to water (Ward & Esler 2011), evenly distributed precipitation is still a requirement to compensate for the loss of water through transpiration (Mordelet *et al.* 1997).

Warmer temperatures increase rates of virtually all chemical and biochemical processes in plants and soils (Saxe *et al.* 2001). Resultant increases in photosynthetic capability and overall metabolic activity, may prove favourable to woody plants. Way and Oren (2010) used data from 63 studies to examine the difference in growth response of plants to a change in temperature. These authors found that elevated temperatures enhanced growth (measured as shoot height, stem diameter and biomass) in deciduous species (Way & Oren 2010). Changes to climates across the globe may have long-term effects on plant communities because temperature plays an important role in regulating ecosystem processes, such as the activity of soil microbes (Rustad *et al.* 2001), nutrient availability through N mineralization (Reich *et al.* 1997) and trace-gas emissions (Cantarel *et al.* 2012), as well as root growth and turnover (Bai *et al.* 2010). The extent to which increased temperatures will affect ecosystems may depend on water availability. However, long-term predictions of precipitation changes are difficult to make because precipitation is expected to increase in some areas and decrease in others (IPCC 2014). Few South African studies have investigated independent and interactive effects of altered precipitation and warming on the performance of tree and grass saplings.

### **1.3 Woody plant growth and performance: Land-use drivers of encroachment**

#### ***Effects of nutrient availability***

Plant productivity and above-ground biomass are thought to increase with higher soil resource availability (e.g. nitrogen, water, phosphorus) (Barbosa *et al.* 2014). However, higher nutrient availability may have direct positive effects and indirect negative effects on savanna tree sapling recruitment, by influencing the competitive and facilitative interactions between trees and grasses (Vadigi & Ward 2012). Availability of nutrients may directly enhance seedling growth (Wakeling *et al.* 2010) or suppress seedling establishment by increasing grass competition (Kraaij & Ward 2006, Pillay & Ward 2014; Barbosa *et al.* 2014). Because N is usually limiting across grasslands, particularly in southern Africa, N fertilization is often associated with increased productivity (Gough *et al.* 2000; Ward *et al.* 2017). Furthermore, increased nutrient availability may have a negative effect on species diversity. Bustamante *et al.* (2012) found that nitrogen and phosphorus addition reduced plant diversity. A recent study by Ward *et al.* (2017) compared the results of a long-term (65 years) experiment in a South African grassland with the world's longest-running ecological experiment, the Park Grass study at Rothamsted, United Kingdom. The results showed an apparently universal negative relationship between species richness and ANPP, as well as the negative impact of nitrogen addition on species richness.

#### ***Effects of fire and grazing***

The occurrence of fire in African savannas is highly variable, ranging from frequent to sporadic, and even rare in some instances (Meyer *et al.* 2005). Although fire is a major disturbance factor in many biomes, from savannas to tropical rain forests, other factors such

as season, intensity and frequency of fire are key determinants of its impacts on vegetation (Bond & Keeley 2005; Archibald *et al.* 2012; Twidwell *et al.* 2016). Smit *et al.* (2016) and Case & Staver (2017) found that further encroachment can only be managed with increased fire frequency. However, many savanna trees are able to persist despite repeated burning (Bond & Midgley 2001; Bond & Keeley 2005; Lawes *et al.* 2012). One of the ways that woody plants persist after a fire is through the ability to resprout from the base of the plant following the death of the above-ground stem (Lawes *et al.* 2012). Given the high flammability of mesic savannas, disturbance due to fire is of particular importance (Bond & Keeley 2005; Archibald & Hempson 2016). Furthermore, fire is known to cause large amounts of soil nutrient loss (particularly N loss) to the atmosphere through the process of volatilization (Aranibar *et al.* 2003). Data from long-term fire experiments in grasslands and savannas indicate that frequent fires over long periods lead to a reduction in total soil N pools (Coetsee *et al.* 2010). Hence, fire may not only influence tree sapling mortality, but also significantly influence soil nutrient cycles.

Grazing as a disturbance has been shown to reduce water and nutrient uptake in grasses, resulting in an increase in available resources for the woody vegetation (Kambatuku *et al.* 2011). Therefore, more resources are obtained by the trees than by grasses, and as a consequence, this leads to the recruitment and encroachment of woody plants in savannas, especially in high-precipitation areas (Van Langevelde *et al.* 2003; Tjelele *et al.* 2015). Furthermore, effects of large herbivores may also interact with fire activity because high levels of grazing typically reduce fire frequency, which can enable woody plants to escape the “fire trap”, and increase in dominance (Werner & Peacor 2003; Archibald & Hempson 2016). An increase in grazing pressure has been noted as a main cause for reduced fire frequency, leading to mass tree recruitment or woody plant encroachment (Van Auken 2000; Gordijn *et al.* 2012).



### *Nitrogen fixation as a driver of encroachment*

In N-limited systems such as savannas, one would assume that the ability of leguminous trees to biologically fix nitrogen (N<sub>2</sub>) would increase their competitive effect over the grass component (Cramer *et al.* 2007; Kambatuku *et al.* 2013). The encroachment of woody legumes may then be attributed in part to their ability to fix nitrogen, especially during the tree seedling stage, when grasses are usually superior competitors (Knoop & Walker 1985, Van Der Waal *et al.* 2009). Species from the plant family Leguminosae are well known for forming a symbiotic relationship with nitrogen-fixing soil bacterium in the genera *Azorhizobium*, *Bradyrhizobium* and *Rhizobium* (Crews 1999). Through this association, the legume receives otherwise inaccessible biologically fixed N<sub>2</sub> from the soil bacteria (Liu *et al.* 2010). Global biological N fixation (BNF) in terrestrial ecosystems has been estimated at 128 Tg.N .yr<sup>-1</sup>, supplying approximately 15% of the N requirement across all biome types (Galloway *et al.* 2004). According to this estimate, about 70% of BNF occurs in regions with warmer climates – with Africa contributing approximately 25.9 Tg.N.yr<sup>-1</sup> (Bustamante *et al.* 2012). Indeed, tropical savanna ecosystems are considered ‘hot spots’ of biological nitrogen fixation (BNF) due to high densities of fixing legumes (Vitousek *et al.* 2013).

Under low soil-nitrogen conditions, the nitrogen-fixing trees have a competitive advantage over other non-N-fixing plants and, given enough precipitation, may germinate *en masse* (Kraaij & Ward 2006). However, the tree-legume species in savanna systems do not always fix N<sub>2</sub> (Aranibar *et al.* 2003, Kambatuku *et al.* 2013). Despite the few studies directly documenting N-fixation by native legumes under natural conditions in African savannas, there is some indirect evidence suggesting N fixation for a few species (Ndoye 1995; Cramer *et al.* 2007). However, little is known about the effects of nutrients, fire and grazing on the rate of BNF, with relevance to the expansion of woody plants into savannas and grasslands.

For example, if N-fixation carries a high carbon cost, one might expect low rates of fixation under low resource-availability scenarios, or under intense intra-specific competition (Kambatuku *et al.* 2013).

#### **1.4 Aims and objectives of the study**

This study sought to understand the mechanisms (climate and land-use changes) that drive woody plant encroachment of grasslands and savannas, and the biogeochemical consequences of woody plant encroachment across a precipitation gradient (300 mm to 1500 mm, MAP) in South Africa.

The specific objectives of the study were to:

- (1) Quantify the independent and interactive effects of altered water availability and warming on the growth and performance of savanna grasses and tree saplings.
- (2) Determine the effects of fertilizer, fire and grazing on the survival, growth and biomass of a dominant encroaching savanna tree species, and the effects of competition from grasses on tree growth and survival.
- (3) Examine the effects of fertilization and woody plant encroachment on soil, litter and plant dynamics across a precipitation gradient.
- (4) Biological nitrogen fixation (BNF) is an important factor differentiating trees from grasses in many southern African environments (Cramer *et al.* 2010; Kambatuku *et al.* 2013). We investigated the effects of BNF in a dominant leguminous encroaching species, *Acacia sieberiana*, on competition with grasses.

Studying interactions between savanna trees and grasses may be difficult for various reasons, such as long lifespans, during which the performance of an individual plant may fluctuate. Perennial savanna trees typically reach reproductive maturity at around 10 years old (Rohner

& Ward 1999). Therefore, while reproductive fitness is the most relevant measure of the performance (Goldberg et al. 1999), it is not always possible to measure a metric such as fecundity in savanna trees. Hence, for the purpose of this thesis, we have used physiological traits as indices of performance (Lavorel et al. 2007; Pérez-Harguindeguy et al. 2013).

## 1.5 Study sites

The study was carried out in South Africa at four study sites along a precipitation gradient (Fig. 1). The spatial variation in precipitation in southern Africa displays an increasing gradient of precipitation from west to east, coinciding with the precipitation gradient of our study sites. Mean annual precipitation ranged from 300 mm to 1500 mm. The four sites were Middelburg (300 mm MAP), Pniel (350 mm MAP), Bergville (700 mm MAP) and KwaMbonambi (1500 mm MAP) (Table 1).

## 1.6 Thesis structure

**Chapter 1** is a brief introduction of the thesis that outlines the key questions, objectives, study sites, and structure of the thesis. In **Chapter 2**, I present the results of a greenhouse experiment in which several factors were controlled to simulate possible climate-change scenarios of altered precipitation and temperature. This study showed that drought has important negative implications for grass productivity, and may reduce the competitive effects of grasses on trees. Coupled with increased performance of trees under warmed conditions, future climate change may further promote tree establishment, especially in areas where precipitation is limiting. **Chapter 3** represents a greenhouse study on the effects of fertilizer, fire and grazing on the survival, growth and biomass of a dominant encroaching savanna tree species, *Acacia sieberiana*, in KwaZulu-Natal. The study showed that

fertilization was beneficial to grasses only, increasing their total biomass, and consequently, resulting in high tree sapling mortality and reduced photosynthetic ability. This study also demonstrated the post-fire resprouting response of *Acacia sieberiana* as a possible mechanism promoting its successful encroachment. **Chapter 4** presents a field study on the effects of fertilizer addition and shrub encroachment on soil, litter and plant dynamics on a precipitation gradient from the dry central Karoo (ca. 300 mm MAP) to the humid eastern seaboard of South Africa (1500 mm MAP)(Fig. 1). Here we found that increased nutrient availability was of importance in high-precipitation sites only, possibly due to water scarcity at the low-precipitation sites. The overall purportedly beneficial effects of encroachment on litter and plant species richness was more pronounced at the high-precipitation sites. **Chapter 5** reports on a greenhouse study investigating the effects of fertilizer addition, simulated fire and grazing on the rate of BNF in a common leguminous woody invader. In this study, we showed that fertilizer addition suppresses BNF because the presence of readily available N negates the costs of fixation. We also found that tree saplings invest heavily in BNF post-fire, possibly to compensate for soil N lost after a fire. Finally, **Chapter 6** presents general conclusions based on the experimental results obtained over the duration of the study. New avenues for future research are also proposed in this final chapter.

All chapters in the thesis, with the exception of the Introduction (**Chapter 1**) and the Conclusions (**Chapter 6**), are formatted according to the style of a particular targeted journal, as indicated on the title page of that chapter. As a result, some repetition and inconsistency of format is unavoidable. The chapters and their targeted journals are as follows:

**Chapter 2:** “Drought suppresses grass productivity while warming promotes tree performance in a savanna”. Formatted for *Global Change Biology*.

**Chapter 3:** “Interactive effects of fire, fertilizer and grazing on an encroaching woody species: the importance of grass competition in limiting sapling growth and performance”.

Submitted to *Plant Ecology*.

**Chapter 4:** “Soil, litter and plant dynamics across a precipitation gradient: the effects of fertilization and woody plant encroachment”. Formatted for *Ecosystems*.

**Chapter 5:** “Fertilizer and fire influences biological nitrogen fixation and soil respiration in *Acacia sieberiana*”. Submitted to *Plant and Soil*.

Table 1.1 Study site description (Mucina & Rutherford 2006)

Site	GPS coordinates	Annual Precipitation (mm)	Temperature (°C) Min      Max	Biome	Soil Characteristics	Major plant species
KwaMbonambi	28° 49' 60.61"S 32° 16' 96.92"E	1500	3.5      35	Maputaland wooded grasslands	Quaternary redistributed sands supporting yellowish redistributed sands of the Berea formation	<i>Sporobolus fimbriatus</i> , <i>Digitaria natalensis</i> (grasses); <i>Diospyros lycioides</i> (shrub); <i>Terminalia sericea</i> (tree)
Bergville	28° 79' 06.30" S 29° 38' 98.40"E	700	5.8      32.6	KwaZulu-Natal moist grasslands	Ordovician Natal group sandstone	<i>Themeda triandra</i> , <i>Hyparrhenia hirta</i> (grasses); <i>Acacia karroo</i> , <i>A sieberiana</i> (trees)
Pniel	28° 34' 50.00"S 24° 30' 30.70"E	350	-4.1      37.5	Kimberley thornveld	Sandy to loam soils of the Hutton soil form	<i>Eragrotis curvula</i> , <i>Schmidtia pappophoroides</i> (grasses); <i>Acacia erioloba</i> , <i>A tortilis</i> , <i>A. karroo</i> , <i>A. mellifera</i> , <i>Tarchonanthus camphoratus</i> (trees)
Middelburg	31° 25' 98.83"S 24° 58' 82.10"E	300	-7.2      36.1	Eastern Upper Karoo	Sandy to loam soils of the Hutton soil form	<i>Aristida</i> and <i>Eragrostis</i> (grasses); <i>Searsia erosa</i> , <i>S. burchellii</i> , <i>Diospyros lycioides</i> and <i>Eriocephalus ericoides</i> (shrubs)

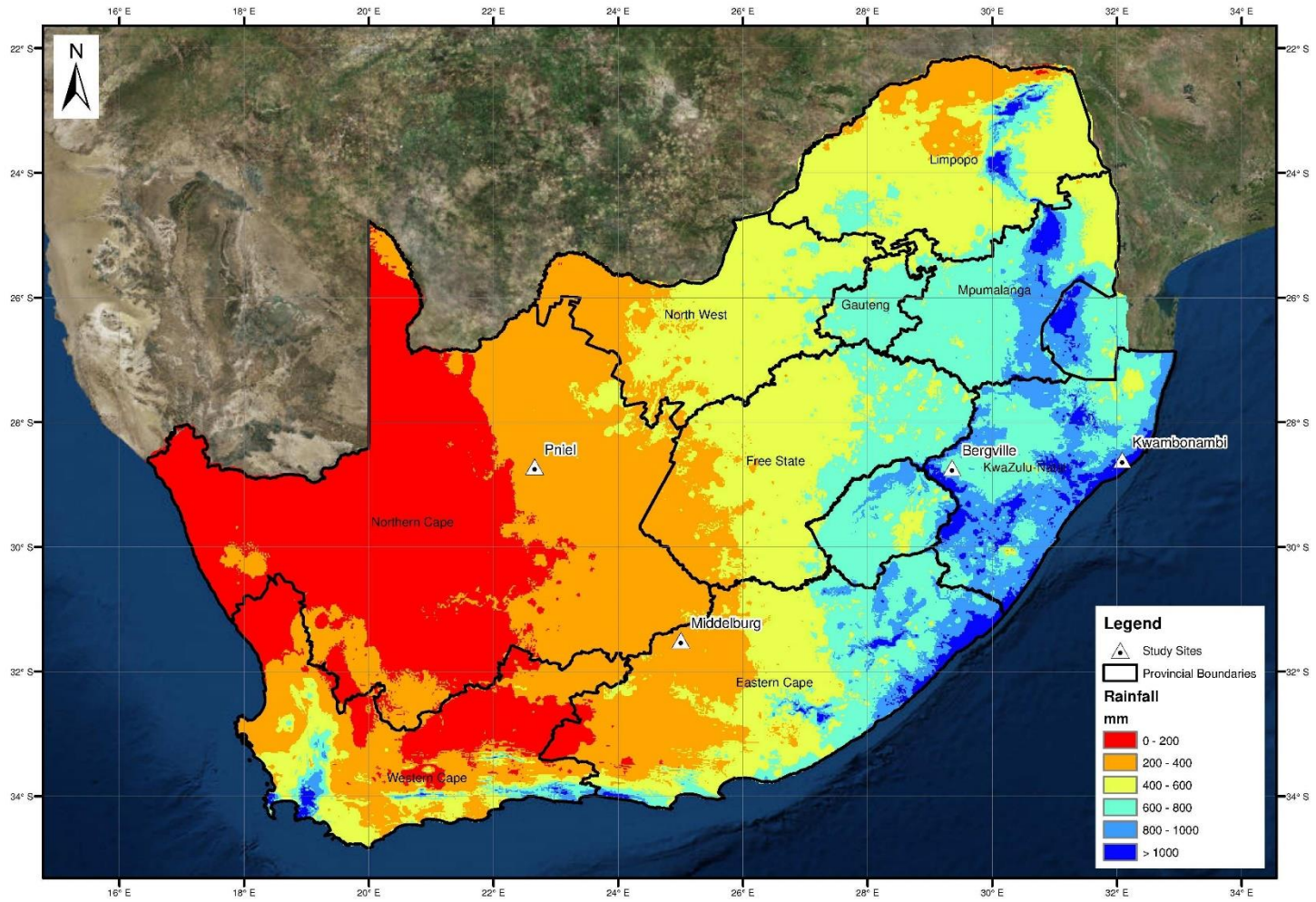


Fig. 1.2 Four study sites in South Africa, representing a precipitation gradient from 300 mm – 1500 mm mean annual precipitation.

## 1.7 References

- Accatino F., De Michele C., Vezzoli R., Donzelli D. and Scholes R.J. 2010. Tree–grass co-existence in savanna: interactions of rain and fire. *Journal of Theoretical Biology* 267: 235-242.
- Alpert P. and Simms E.L. 2002. The relative advantages of plasticity and fixity in different environments: when is it good for a plant to adjust? *Evolutionary Ecology* 16: 285-297.
- Aranibar J., Macko S., Anderson I., Potgieter A., Sowry R. and Shugart H. 2003. Nutrient cycling responses to fire frequency in the Kruger National Park (South Africa) as indicated by stable isotope analysis. *Isotopes in Environmental and Health Studies* 39: 141-158.
- Archer S., Schimel D.S. and Holland E.A. 1995. Mechanisms of shrubland expansion: land use, climate or CO<sub>2</sub>? *Climatic Change* 29: 91-99.
- Archer S.R., Andersen E.M., Predick K.I., Schwinning S., Steidl R.J. and Woods S.R. 2017. Woody plant encroachment: causes and consequences. In: Briske D. D. (ed), *Rangeland Systems: Processes, Management and Challenges*. Springer International Publishing, New York, pp. 25-84.
- Archibald S. and Hempson G.P. 2016. Competing consumers: contrasting the patterns and impacts of fire and mammalian herbivory in Africa. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 371: 20150309. [doi: 10.1098/rstb.2015.0309].
- Archibald S., Staver A.C. and Levin S.A. 2012. The evolution of human-driven fire regimes in Africa. *Proceedings of the National Academy of Sciences, U.S.A.* 109: 847-852.
- Bai W., Wan S., Niu S., Liu W., Chen Q., Wang Q., Zhang W., Han X. and Li L. 2010. Increased temperature and precipitation interact to affect root production, mortality, and turnover in a temperate steppe: implications for ecosystem C cycling. *Global Change Biology* 16: 1306-1316.
- Barbosa E.R., Tomlinson K.W., Carvalheiro L.G., Kirkman K., de Bie S., Prins H.H. and van Langevelde F. 2014. Short-term effect of nutrient availability and rainfall distribution on biomass production and leaf nutrient content of savanna tree species. *PLoS One* 9: [doi: 10.1371/journal.pone.0092619].
- Barbosa E.R.M., van Langevelde F., Tomlinson K.W., Carvalheiro L.G., Kirkman K., de Bie S., Prins H.H.T. 2014. Tree species from different functional groups respond differently to environmental changes during establishment. *Oecologia* 174: 1345-1357.



- Bond W.J. 2008. What limits trees in C<sub>4</sub> grasslands and savannas? *Annual Review of Ecology, Evolution and Systematics* 39: 641-659.
- Bond, W.J. and Parr, C.L. 2010. Beyond the forest edge: ecology, diversity and conservation of grassy biomes. *Biological Conservation* 143: 2395-2404.
- Bond W.J. and Keeley J.E. 2005. Fire as a global 'herbivore': the ecology and evolution of flammable ecosystems. *Trends in Ecology and Evolution* 20: 387-394.
- Bond W.J. and Midgley G.F. 2012. Carbon dioxide and the uneasy interactions of trees and savannah grasses. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 367: 601-612.
- Bond W.J. and Midgley J.J. 2001. Ecology of sprouting in woody plants: the persistence niche. *Trends in Ecology and Evolution* 16: 45-51.
- Bond W.J., Staver A.C., Cramer M.D., Wakeling J.L., Midgley J.J. and Balfour D.A. 2017. Demographic bottlenecks and savanna tree abundance. In: Cromsigt J.P.G.M, Archibald S. and Owen-Smith N. (eds.), *Conserving Africa's Mega-Diversity in the Anthropocene: The Hluhluwe-iMfolozi Park story*. Cambridge University Press. pp. 161-188.
- Bustamante M.C., de Brito D., Kozovits A., Luedemann G., de Mello T.B., de Siqueira Pinto A., Munhoz C.R. and Takahashi F.C. 2012. Effects of nutrient additions on plant biomass and diversity of the herbaceous-subshrub layer of a Brazilian savanna (Cerrado). *Plant Ecology* 213: 795-808.
- Calabrese J.M., Vazquez F., Lopez C., S. M.M. and Grimm V. 2010. The independent and interactive effects of tree-tree establishment, competition and fire on savanna structure and dynamics. *American Naturalist* 175: 44-65.
- Cantarel A.A., Bloor J.M., Pommier T., Guillaumaud N., Moiro C., Soussana J.F. and Poly F. 2012. Four years of experimental climate change modifies the microbial drivers of N<sub>2</sub>O fluxes in an upland grassland ecosystem. *Global Change Biology* 18: 2520-2531.
- Carlyle C.N., Fraser L.H. and Turkington R. 2014. Response of grassland biomass production to simulated climate change and clipping along an elevation gradient. *Oecologia* 174: 1065-1073.
- Case M.F., Staver A.C. and James J. 2017. Fire prevents woody encroachment only at higher-than-historical frequencies in a South African savanna. *Journal of Applied Ecology* 54: 955-962.
- Coetsee C., Bond W.J. and February E.C. 2010. Frequent fire affects soil nitrogen and carbon in an African savanna by changing woody cover. *Oecologia* 162: 1027-1034.

- Cramer M.D., Chimpango S.B.M., Van Cauter A., Waldram M.S. and Bond W.J. 2007. Grass competition induces N<sub>2</sub> fixation in some species of African *Acacia*. *Journal of Ecology* 95: 1123-1133.
- Cramer M.D., Van Cauter A. and Bond W.J. 2010. Growth of N<sub>2</sub>-fixing African savanna *Acacia* species is constrained by below-ground competition with grass. *Journal of Ecology* 98: 156-167.
- Crews T.E. 1999. The presence of nitrogen fixing legumes in terrestrial communities: evolutionary vs ecological considerations. *Biogeochemistry* 46: 233-246.
- Devine, A. P., McDonald, R., Quaife, T. and Maclean I.D.M. 2017. Determinants of woody encroachment and cover in African savannas. *Oecologia* 183: 939–951.
- Eldridge D.J., Bowker M.A., Maestre F.T., Roger E., Reynolds J.F. and Whitford W.G. 2011. Impacts of shrub encroachment on ecosystem structure and functioning: towards a global synthesis. *Ecology Letters* 14: 709–722.
- Galloway J.N., Dentener F.J., Capone D.G., Boyer E.W., Howarth R.W., Seitzinger S.P., Asner G.P., Cleveland C., Green P. and Holland E. 2004. Nitrogen cycles: past, present, and future. *Biogeochemistry* 70: 153-226.
- Gignoux J., Lahoreau G., Julliard R. and Barot S. 2009. Establishment and early persistence of tree seedlings in an annually burned savanna. *Journal of Ecology* 97: 484-495.
- Goldberg, D.E., Rajaniemi, T., Gurevitch, J. and Stewart-Oaten, A. 1999. Empirical approaches to quantifying interaction intensity: competition and facilitation along productivity gradients. *Ecology*, 80: 1118-1131.
- Gordijn P.J., Rice E. and Ward D. 2012. The effects of fire on woody plant encroachment are exacerbated by succession of trees of decreased palatability. *Perspectives in Plant Ecology, Evolution and Systematics* 14: 411-422.
- Gough L., Osenberg C.W., Gross K.L. and Collins S.L. 2000. Fertilization effects on species density and primary productivity in herbaceous plant communities. *Oikos* 89: 428-439.
- Gray E.F. and Bond W.J. 2013. Will woody plant encroachment impact the visitor experience and economy of conservation areas? *Koedoe* 55: art. #1106. [doi: 10.4102/koedoe.v55i1.1106].
- Hibbard K.A., Archer S., Schimel D.S. and Valentine D.W. 2001. Biogeochemical changes accompanying woody plant encroachment in a subtropical savanna. *Ecology* 82: 1999-2011.
- Hoffman, M.T., and Ashwell A. 2001. *Nature Divided: Land Degradation in South Africa*. Cape Town, South Africa: University of Cape Town Press.

- Hudak A.T. and Wessman C.A. 2001. Textural analysis of high resolution imagery to quantify bush encroachment in Madikwe Game Reserve, South Africa, 1955-1996. *International Journal of Remote Sensing* 22: 2731-2740.
- IPCC. 2014. *Climate Change 2014—Impacts, Adaptation and Vulnerability: Regional Aspects*. Cambridge University Press.
- Kambatuku J., Cramer M. and Ward D. 2013. Nitrogen fertilisation reduces grass-induced N<sub>2</sub> fixation of tree seedlings from semi-arid savannas. *Plant and Soil* 365: 307-320.
- Kambatuku J.R., Cramer M.D. and Ward D. 2011. Savanna tree–grass competition is modified by substrate type and herbivory. *Journal of Vegetation Science* 22: 225-237.
- Kgope B.S., Bond W.J. and Midgley G.F. 2010. Growth responses of African savanna trees implicate atmospheric [CO<sub>2</sub>] as a driver of past and current changes in savanna tree cover. *Austral Ecology* 35: 451-463.
- Knoop W.T. and Walker B.H. 1985. Interactions of woody and herbaceous vegetation in a southern African savanna. *Journal of Ecology* 73: 235-253.
- Kraaij T. and Ward D. 2006. Effects of rain, nitrogen, fire and grazing on tree recruitment and early survival in bush-encroached savanna, South Africa. *Plant Ecology* 186: 235-246.
- Lavorel, S., Diaz, S., Cornelissen, J.H.C., Garnier, E., Harrison, S.P., McIntyre, S., Pausas, J.G., Perez-Harguindeguy, N., Roumet, C. and Urcelay, C. 2007. Plant functional types: Are we getting any closer to the Holy Grail? In: *Terrestrial Ecosystems in a Changing World* (eds J.G. Canadell, D.D.E. Pataki & L.F. Pitelka), pp. 171-186. Springer, Berlin, Germany.
- Lawes M.J., Midgley J.J. and Clarke P.J. 2012. Costs and benefits of relative bark thickness in relation to fire damage: a savanna/forest contrast. *Journal of Ecology* 101: 1-8.
- Leakey A.D.B., Bishop K.A. and Ainsworth E.A. 2012. A multi-biome gap in understanding of crop and ecosystem responses to elevated CO<sub>2</sub>. *Current Opinion in Plant Biology* 15: 1-9.
- Liu Y., Wu L., Baddeley J.A. and Watson C.A. 2010. Models of biological nitrogen fixation of legumes. A review. *Sustainable Agriculture* 2: 883-905.
- Ludwig F., de Kroon H., Berendse F. and Prins H.H. 2004. The influence of savanna trees on nutrient, water and light availability and the understory vegetation. *Plant Ecology* 170: 93-105.
- Meyer K., Ward D., Moustakas A. and Wiegand K. 2005. Big is not better: small *Acacia mellifera* shrubs are more vital after fire. *African Journal of Ecology* 43: 131-136.

- Meyer K.M., Ward D., Wiegand K. and Moustakas A. 2008. Multi-proxy evidence for competition between savanna woody species. *Perspectives in Plant Ecology, Evolution and Systematics* 10: 63-72.
- Meyer K.M., Wiegand K., Ward D. and Moustakas A. 2007. SATCHMO: a spatial simulation model of growth, competition, and mortality in cycling savanna patches. *Ecological Modelling* 209: 377-391.
- Mordelet P., Menaut J.C. and Mariotti A. 1997. Tree and grass rooting patterns in an African humid savanna. *Journal of Vegetation Science* 8: 65-70.
- Mucina L. and Rutherford M.C editors. 2006. *The Vegetation of South Africa, Lesotho and Swaziland*. Pretoria, South Africa: South African National Biodiversity Institute.
- Ndoye I.G., M; Danso, S. K. A; Dreyfus, B. 1995. Nitrogen fixation in *Faidherbia albida*, *Acacia raddiana*, *Acacia senegal* and *Acacia seyal* estimated using the <sup>15</sup>N isotope dilution technique. *Plant and Soil* 172: 175-180.
- Parr, C.L., Lehmann, C.E.R., Bond, W.J., Hoffmann, W.A. and Andersen, A.N. 2014. Tropical grassy biomes: misunderstood, neglected, and under threat. *Trends in Ecology and Evolution* 29: 205-213.
- Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M.S., Cornwell, W.K., Craine, J.M., Gurvich, D.E., Urcelay, C., Veneklaas, E.J., Reich, P.B., Poorter, L., Wright, I.J., Ray, P., Enrico, L., Pausas, J.G., De Vos, A.C., Buchmann, N., Funes, G., Quétier, F., Hodgson, J.G., Thompson, K., Morgan, H.D., Ter Steege, H., Van Der Heijden, M.G.A., Sack, L., Blonder, B., Poschlod, P., Vaieretti, M.V., Conti, G., Staver, A.C., Aquino, S. and Cornelissen, J.H.C. 2013. New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany* 61: 167-234.
- Pillay T. and Ward D. 2014. Competitive effect and response of savanna tree seedlings: comparison of survival, growth and associated functional traits. *Journal of Vegetation Science* 25: 226-234.
- Reich P.B., Grigal D.F., Aber J.D. and Gower S.T. 1997. Nitrogen mineralization and productivity in 50 hardwood and conifer stands on diverse soils. *Ecology* 78: 335-347.
- Riginos C. and Grace J.B. 2008. Savanna tree density, herbivores, and the herbaceous community: bottom-up vs. top-down effects. *Ecology* 89: 2228-2238.
- Rohner, C. and Ward, D. 1999. Large mammalian herbivores and the conservation of arid *Acacia* stands in the Middle East. *Conservation Biology* 13: 1162-1171.

- Rustad L.E., Campbell J.L., Marion G.M., Norby R.J., Mitchell M.J., Hartley A.E., Cornelissen J.H.C., Gurevitch J. and Gcte-News. 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* 126: 543-562.
- Sankaran M., Hanan N.P., Scholes R.J., Ratnam J., Augustine D.J., Cade B.S., Gignoux J., Higgins S.I., Le Roux X., Ludwig F., Ardo J., Banyikwa F., Bronn A., Bucini G., Caylor K.K., Coughenour M.B., Diouf A., Ekaya W., Feral C.J., February E.C., Frost P.G.H., Hiernaux P., Hrabar H., Metzger K.L., Prins H.H.T., Ringrose S., Sea W., Tews J., Worden J. and Zambatis N. 2005. Determinants of woody cover in African savannas. *Nature* 438: 846-849.
- Saxe H., Cannell M.G., Johnsen O., Ryan M.G. and Vourlitis G. 2001. Tree and forest functioning in response to global warming. *New Phytologist* 149: 369-399.
- Smit, I.P., Asner, G.P., Govender, N., Vaughn, N.R. and Van Wilgen, B.W. 2016. An examination of the potential efficacy of high-intensity fires for reversing woody encroachment in savannas. *Journal of Applied Ecology* 53: 1623-1633.
- Throop H.L., Reichmann L.G., Sala O.E. and Archer S.R. 2012. Response of dominant grass and shrub species to water manipulation: an ecophysiological basis for shrub invasion in a Chihuahuan Desert grassland. *Oecologia* 169: 373-383.
- Tjelele J., Ward D. and Dziba L. 2015. The effects of seed ingestion by livestock, dung fertilization, trampling, grass competition and fire on seedling establishment of two woody plant species. *PLoS One* 10: e0117788. [doi: 10.1371/journal.pone.0117788].
- Twidwell D., Rogers W.E., Wonkka C.L., Taylor C.A. and Kreuter U.P. 2016. Extreme prescribed fire during drought reduces survival and density of woody resprouters. *Journal of Applied Ecology* 53: 1585-1596.
- Vadigi S. and Ward D. 2012. Fire and nutrient gradient effects on the sapling ecology of four *Acacia* species in the presence of grass competition. *Plant Ecology* 213: 1793-1802.
- Van Auken O.W. 2000. Shrub invasions of North American semiarid grasslands. *Annual Review of Ecology and Systematics* 31: 197-215.
- Van Der Waal C., De Kroon H., De Boer W.F., Heitkönig I.M.A., Skidmore A.K., De Knegt H.J., Van Langevelde F., Van Wieren S.E., Grant R.C., Page B.R., Slotow R., Kohi E.M., Mwakiwa E. and Prins H.H.T. 2009. Water and nutrients alter herbaceous competitive effects on tree seedlings in a semi-arid savanna. *Journal of Ecology* 97: 430-439.

- Van Langevelde F., Van de Vijver C., Kumar L., Van de Koppel J., De Ridder N., Van Andel J., Skidmore A., Hearne J., Stroosnijder L., Bond W.J., Prins H.H.T. and Rietkerk M. 2003. Effects of fire and herbivory on the stability of savanna ecosystems. *Ecology* 84: 337-350.
- Violle C. and Jiang L. 2009. Towards a trait-based quantification of species niche. *Journal of Plant Ecology* 2: 87-93.
- Vitousek P.M., Menge D.N.L., Reed S.C. and Cleveland C.C. 2013. Biological nitrogen fixation: rates, patterns and ecological controls in terrestrial ecosystems. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 368: 20130119. [doi: 10.1098/rstb.2013.0119].
- Volder A., Briske D.D. and Tjoelker M.G. 2013. Climate warming and precipitation redistribution modify tree–grass interactions and tree species establishment in a warm-temperate savanna. *Global Change Biology* 19: 843-857.
- Wakeling J.L., Cramer M.D. and Bond W.J. 2010. Is the lack of leguminous savanna trees in grasslands of South Africa related to nutritional constraints? *Plant and Soil* 336: 173-182.
- Walter H. 1971. *Ecology of Tropical and Subtropical Vegetation*. Oliver and Boyd, Edinburgh, UK.
- Ward D. and Esler K.J. 2011. What are the effects of substrate and grass removal on recruitment of *Acacia mellifera* seedlings in a semi-arid environment? *Plant Ecology* 212: 245-250.
- Ward D., Hoffman M.T. and Collocott S.J. 2014. A century of woody plant encroachment in the dry Kimberley savanna of South Africa. *African Journal of Range and Forage Science* 31: 107-121.
- Ward D., Kirkman K. and Tsvuura Z. 2017. An African grassland responds similarly to long-term fertilization to the Park Grass experiment. *PLoS One* 12: e0177208. [doi: 10.1371/journal.pone.0177208].
- Ward D., Wiegand K. and Getzin S. 2013. Walter's two-layer hypothesis revisited: back to the roots! *Oecologia* 172: 617-630.
- Way D.A. and Oren R. 2010. Differential responses to changes in growth temperature between trees from different functional groups and biomes: a review and synthesis of data. *Tree Physiology* 30: 669-88.
- Weltzin J.F., Loik M.E., Schwinning S., Williams D.G., Fay P.A., Haddad B.M., Harte J., Huxman T.E., Knapp A.K. and Lin G. 2003. Assessing the response of terrestrial ecosystems to potential changes in precipitation. *Bioscience* 53: 941-952.

- Wenig M., Spichtinger N., Stohl A., Held G., Beirle S., Wagner T., Jähne B. and Platt U. 2003. Intercontinental transport of nitrogen oxide pollution plumes. *Atmospheric Chemistry and Physics* 3: 387-393.
- Werner E.E. and Peacor S.D. 2003. A review of trait-mediated indirect interactions in ecological communities. *Ecology* 84: 1083-1100.
- White S.R., Carlyle C.N., Fraser L.H. and Cahill J.F. 2012. Climate change experiments in temperate grasslands: synthesis and future directions. *Biology Letters* 8: 484-487.
- Wiegand K., Saltz D. and Ward D. 2006. A patch dynamics approach to savanna dynamics and bush encroachment – insights from an arid savanna. *Perspectives in Plant Ecology, Evolution and Systematics* 7: 229-242.
- Wiegand K., Ward D. and Saltz D. 2005. Multi-scale patterns and bush encroachment in an arid savanna with a shallow soil layer. *Journal of Vegetation Science* 16: 311-320.

## **CHAPTER 2: Drought suppresses grass productivity and warming promotes tree performance**

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## 2.1 Abstract

We examined the independent and interactive effects of altered water availability and warming on the growth and performance of savanna grasses and tree saplings in the face of predictions of altered global climate change. Using a completely randomized experimental design in the greenhouse, we investigated the effects of manipulated water availability and passive warming on the productivity of the commonly occurring grass species *Eragrostis curvula*, and the emergence and performance of seedlings of the native woody encroacher, *Acacia sieberiana*. We simulated a typical savanna microhabitat by planting tree species within a grass matrix, in 45 L black plastic bags. Watering treatments were calculated using the long-term (50 year) monthly mean precipitation, and applied weekly as drought (minus 40% of the long-term average), normal (same amount as the long-term average of the area), and excess (addition of 40% of the long-term average). Passive warming was achieved using open-top chambers. Tree-seedling emergence was monitored in the first two weeks. Thereafter, weekly tree growth-rate measurements were taken. After 24 weeks the experiment was terminated, and the final above-ground grass biomass, tree root: shoot ratio and tree specific leaf area were quantified.

We found that water availability was the main limiting factor for grass productivity, with low grass biomass attained under the drought treatments. Warming had no significant effect on grass productivity, but had a large positive effect on tree growth and performance when coupled with excess or normal water treatments. Tree seedling root:shoot ratios were highest in the drought treatment, possibly as an adaptation to low water-supply. Higher specific leaf area was recorded with warming, further elucidating the positive effect of increased temperature for tree growth. Drought has important negative implications for grass productivity, and may reduce the competitive effects of grasses on trees. Coupled with

increased performance of trees under warmed conditions, future climate change may further promote tree establishment, especially in areas where precipitation is limiting.

**Key-words**

*Acacia sieberiana*, *Vachellia*, grass, precipitation, warming, drought, savanna

## 2.2 Introduction

Climate-change models predict future global alterations in mean temperature and precipitation patterns, which are likely to affect ecosystem function (Carlyle *et al.*, 2014). Land areas may warm by as much as 1.6°C over the semi-arid parts of southern Africa by 2050, with a predicted 10% reduction in precipitation (IPCC 2014). Both natural and experimental warming have been shown to generally increase productivity in plant communities (Hudson & Henry, 2009) and alter species interactions (Wu *et al.*, 2011; Carlyle *et al.*, 2014). Warmer temperatures increase rates of virtually all chemical and biochemical processes in plants and soils (Saxe *et al.*, 2001). Resultant increases in photosynthetic capability and overall metabolic activity, may prove favourable to woody plants. Way & Oren (2010) used data from 63 studies to examine the difference in growth response to a change in temperature. They found that elevated temperatures enhanced growth (measured as shoot height, stem diameter and biomass) in deciduous species (Way & Oren, 2010). Wu *et al.* (2011) found that experimental warming and increased precipitation generally stimulated plant growth, whereas decreased precipitation had the opposite effects. Volder *et al.* (2010) consider climate warming and drought to alter tree establishment in savannas through differential responses of tree seedlings and grass to intermittent precipitation events.

Water availability, being the most limiting factor in most ecosystems (Ludwig *et al.*, 2004), is likely to have the greatest impact on plant communities in arid environments (Weltzin *et al.*, 2003). Changes in precipitation event size and distribution may have larger impacts on plant performance and productivity than the effects of elevated CO<sub>2</sub> in grassland and savanna ecosystems, yet responses have been highly variable across experiments (White *et al.*, 2012; Carlyle *et al.*, 2014). For example, Gherardi & Sala (2015) conducted a field experiment where they experimentally manipulated precipitation, and found that total

productivity of grasses declined in response to increased precipitation variability. However, shrubs benefited, suggesting a potential shift from grassland to shrubland in the future. Kraaij & Ward (2006) showed empirically that the main driver of woody plant encroachment at a semi-arid site of South Africa was water availability, with responses to the maximum precipitation leading to encroachment by *Acacia mellifera*. Mostly, encroachment was due to a change in the frequency of precipitation – simulated precipitation events of at least 15 mm every two weeks led to tree invasion. Fay *et al.*, (2003) have also shown through a long-term precipitation manipulation experiment in a mesic tallgrass prairie, the negative effects of changes in water availability on above-ground net primary productivity. Furthermore, Joubert *et al.*, 2017 have shown that even long-lived species such as *Acacia mellifera*, living for well over a century, is susceptible to drought.

Tree–grass interactions are well represented in the literature, but empirical studies investigating future climate change scenarios are lacking, particularly in Africa (Leakey *et al.*, 2012). Indeed, savanna tree–grass interactions may be particularly sensitive to climate change (Volder *et al.*, 2013), and of great importance considering the vast worldwide expansion of woody plant density (termed bush or shrub encroachment) in grasslands and savannas (Archer *et al.*, 2017). Determining the relative responses of trees and grasses may also help predict shifts in vegetation structure, or plasticity in traits favouring future climate change (Alpert & Simms, 2002; Bond 2008). The aim of this study was to investigate the effects of altered precipitation and warming on the performance of tree and grass saplings. We used a greenhouse experiment to simulate altered precipitation patterns and passive warming using open-top chambers (OTC). Temperature increases of less than 1°C are sufficient to induce changes in soil respiration (Rustad *et al.*, 2001), nitrogen mineralization and above-ground plant biomass (Volder *et al.*, 2013). We measured grass productivity and

tree seedling emergence, subsequent tree-sapling growth rates and changes in functional traits, viz. root:shoot ratio and specific leaf area. We predicted that:

1. Reduced watering would have a more profound negative effect on grass biomass than would the warming treatment because grasses are known to respond more rapidly to water availability in the upper soil layers (Walter 1939; Knoop & Walker, 1985; Ward *et al.*, 2013).
2. Similarly, we anticipated that watering would be more important than warming for tree seedling emergence because water is the main requirement for germination (Kraaij & Ward, 2006).
3. We expect that warming would be an important factor for tree performance, with positive effects of warming on tree growth rates and functional traits, attributed to increased metabolic rates under warmed conditions (Saxe *et al.*, 2001; Way & Oren, 2010). Furthermore, Stevens *et al.*, (2014) showed that warmer temperatures accelerate the rate of radicle extension in trees and increase the frequency of seedling establishment events.

## **2.3 Methods**

### ***Study species***

*Acacia sieberiana* DC. var. *woodii* (Burt Davy) Keay & Brenan (recently renamed *Vachellia sieberiana*; commonly referred to as the paperbark thorn) is a medium-sized (7-15 m), deciduous, flat-topped tree, found in southern Africa, north to Ethiopia. This tree species is known to encroach mountainous grasslands and savannas, particularly in the mesic-humid areas of southern Africa (Grellier *et al.*, 2013). The grass species selected for this study was *Eragrostis curvula* (Schrad.) Nees which is a widespread savanna grass species, common to KwaZulu-Natal province (South Africa), where the study was conducted.

### ***Experimental setup***

The experiment was carried out at the greenhouse of the Botanical Gardens of the University of KwaZulu-Natal, Pietermaritzburg, South Africa. A tree-grass matrix was used to mimic a typical savanna microhabitat. We used 60 45 L bags (0.5 m W x 0.4 m L) in a completely randomized design. To replicate the grass component of the savanna, we sowed *Eragrostis curvula* into 45 L bags (approximately 50 grass seeds per bag) containing Umgeni grit, an alluvial soil characteristic of the Pietermaritzburg area, with a particle diameter of approximately 1.5 to 2mm. *Acacia sieberiana* was used to replicate the woody savanna component. Three levels of water treatment (drought, normal and excess), and two levels of temperature (ambient and warmed), in all combinations were replicated five times during the 24-week course of the experiment. We rotated these bags on a biweekly basis to minimize edge effects.

Water availability was manipulated by hand watering bags weekly for the duration of the experiment. The long-term (50-year, 1950-2000) mean annual precipitation for the area is 750 mm. Once each week, each bag received water equivalent to the mean annual precipitation (*normal*), while others we increased the monthly precipitation average by 40 %, as the *excess* treatment. Similarly, the average precipitation was decreased by 40% for the *drought* treatment. Bags were hand-watered slowly to ensure minimal runoff; locally collected rainwater was used for the watering. An increase in globally averaged surface temperatures within the next century is predicted to result in fewer, but larger precipitation events (IPCC 2014). Additionally, models indicate that greater evaporation in response to warmer atmospheric temperatures will likely increase the frequency, duration, and intensity of droughts (Liu et al. 2017). Hence, we used the long-term mean annual precipitation for the

area (750 mm MAP), to mimic the normal, excess (average plus 40 %), and drought (normal minus 40%). This methodology was comparable to that of Volder et al. (2010).

Passive open-top chambers (OTC) are used to experimentally increase temperature (Marion *et al.*, 1997; Fraser *et al.*, 2009; Carlyle *et al.*, 2014). Each OTC (sixty in total) was constructed with a hexagonal base and a top opening of 0.6 m. Plastic was secured to eight wooden stakes driven into the soil, angled so that the top opening was square, 0.8 m above the soil surface. To ensure efficacy of the OTC, soil temperature was measured at 5 cm depth in a subset of bags in all treatment bags, using the Thermochron iButton® temperature logger (n = 20). Temperature measurements were taken over a 7 day period. OTC temperatures were shown to be higher ( $21.6 \pm 1.1^\circ\text{C}$ ) than that of the control bags ( $20.7 \pm 1.3^\circ\text{C}$ ).

At the beginning of the experiment, 300 seeds of *A. sieberiana* were chemically scarified for one hour by soaking in 1 M hydrochloric acid (HCl). Seeds were then rinsed repeatedly using distilled water, and soaked in boiling water overnight. Five tree seeds per bag were sowed. We monitored seedling emergence after 14 days, and thereafter thinned the tree seedlings to one per bag. Weekly measurements of tree height were recorded and subsequently used to calculate relative growth rate ( $\text{RGR}_{\text{height}}$ ). Calculations for relative growth rate of tree sapling stem height ( $\text{RGR}_{\text{height}}$ ) were based on the natural logarithm measured in  $\text{mm}\cdot\text{mm}^{-1}\cdot\text{week}^{-1}$  (Kohi *et al.*, 2009). After 24 weeks, specific leaf area of three randomly selected leaves per sapling were recorded. Thereafter, all trees and grasses were subsequently harvested. Tree roots were carefully washed free of sand to minimize loss of fine root material. Tree shoots and roots were oven dried at  $70^\circ\text{C}$  for 24 hours. Tree root and shoot dry biomasses and above-ground grass biomass were quantified.

## *Statistical analysis*

All statistical analyses were conducted in SPSS (version 18.0 for Windows). Effects of treatment variables (watering and warming) on tree and grass growth parameters were analyzed using Multivariate Analysis of Variance (MANOVA) to control for Type I error caused by the analysis of multiple dependent variables. Significant treatment effects were then assessed using Bonferroni *post hoc* tests.

## **2.4 Results**

We found overall significant effects of watering treatments (MANOVA: Wilk's lambda = 0.017,  $p < 0.001$ ), the warming treatment (MANOVA: Wilk's lambda = 0.016,  $p < 0.001$ ) and the interaction between watering and warming treatments (MANOVA: Wilk's lambda = 0.033,  $p < 0.001$ ).

We found that above-ground grass biomass was significantly affected by watering regime (Table 1), with the highest biomass attained in the excess watering treatment ( $347.1 \text{ g} \pm 33.7 \text{ g}$ ) as compared to the drought treatment ( $41.5 \text{ g} \pm 15.3 \text{ g}$ , Fig. 1). Warming had no significant effect on final grass biomass (Table 1). There were no significant interaction effects on above-ground grass biomass (Table 1).

Significant interaction effects of watering regime and warming were observed for percentage tree seedling emergence (Table 1, Fig. 2). We found that percentage tree seedling emergence was significantly higher (Table 1) in the normal and excess watering treatment when the temperature was warmer (mean =  $97.7 \% \pm 4.40$  and  $89.0 \% \pm 4.12$ , respectively). In the drought treatment, tree seedling emergence was significantly lower ( $p < 0.005$ ) than the normal and excess watering treatment, irrespective of temperature (Fig. 2). There was a



marginally significant difference ( $p = 0.059$ ) in percentage emergence between the normal and excess watering regimes.

Tree sapling growth parameters ( $RGR_{\text{height}}$  and root:shoot ratio) showed significant main and interaction effects of watering regime and temperature treatment (Table 1). Consistently higher  $RGR_{\text{height}}$  was attained in the warmed treatment, across watering regimes. Excess watering resulted in significantly higher  $RGR_{\text{height}}$  (mean=  $0.06 \pm 0.004 \text{ mm}\cdot\text{mm}^{-1}\cdot\text{week}^{-1}$ ), as compared to the drought treatment (mean=  $0.02 \pm 0.002 \text{ mm}\cdot\text{mm}^{-1}\cdot\text{week}^{-1}$ ) when both were warmed (Fig. 3). There were no significant differences in  $RGR_{\text{height}}$  between the normal and excess watering regimes ( $p = 0.129$ ).

Tree sapling root:shoot ratio was highest in the ambient temperature and drought treatment (mean =  $0.47 \pm 0.01$ ,  $p < 0.05$ , Fig. 4). Tree specific leaf area (SLA) was only significantly affected by warming (Table 1), with consistently higher SLA in plants under the warming treatment than those under ambient conditions (Fig. 5). There was a marginally significant difference ( $p = 0.052$ ) among watering treatments, with the excess watering treatment having the highest mean SLA (mean=  $8.26 \pm 0.88 \text{ g}\cdot\text{cm}^{-2}$ ).

## 2.5 Discussion

Based on predicted climate models for Africa, decreased precipitation and higher average temperatures are expected in the next half-century (Engelbrecht & Engelbrecht, 2016), thereby altering ecosystem functioning in savannas and grasslands worldwide (Moncrieff *et al.*, 2015). We found that above-ground grass biomass increased with greater water availability, indicating that water is the most limiting resource for grass persistence. Following Walters's (1939) two-layer hypothesis, both trees and grasses are water-limited and acquire moisture from different soil depths. Grasses, which have shallow roots, rely on

water from the topsoil, while trees which also have deeper roots, use water from the subsoil (Walter 1939; Ward *et al.* 2013). Consequently, grasses may be affected more severely by reduced water availability, particularly over a single growing season, as demonstrated in this study. Wu *et al.*, (2011) conducted an experimental manipulation of precipitation and temperature in grasslands, and also found decreases in grass shoot biomass with reduced precipitation. This has also been demonstrated by Kraaij & Ward (2006), who showed that tree recruitment was constrained by both precipitation amount and frequency (see also Ward & Esler, 2011). A study by February *et al.*, (2013) in the Kruger National Park, South Africa, also showed that grass biomass was significantly and positively influenced by precipitation addition. The strong limitation of water availability on grass productivity may also imply that during drought periods, the competitive effect of grasses is reduced to an extent where recruitment of woody plants may be favoured (February *et al.*, 2013).

As predicted, we found that increased water availability and warming promoted tree seedling emergence. The germination, emergence and establishment phase is critical in the life cycle of plants, particularly savanna tree species (Higgins *et al.*, 2000; Vadigi & Ward, 2012). Survival of tree seedlings that emerge during a particular season depends on water availability in the topsoil, which is also where competition for soil moisture is strongest, particularly for young trees (Knoop & Walker, 1985; Kambatuku *et al.*, 2013; Ward *et al.*, 2013). Increased water availability may promote tree seedling establishment by reducing competition with grasses, thereby allowing successful emergence of tree saplings.

Germination of cohorts of tree saplings during high precipitation years has been recorded by several studies (Wilson & Witkowski, 1998; Ward 2005; Ward *et al.*, 2014). O'Connor (1995) found that *Acacia karroo* seedling survival was related to moisture availability with little or no survival when precipitation was below 500 mm per annum. Joubert *et al.*, (2008; 2012) found that three or more years of precipitation in excess of 500 mm were necessary for

survival of *A. mellifera* in Namibia. This empirical evidence shows that a decline in soil available moisture below the average requirement for a particular species, may result in significant mortality, or at least reduced performance.

Increased metabolic activity caused by warmer temperatures may also promote seedling establishment (Saxe *et al.*, 2001). However, increased temperature coupled with drought significantly reduced the emergence of tree seedlings in our study, possibly due to the increase in water deficit with warming (del Cacho *et al.*, 2012). Conversely, increased temperature in the presence of excess watering resulted in the highest relative-growth rate of trees in our study. This result indicates a strong coupling of water availability and temperature in determining the success of woody plants under predicted climate change (Sankaran *et al.*, 2005; Volder *et al.*, 2013). In addition, the apparent overlap in tree growth parameters between the normal and excess watering treatments highlights the important negative effect of drought on tree survival and persistence.

While future climate change may alter the emergence of tree seedlings, established populations may also display a shift in functional traits. Plasticity of functional traits enables plants to respond to variation in resource availability (Alpert & Simms, 2002). Root:shoot ratios provide insights into plant allocation, and differing strategies to cope with climatic variables (Cornelissen *et al.*, 2003b). For example, Holdo (2013) showed that in *Combretum hereroense*, a common savanna species, shoot:root ratios exhibited lower allocation to root tissue under elevated nutrient conditions (Holdo, 2013). Our study found a dramatic increase in root:shoot ratio of tree saplings under drought conditions. We suspect that tree saplings invested more in root production in response to the drought treatment to better intercept available soil moisture. This has been substantiated elsewhere: Ansley *et al.* (2014) found an increase in coarse root growth during extended drought. Hoffmann *et al.* (2004) reported an increased competitive advantage of savanna trees as compared to forest species, due to a

higher root:shoot ratio. This greater investment in roots, presumably in root length, allowed savanna trees to survive droughts by accessing water deeper in the soil profile (Hoffmann *et al.*, 2004).

Specific leaf area (SLA) is an easily measured functional trait that correlates strongly with photosynthetic capacity, leaf lifespan and leaf nitrogen content (Morin *et al.*, 2010). Some of the best-documented effects of climate change have been shifts in leaf phenology (Morin *et al.*, 2010; Primack & Miller-Rushing, 2012), with SLA identified as a key trait for the investigation of adaptive phenotypic plasticity under altered climatic conditions (Nicotra *et al.*, 2010). Yin (2002) conducted a meta-analysis based on 170 data cases including 62 different species, and found that leaves that have been acclimated to low temperatures have reduced SLA. Conversely, higher temperatures should be associated with larger SLA, which indicates larger leaf area for a given mass (Reich, 2014). Plants with traits associated with high investment in photosynthesis and fast growth generally display a high SLA, and serve as an indicator of plant performance with environmental change (Cornelissen *et al.*, 2003a; Pérez-Harguindeguy *et al.* 2013). In our study, we found that temperature warming resulted in higher specific leaf area. This indicates that encroaching woody trees, such as *A. sieberiana*, may benefit from future climate warming through a more extensive foliar display that captures more light for constant biomass investment (Wang *et al.*, 2012).

## **2.6 Conclusions**

Temperature had no effect on grass productivity in this study. However, drought conditions exerted a strong negative pressure on above-ground grass production. Future predicted drought scenarios may decrease the competitive effect of grasses on trees in savannas (February *et al.*, (2013). Reduced grass competition, coupled with the strong positive effect of

warming on tree growth variables, such as increased relative growth rate and higher specific leaf area, may allow tree proliferation to pose a continual threat to grassland and savanna ecosystems. The positive effect of warming on tree performance is linked to higher water availability. With future climate models predicting a reduction in precipitation over southern Africa (IPCC, 2014), extreme high precipitation events may provide opportunities for *en masse* germination and establishment of woody encroachers. Nevertheless, more long-term field experiments with more extensive warming and watering-level treatments in savannas and grasslands are urgently needed to obtain further understanding of the responses of encroaching species across life stages to climate change.

## 2.7 References

- Alpert P, Simms EL (2002) The relative advantages of plasticity and fixity in different environments: when is it good for a plant to adjust? *Evolutionary Ecology*, **16**, 285-297.
- Ansley RJ, Boutton TW, Jacoby PW (2014) Root biomass and distribution patterns in a semi-arid mesquite savanna: responses to long-term rainfall manipulation. *Rangeland Ecology & Management*, **67**, 206-218.
- Archer SR, Andersen EM, Predick KI, Schwinning S, Steidl RJ, Woods SR (2017) Woody plant encroachment: causes and consequences. In: Briske D. D. (ed), *Rangeland Systems: Processes, Management and Challenges*. Springer International Publishing, New York, pp. 25-84.
- Bond WJ (2008) What limits trees in C<sub>4</sub> grasslands and savannas? *Annual Review of Ecology, Evolution and Systematics*, **39**, 641-659.
- Carlyle CN, Fraser LH, Turkington R (2014) Response of grassland biomass production to simulated climate change and clipping along an elevation gradient. *Oecologia*, **174**, 1065-1073.
- Cornelissen JHC, Cerabolini B, Castro-Díez P, Villar-Salvador P, Montserrat-Martí G, Puyravaud JP, Maestro M, Werger MJA, Aerts R (2003a) Functional traits of woody plants: correspondence of species rankings between field adults and laboratory-grown seedlings? *Journal of Vegetation Science*, **14**, 311-322.
- Cornelissen JHC, Lavorel S, Garnier E, Diaz S, Buchmann N, Gurvich DE, Reich PB, Ter Steege H, Morgan HD, Van Der Heijden MGA, Pausas JG, Poorter H (2003b) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany*, **51**, 335-380.
- del Cacho M, Saura-Mas S, Estiarte M, Peñuelas J, Lloret F (2012) Effect of experimentally induced climate change on the seed bank of a Mediterranean shrubland. *Journal of Vegetation Science*, **23**, 280-291.
- Engelbrecht CJ, Engelbrecht FA (2016) Shifts in Köppen-Geiger climate zones over southern Africa in relation to key global temperature goals. *Theoretical and Applied Climatology*, **123**, 247-261.
- Fay PA, Carlisle JD, Knapp AK, Blair JM, Collins SL (2003) Productivity responses to altered rainfall patterns in a C<sub>4</sub>-dominated grassland. *Oecologia*, **137**, 245-251.

- February EC, Higgins SI, Bond WJ, Swemmer L (2013) Influence of competition and rainfall manipulation on the growth responses of savanna trees and grasses. *Ecology*, **94**, 1155-1164.
- Fraser LH, Greenall A, Carlyle C, Turkington R, Friedman CR (2009) Adaptive phenotypic plasticity of *Pseudoroegneria spicata*: response of stomatal density, leaf area and biomass to changes in water supply and increased temperature. *Annals of Botany*, **103**, 769–775.
- Gherardi LA, Sala OE (2015) Enhanced precipitation variability decreases grass- and increases shrub-productivity. *Proceedings of the National Academy of Sciences, U.S.A.*, **112**, 12735-12740.
- Grellier S, Ward D, Janeau JL, Podwojewski P, Lorentz S, Abbadie L, Valentin C, Barot S (2013) Positive versus negative environmental impacts of tree encroachment in South Africa. *Acta Oecologica*, **53**, 1-10.
- Higgins SI, Bond WJ, Trollope WSW (2000) Fire, resprouting and variability: a recipe for grass-tree coexistence in savanna. *Journal of Ecology*, **88**, 213-229.
- Hoffmann WA, Orthen B, Franco AC (2004) Constraints to seedling success of savanna and forest trees across the savanna-forest boundary. *Oecologia*, **140**, 252-260.
- Holdo RM (2013) Effects of fire history and N and P fertilization on seedling biomass, specific leaf area, and root: shoot ratios in a South African savannah. *South African Journal of Botany*, **86**, 5-8.
- Hudson JM, Henry G (2009) Increased plant biomass in a High Arctic heath community from 1981 to 2008. *Ecology*, **90**, 2657-2663.
- Intergovernmental Panel on Climate Change IPCC (2014) Climate change 2014–Impacts, adaptation and vulnerability: regional aspects. Cambridge, UK: Cambridge University Press.
- Joubert DF, Rothauge A, Smit GN (2008) A conceptual model of vegetation dynamics in the semiarid highland savanna of Namibia, with particular reference to bush thickening by *Acacia mellifera*. *Journal of Arid Environments*, **72**, 2201-2210.
- Joubert DF, Smit GN, Hoffman MT (2012) The role of fire in preventing transitions from a grass dominated state to a bush thickened state in arid savannas. *Journal of Arid Environments*, **87**, 1-7.

- Joubert D, Rust A, Smit G, Hoffman M (2017) Growth rates and mortality patterns of *Acacia mellifera* subsp. *detinens* in the semi-arid Highland Savanna, Namibia: encroachment is not as rapid as previously believed. *Namibian Journal of Environment* **1**: A-5.
- Kambatuku JR, Cramer MD, Ward D (2013) Overlap in soil water sources of savanna woody seedlings and grasses. *Ecohydrology*, **6**, 464-473.
- Knoop WT, Walker BH (1985) Interactions of woody and herbaceous vegetation in a southern African savanna. *Journal of Ecology*, **73**, 235-253.
- Kohi EM, de Boer FW, Slot M, van Wieren SE, Ferwerda JG, Grant RC, Heitkonig IMA, de Knecht HJ, van der Waal C, Prins HHT (2009) Effects of simulated browsing on growth and leaf chemical properties in *Colophospermum mopane* saplings. *African Journal of Ecology*, **48**, 190–196.
- Kraaij T, Ward D (2006) Effects of rain, nitrogen, fire and grazing on tree recruitment and early survival in bush-encroached savanna, South Africa. *Plant Ecology*, **186**, 235-246.
- Leakey ADB, Bishop KA, Ainsworth EA (2012) A multi-biome gap in understanding of crop and ecosystem responses to elevated CO<sub>2</sub>. *Current Opinion in Plant Biology*, **15**, 1-9.
- Liu B, Yan Z, Sha J, Li S (2017) Drought evolution due to climate change and links to precipitation intensity in the Haihe River Basin. *Water* **9**: 878.
- Ludwig F, de Kroon H, Berendse F, Prins HH (2004) The influence of savanna trees on nutrient, water and light availability and the understory vegetation. *Plant Ecology*, **170**, 93-105.
- Marion G, Henry G, Freckman D, Johnstone J, Jones G, Jones M, Levesque E, Molau U, Mølgaard P, Parsons A (1997) Open-top designs for manipulating field temperature in high-latitude ecosystems. *Global Change Biology*, **3**, 20-32.
- Moncrieff GR, Scheiter S, Slingsby JA, Higgins SI (2015) Understanding global change impacts on South African biomes using dynamic vegetation models. *South African Journal of Botany*, **101**, 16-23.
- Morin X, Roy J, Sonié L, Chuine I (2010) Changes in leaf phenology of three European oak species in response to experimental climate change. *New Phytologist*, **186**, 900-910.
- Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan E, Mathesius U, Poot P, Purugganan MD, Richards C, Valladares F (2010) Plant phenotypic plasticity in a changing climate. *Trends in Plant Science*, **15**, 684-692.



- Pérez-Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte, MS, Cornwell WK, Craine JM, Gurvich DE, Urcelay C, Veneklaas EJ, Reich PB, Poorter L, Wright IJ, Ray P, Enrico L, Pausas JG, De Vos AC, Buchmann N, Funes, G, Quétier F, Hodgson JG, Thompson K, Morgan HD, Ter Steege H, Van Der Heijden MGA, Sack L, Blonder B, Poschlod P, Vaieretti MV, Conti G, Staver AC, Aquino, S and Cornelissen JHC (2013) New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany*, **61**, 167-234.
- Primack RB, Miller-Rushing AJ (2012) Uncovering, collecting, and analyzing records to investigate the ecological impacts of climate change: a template from Thoreau's Concord. *BioScience*, **62**, 170-181.
- Reich PB (2014) The world-wide 'fast-slow' plant economics spectrum: a traits manifesto. *Journal of Ecology*, **102**, 275-301.
- Rustad LE, Campbell JL, Marion GM, Norby RJ, Mitchell MJ, Hartley AE, Cornelissen JHC, Gurevitch J (2001) A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia*, **126**, 543-562.
- Sankaran M, Hanan NP, Scholes RJ, Ratnam J, Augustine DJ, Cade BS, Gignoux J, Higgins SI, Le Roux X, Ludwig F, Ardo J, Banyikwa F, Bronn A, Bucini G, Caylor KK, Coughenour MB, Diouf A, Ekaya W, Feral CJ, February EC, Frost PGH, Hiernaux P, Hrabar H, Metzger KL, Prins HHT, Ringrose S, Sea W, Tews J, Worden J, Zambatis N (2005) Determinants of woody cover in African savannas. *Nature*, **438**, 846-849.
- Saxe H, Cannell MG, Johnsen O, Ryan MG, Vourlitis G (2001) Tree and forest functioning in response to global warming. *New Phytologist*, **149**, 369-399.
- Stevens N, Seal CE, Archibald S, Bond W (2014) Increasing temperatures can improve seedling establishment in arid-adapted savanna trees. *Oecologia*, **175**, 1029-1040.
- Vadigi S, Ward D (2012) Fire and nutrient gradient effects on the sapling ecology of four *Acacia* species in the presence of grass competition. *Plant Ecology*, **213**, 1793-1802.
- Volder A, Briske DD, Tjoelker MG (2013) Climate warming and precipitation redistribution modify tree-grass interactions and tree species establishment in a warm-temperate savanna. *Global Change Biology*, **19**, 843-857.
- Walter H (1939) Grassland, savanne und busch der arideren teile Afrikas in ihrer ökologischen bedingtheit. *Jahrbucher für Wissenschaftliche Botanik*, **87**, 750-860.

- Wang D, Heckathorn SA, Wang X, Philpott SM (2012) A meta-analysis of plant physiological and growth responses to temperature and elevated CO<sub>2</sub>. *Oecologia*, **169**, 1-13.
- Ward D (2005) Do we understand the causes of bush encroachment in African savannas? *African Journal of Range and Forage Science*, **22**, 101-105.
- Ward D, Esler KJ (2011) What are the effects of substrate and grass removal on recruitment of *Acacia mellifera* seedlings in a semi-arid environment? *Plant Ecology*, **212**, 245-250.
- Ward D, Hoffman MT, Collocott SJ (2014) A century of woody plant encroachment in the dry Kimberley savanna of South Africa. *African Journal of Range and Forage Science*, **31**, 107-121.
- Ward D, Wiegand K, Getzin S (2013) Walter's two-layer hypothesis revisited: back to the roots! *Oecologia*, **172**, 617-630.
- Way DA, Oren R (2010) Differential responses to changes in growth temperature between trees from different functional groups and biomes: a review and synthesis of data. *Tree Physiology*, **30**, 669–688.
- Weltzin JF, Loik ME, Schwinning S, Williams DG, Fay PA, Haddad BM, Harte J, Huxman TE, Knapp AK, Lin G (2003) Assessing the response of terrestrial ecosystems to potential changes in precipitation. *BioScience*, **53**, 941-952.
- White SR, Carlyle CN, Fraser LH, Cahill JF (2012) Climate change experiments in temperate grasslands: synthesis and future directions. *Biology Letters*, **8**, 484-487.
- Wilson TB, Witkowski ETF (1998) Water requirements for germination and early seedling establishment in four African savanna woody plant species. *Journal of Arid Environments*, **38**, 541–550.
- Wu Z, Dijkstra P, Koch GW, Penuelas J, Hungate BA (2011) Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. *Global Change Biology*, **17**, 927-942.
- Yin X (2002) Responses of leaf nitrogen concentration and specific leaf area to atmospheric CO<sub>2</sub> enrichment: a retrospective synthesis across 62 species. *Global Change Biology*, **8**, 631-642.

Table 2.1 Univariate statistics based on the significant MANOVA results, showing the effects of watering regime and warming treatments on several grass and tree growth parameters. Bold values indicate significant differences ( $p < 0.05$ ).

Treatments	<i>df</i>	Final above-ground grass biomass (g)		Percentage tree seedling emergence (%)		Tree relative growth rate height (mm.mm <sup>-1</sup> .week <sup>-1</sup> )		Tree root: shoot		Tree specific leaf area (g/cm <sup>2</sup> )	
		<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Watering	2	<b>12.522</b>	<b>&lt;0.005</b>	<b>9.320</b>	<b>0.0151</b>	<b>8.826</b>	<b>0.008</b>	<b>15.841</b>	<b>&lt;0.005</b>	<b>14.381</b>	<b>&lt;0.005</b>
Warming	1	0.034	0.855	<b>6.946</b>	<b>0.0110</b>	<b>13.801</b>	<b>&lt;0.005</b>	<b>16.91</b>	<b>0.007</b>	<b>12.586</b>	<b>&lt;0.005</b>
Watering* Warming	2	0.187	0.865	1.703	0.309	<b>4.408</b>	<b>0.024</b>	<b>12.402</b>	<b>&lt;.005</b>	<b>8.697</b>	<b>&lt;0.005</b>
<i>Error df</i>		<i>53</i>		<i>53</i>		<i>53</i>		<i>53</i>		<i>53</i>	

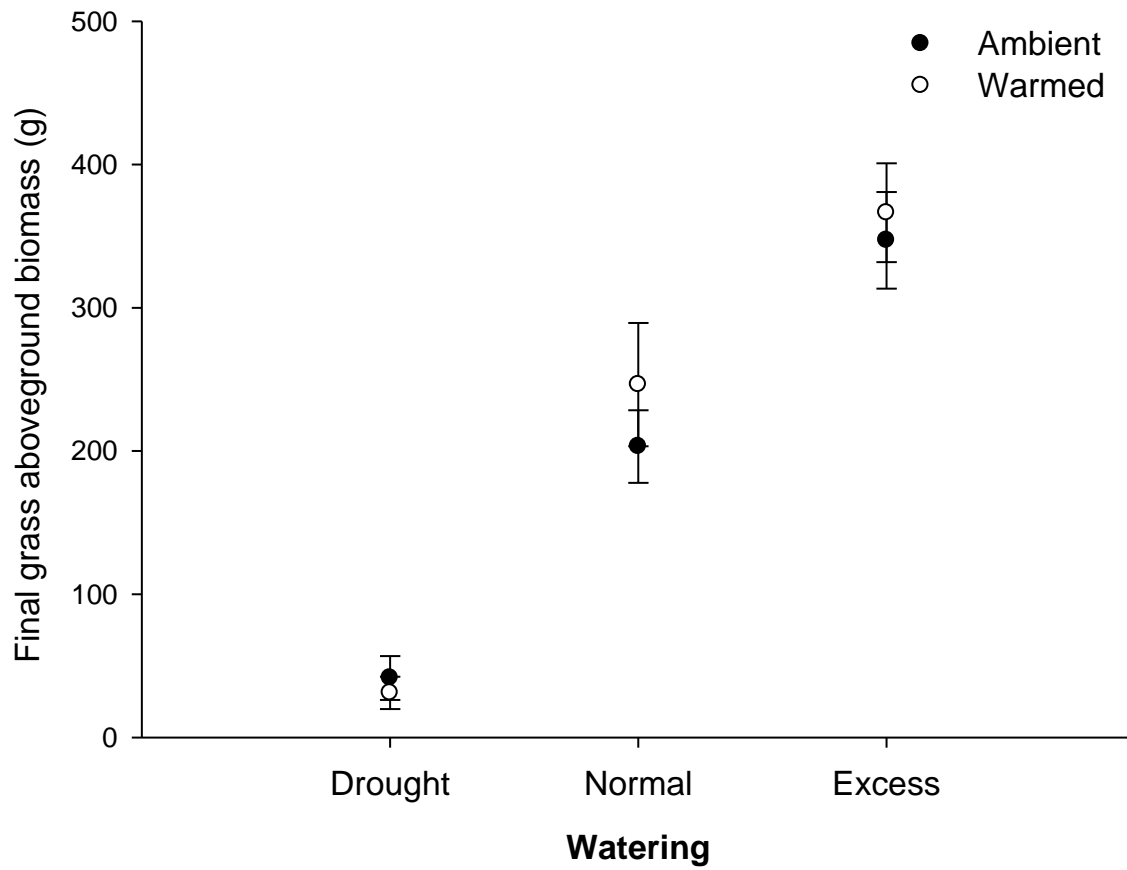


Figure 2.1 Mean above-ground grass biomass  $\pm$  SE after 14 days for the three watering regimes (drought, normal and excess) and two temperature treatments (● ambient and ○ warmed).

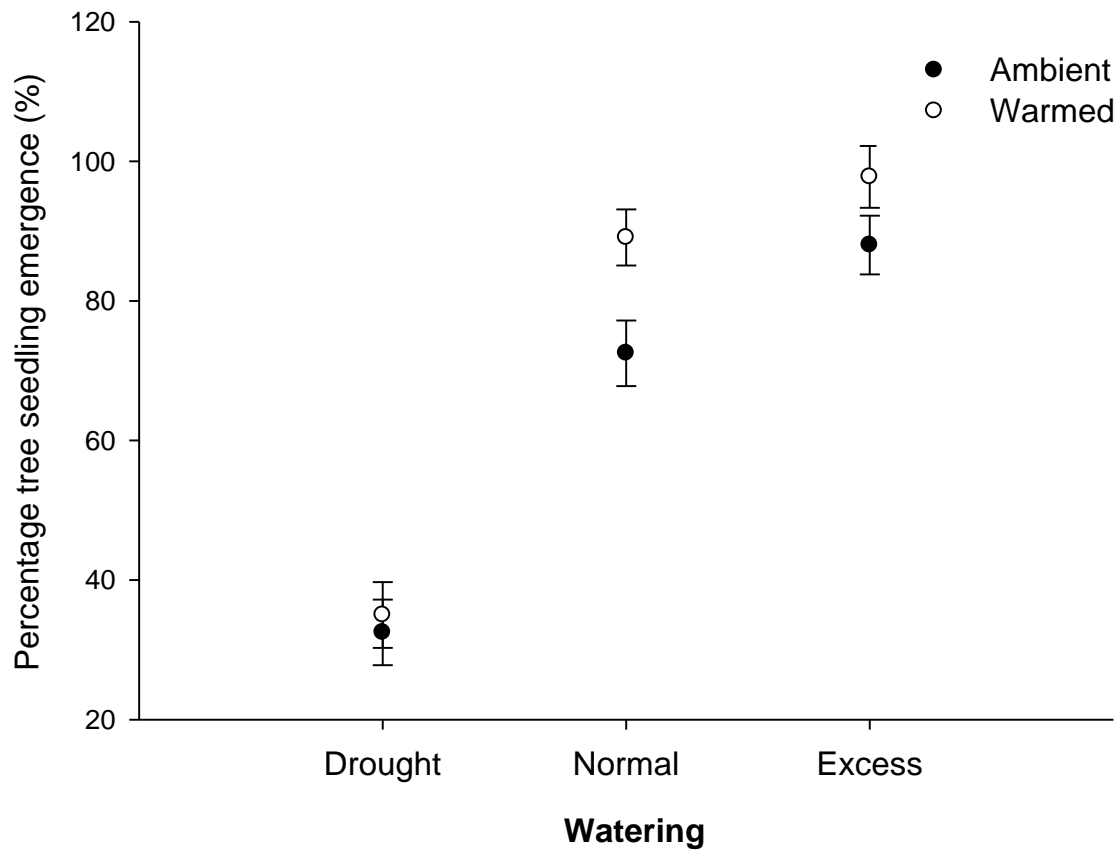


Figure 2.2 Mean percentage tree seedling emergence  $\pm$  SE after 14 days for the three watering regimes (drought, normal and excess) and two temperature treatments ( $\bullet$  ambient and  $\circ$  warmed).

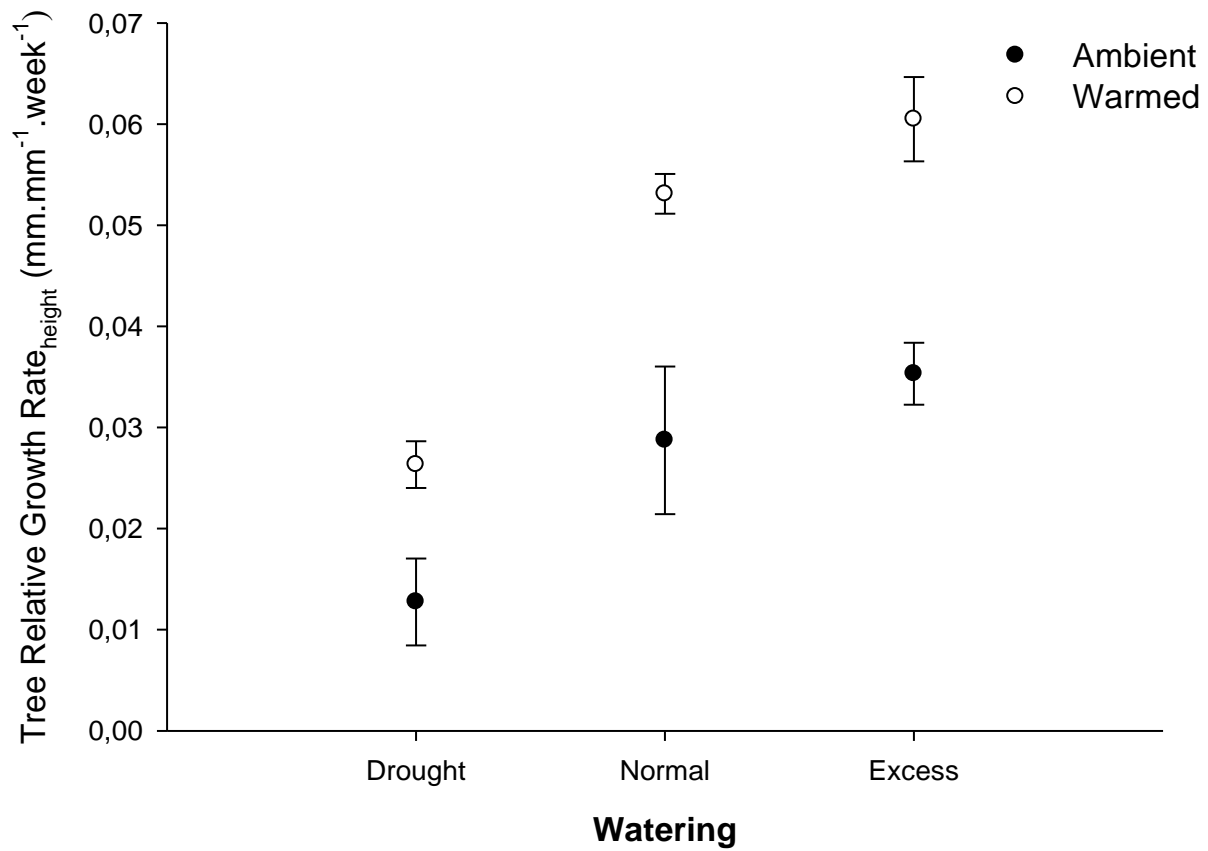


Figure 2.3 Mean tree relative growth rate in height (mm.mm<sup>-1</sup>.week<sup>-1</sup>) ± SE after 24 weeks, for the three watering regimes (drought, normal and excess) and two temperature treatments (● ambient and ○ warmed).

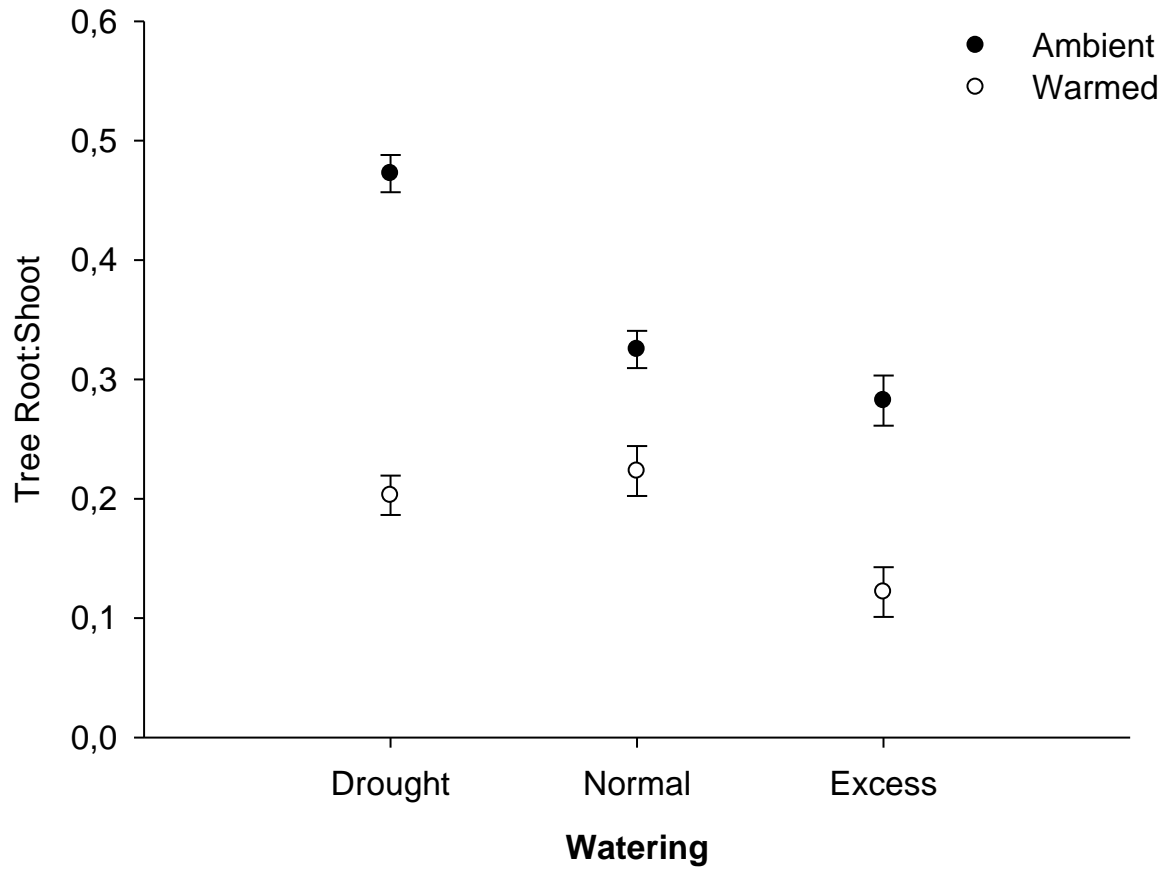


Figure 2.4 Mean tree root:shoot ratio  $\pm$  SE after 14 days, for the three watering regimes (drought, normal and excess) and two temperature treatments (● ambient and ○ warmed).

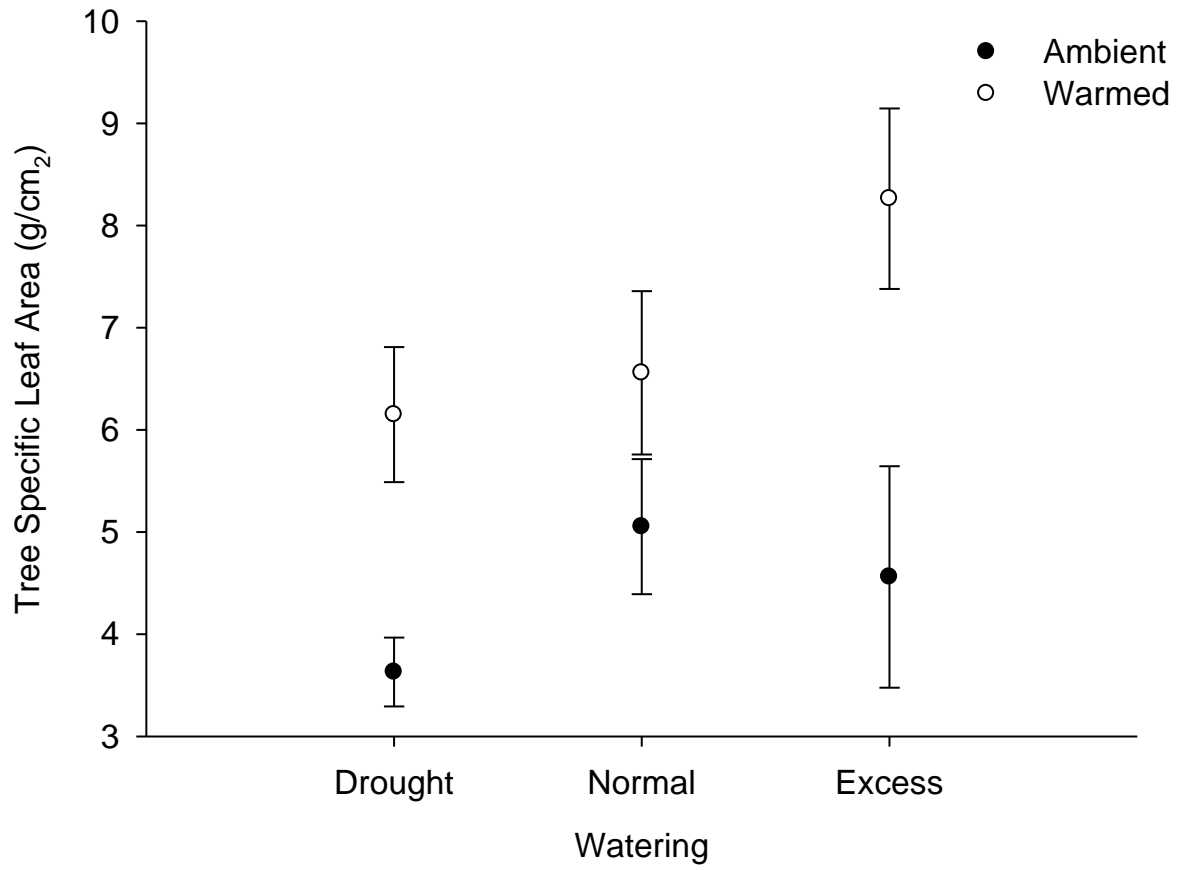


Figure 2.5 Mean tree SLA  $\pm$  SE after 14 days, for the three watering regimes (drought, normal and excess) and two temperature treatments (● ambient and ○ warmed).



**CHAPTER 3: Interactive effects of fire, fertilizer and grazing on an  
encroaching woody species: The importance of grass competition in limiting  
sapling growth and performance**

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### 3.1 Abstract

Understanding the factors controlling tree sapling recruitment is an integral research priority for savanna ecosystems, particularly for the management of woody plant encroachment. We investigated the effects of fertilizer, fire and grazing on the survival, growth and biomass of *Acacia sieberiana* using a completely randomized experimental design in the greenhouse. We simulated a typical savanna microhabitat by planting a common savanna grass species, *Eragrostis curvula*, together with saplings of *A. sieberiana*. The trees and grasses were allowed to establish for 12 weeks. Thereafter, treatments of fertilizer (100 kg N/ha), simulated grazing and fire were applied. Weekly measurements of relative growth rate ( $RGR_{\text{height}}$  and  $RGR_{\text{diameter}}$ ) were recorded. At the end of 24 weeks, survival, total above- and below-ground biomass, and key functional traits of specific leaf area and average thorn length were recorded to assess differences in tree performance. We found that fertilization was beneficial to grasses only, increasing their total biomass. We also observed increased mortality of tree saplings with higher grass biomass. Fire increased the tree saplings' investment in shoot growth and stem diameter. However, we found no evidence of differential shoot growth and stem diameter (post-fire allocation) when grass competition was absent. The presence of higher grass biomass was found to result in decreased specific leaf area of tree saplings. We conclude that grass competition is important for controlling the encroachment of *Acacia sieberiana*. High grass biomass reduces tree sapling survival and specific leaf area which is an indicator of photosynthetic ability.

#### Key-words

*Acacia sieberiana*, *Vachellia*, fire, fertilizer, grazing, competition, biomass, sapling survival

### 3.2 Introduction

The persistence of trees and grasses in savannas can be explained using either competition-based models or demographic bottleneck models (Chesson 2000; Sankaran et al. 2004). Both model types highlight that the factors affecting tree sapling recruitment have a key impact on savanna plant dynamics. In competition-based models, precipitation and nutrient availability are seen as key drivers of the tree-grass continuum (Walter 1971; Walker et al. 1981). Demographic bottleneck models, on the other hand, focus on factors that limit the establishment and subsequent survival of the woody plants in savanna ecosystems (Higgins et al. 2000; Sankaran et al. 2004). Disturbances such as fire and herbivory are factors that are emphasized in demographic bottleneck models (Bond et al. 2017). More importantly, the factors affecting the large-scale shifts in dominance and range expansion of woody plants (termed woody plant/shrub encroachment) (Bond and Midgley 2000; Ward 2005), experienced globally in savannas and grasslands, remain largely unclear: chronic cattle grazing and fire suppression are noted as important drivers in the African context (Bond and Keeley 2005; Stevens et al. 2017).

An additional factor of potential importance is nitrogen deposition, which is predicted to increase in southern Africa during the next few decades due to rising industrial emissions and changes in land use (Phoenix et al. 2006; Miyazaki et al. 2012). Tree sapling recruitment under conditions of increased nutrient availability, as well as changes in fire and grazing pressure, is an integral research priority for understanding savanna ecosystems, as asserted by several other studies (e.g., Higgins et al. 2000; Wiegand et al. 2006; Tjelele et al. 2015).

A meta-analysis of African savanna field sites showed a strong positive but asymptotic relationship between woody plant cover and mean annual precipitation (MAP) (Sankaran et al. 2005). Below 650 mm MAP, herbivory, fire, and soil properties limit woody cover, which depends linearly on MAP (Meyer et al. 2007). Above this threshold, savannas are unstable and

disturbances such as fire or herbivory are necessary to prevent woody plant encroachment or canopy closure (Sankaran et al. 2005). Tjelele et al. (2015) have experimentally demonstrated the positive effect of fire on seedling emergence and recruitment of two encroaching woody savanna species in South Africa (*Acacia nilotica* and *Dichrostachys cinerea*). Furthermore, using multifactorial experiments, Grellier et al. (2012) have shown that the removal of grass by grazing and/or fire had the most important beneficial effect on *Acacia sieberiana* tree seedling recruitment in savannas. Kraaij and Ward (2006) showed that frequent watering, nutrient control (i.e. no nitrogen fertilization) and grazing enhanced tree recruitment in semi-arid savanna with significant interactions between rain, nitrogen and grazing. They concluded that, in contrast to conventional wisdom that grazing alone causes encroachment, there are complex interactions between fire, nutrients, grazing and 'triggering' events such as unusually high precipitation. However, it is still unclear as to whether these factors play a role in regulating woody plant biomass in mesic-humid savannas receiving between 800-1500 mm MAP (Bond 2008).

The aim of this study was to investigate the effects of fertilization, grazing and fire on the early stages of growth, survival and functional traits of an encroaching tree species, *Acacia sieberiana* that occurs in mesic-humid savannas (Coates-Palgrave 2002). In mesic-humid savannas we would not expect precipitation to be a limiting factor. Hence we expect to observe significant independent and interactive effects of fertilization, fire and grazing on tree sapling establishment and growth. We used a completely randomized experimental design in the greenhouse to mimic a typical savanna microhabitat, and applied treatments of fertilizer, fire and grass clipping to simulate grazing. We hypothesized that:

1. N fertilizer would benefit grasses more than tree saplings (Kraaij and Ward 2006; Kambatuku et al. 2013) because these tree saplings are N<sub>2</sub>-fixing legumes which should not be limited by soil nitrogen (Kambatuku et al. 2013).

2. Simulated grazing should increase tree sapling growth and survival by removing the negative pressure of grass competition (Wiegand et al. 2006). Grazing may have a similar effect to fire by creating gaps and making below- and above-ground resources (i.e. water, nutrients, light) available for tree saplings to establish (Grellier et al. 2012; Tjelele et al. 2015).
3. The beneficial effect of burning in reducing above-ground grass biomass means that fires may indirectly enhance woody recruitment (Grellier et al. 2012; Tjelele et al. 2015). Because our study species, *Acacia sieberiana*, is known to resprout after fire (Hean and Ward 2012), and has a thick bark to protect itself against fires (Charles-Dominique et al. 2017), we predict that fire would not decrease the survival of tree saplings.
4. We were also interested in the interactions of these treatments, and the effects of grass biomass on overall performance of tree saplings. For example, we expect that grazing and fire would promote tree sapling growth by eliminating grass biomass and initiating resprouting (Vadigi and Ward 2012).
5. Furthermore, we predict that treatments that favour grasses over tree saplings would have an effect on key functional traits such as specific leaf area (SLA) and thorn length, considering that plants may display shifts in functional response traits due to intense competition (Pillay and Ward 2014). Higher SLA is indicative of greater photosynthetic ability, and greater thorn lengths offer higher levels of defense against herbivory (Cornelissen et al. 2003).

### 3.3 Materials and Methods

#### *Study species*

*Acacia sieberiana* DC. var. *woodii* (Burt Davy) Keay & Brenan (recently renamed *Vachellia sieberiana*; commonly referred to as the paperbark thorn) is a medium-sized (7-15 m), deciduous, flat-topped tree, found in southern Africa, north to Ethiopia. This tree species is known to encroach mountainous grasslands and savannas, particularly in the mesic-humid areas of southern Africa (Grellier *et al.*, 2013). The grass species selected for this study was *Eragrostis curvula* (Schrad.) Nees which is a widespread savanna grass species, common to KwaZulu-Natal province (South Africa) where the study was conducted.

#### *Experimental setup*

The experiment was carried out at the Botanical Gardens of the University of KwaZulu-Natal, Pietermaritzburg, South Africa. A tree-grass matrix was used to mimic a typical savanna microhabitat. To replicate the grass component of the savanna, we sowed *E. curvula*, a widespread savanna grass species, into ninety-six 95 L capacity bins (approximately 50 grass seeds per bin) containing Umgeni grit, an alluvial soil with a particle diameter of approximately 1.5 to 2 mm. At the beginning of the experiment, 200 seeds of *A. sieberiana* were chemically scarified for one hour by soaking in 1 M hydrochloric acid (HCl). Seeds were then rinsed repeatedly using distilled water, and soaked in boiling water overnight. Seeds were then treated with fungicide and germinated on agar plates. A single *A. sieberiana* sapling (height  $\pm$  10 cm) was placed at the centre of each of the 96 bins (Fig. 1).

Three treatments of fertilizer, fire and simulated grazing in all combinations were applied in a completely randomized design during the 24-week course of the experiment. Fertilizer was applied twice, as a standard NPK (nitrogen-phosphorus-potassium) fertilizer, with an application

rate of nitrogen of  $100\text{kg}\cdot\text{ha}^{-1}$ , following the application rate of Cramer et al. (2010). Grasses were cut after 12 weeks to ground level using clippers, to simulate intense grazing. Fire treatments were applied once at 12 weeks, using a butane blowtorch. One week prior to burning, the grass in the bins was cut and weighed. Thereafter, a mean volume of dry grass (60 g dry weight) was placed in each bin to standardize the fuel load and fire intensity per bin. Soil-temperature readings were measured after fire, using the Thermochron iButton® temperature logger, to ensure homogeneity of the fire treatment. Tree seedlings were scorched and sprouted from stems.

Tree survival, tree height and stem diameter were recorded weekly, and subsequently used to calculate relative growth rate (RGR). Calculations for relative growth rate of tree sapling stem height ( $\text{RGR}_{\text{height}}$ ) and relative growth rate of basal stem diameter ( $\text{RGR}_{\text{diameter}}$ ) were based on the natural logarithm measured in  $\text{mm}\cdot\text{mm}^{-1}\cdot\text{week}^{-1}$  (Kohi et al. 2009). After 24 weeks, tree specific leaf area and thorn length of three randomly selected leaves and thorns were recorded. Leave and thorn sampling was randomized by rotating the bins for collection, selecting leaves and thorns from three heights (high, medium and low). Thereafter, all trees and grasses were harvested. Tree roots were carefully washed free of sand to minimize loss of fine root material. Tree shoots and roots were oven dried at  $70\text{ }^{\circ}\text{C}$  for 24 hours. Root biomass is proportional to its carbohydrate content (Schutz et al. 2009) and can act as an indirect quantification of root reserves. Root and shoot dry biomasses were assessed to evaluate sapling resource allocation.

### ***Statistical analysis***

All statistical analyses were conducted in SPSS (version 18.0 for Windows). Differences in survival of tree saplings were assessed using pairwise  $\chi^2$  analysis of contingency tables. Effects of treatment variables (fertilizer, grazing and fire) on grass biomass, specific leaf area and thorn

length were analyzed using Multivariate Analysis of Variance (MANOVA), to control for Type I error caused by the analysis of multiple dependent variables.

We tested for an ‘envelope’ effect using an upper boundary regression technique to determine if tree performance (as measured by percentage survival or specific leaf area) was constrained by the biomass of neighbouring grasses (Lessin et al. 2001). Grass biomass was clustered into 20 g-width classes. This was done to eliminate the variance encountered when correlating ecological data, so that competitive interactions are easily detectable (Goldberg 1991; Lessin et al. 2001). That is, there is a maximal (theoretical) relationship between a dependent variable such as percentage sapling survival and an independent variable such as grass biomass. However, a plethora of factors may skew this relationship upwards at low neighbour densities. No downward skew is likely to occur because there is a constraint placed by neighbouring grass biomass on percentage sapling survival below which additional survival cannot occur. Such a relationship violates the assumption of conventional least-squares regression that there is homogeneity of variance (by definition, an ‘envelope’ results in lower variance at higher values of the independent variable, Ward et al. 2000).

### **3.4 Results**

#### ***Effects of fertilizer addition***

We found that fertilization had a significant effect on the biomass of grasses (Table 1); higher total grass biomass was found with fertilizer application (mean = 276g ± 63.64 SD) than those without fertilizer (mean = 122.4g ± 57.93 SD). As predicted, we observed a significant decrease in the survival of trees with increasing grass biomass ( $r = -0.70$ ,  $F_{1,6} = 14.007$ ,  $p < 0.05$ ; Fig. 2).

There was a significant difference in the mortality of tree saplings (range in  $\chi^2_{1,12} = 22.4-38.5$ ,  $p < 0.05$ ) for all treatments, with a higher percentage of dead plants in treatments with fertilizer



application (mean tree sapling mortality = 43,6 %  $\pm$  1.25 SD) than without fertilizer treatment (mean tree sapling mortality = 16,3 %  $\pm$  1.12 SD; Fig. 3).

### ***Effects of fire and grazing***

The presence of fire had a significant effect on the ratio of relative growth rate in height to relative growth rate in trunk diameter ( $RGR_{\text{height}}:RGR_{\text{diameter}}$ ), as well as the ratio of tree root: shoot biomass (Table 1). We found that the  $RGR_{\text{height}}:RGR_{\text{diameter}}$  of trees was significantly lower with fire treatment (mean = 0.301  $\pm$  0.107 SD) than without fire (mean = 0.559  $\pm$  0.108 SD), indicating that plants invest more in post-fire stem diameter growth. Similarly, the significant two-way interaction between grazing and fire on tree root: shoot biomass (Fig. 4) showed that there was a significant effect of fire on tree root: shoot biomass in the absence of grazing. The tree root: shoot biomass decreased significantly post-fire (without fire: mean = 1.776  $\pm$  0.795 SD; with fire: mean = 0.594  $\pm$  0.243 SD), indicating that trees invested more in shoot growth post-fire (Table 1). When grazing was present, the effect of fire was non-significant ( $p > 0.05$ ) for tree root: shoot biomass.

### ***Functional traits and grass competition***

We found a significant negative relationship between specific leaf area of tree saplings and grass biomass ( $r^2 = 0.68$ ,  $F_{1,62} = 6.600$ ,  $p = 0.01$ ), with higher tree SLA in bins with lower total grass biomass (Fig.5). We found no significant effect of any of the treatments on average thorn length (Table 1).

### 3.5 Discussion

Fertilizer application was beneficial for grasses only, resulting in increased grass biomass and higher tree sapling mortality. This indicates that at the recruitment stage of tree growth, supplementary nutrients will favour grass growth only, as was found by Kraaij and Ward (2006). Improved nutrient availability is more advantageous to grasses, assuming they are superior competitors for soil resources such as water and nutrients because of their fine-root morphology (Aerts et al. 1991; February et al. 2013; Wakeling et al. 2015). Rapid N absorption by C<sub>4</sub> grasses (Busso et al. 2001) such as the *Eragrostis curvula* we used in this experiment could effectively pre-empt soil resources and allow grasses to dominate. Alternatively, dense grass roots may occupy a large proportion of the available rooting space, thereby limiting the establishment of woody sapling roots by spatial exclusion (McConnaughay and Bazzaz 1991). This form of ‘non-resource competition’ could promote mortality of woody saplings.

Competition between plants results in trade-offs between growth, defence and reproduction (Suding et al. 2003). Increased grass biomass places competitive stress on neighboring tree saplings in savannas, adversely affecting their establishment and survival. Competitive interactions between tree saplings and grasses have been known to occur during the early stages of establishment, when rooting niche profiles are shared (Ward and Esler 2011; February et al. 2013), coupled with strong competition for light and nutrients (Vadigi and Ward 2012; Barbosa et al. 2014). The strong competitive effect of grasses on trees is consistent with several studies (Goldberg and Fleetwood 1987; Violle and Jiang 2009; Pillay and Ward 2014), all measuring plant performance against some measure of neighbourhood effect (neighbour height, biomass or density). Cramer et al. (2010), as well as Kraaij and Ward (2006), found that in mesic *Acacia* savannas, in the absence of water limitation, competition for N is the main constraint imposed by grass on growth of *Acacia* seedlings. Reduced grass cover by grazing may

ultimately lead to reduced competition for trees, thereby allowing woody plants to proliferate (Aranda et al. 2015; Tjelele et al. 2015). Tedder et al. (2014) investigated the effects of above- and belowground competition between grasses and two *Acacia* species. They found that root competition appeared to be the major factor influencing sapling growth rates. This is consistent with our findings, showing that grass competition is the major limiting factor for tree sapling recruitment. Our study, however, revealed no direct independent (main) effects of grazing on tree sapling growth and survival. However, we suspect that the treatments of simulated grazing were not able to realistically replicate the direct and indirect effects of herbivores in natural conditions, such as the indirect effects of trampling (Tjelele et al. 2015).

We found that tree saplings displayed a higher investment in post-fire shoot growth as opposed to root growth. This evidence further elucidates the resprouting ability of *Acacia sieberiana* (Hean and Ward 2012), and the inability of fire to constrain its establishment. In another *Acacia* species, Kgope et al. (2010) found that after simulated fire, *A. karroo* plants showed a greater increase in total stem length, total stem diameter and shoot dry weight, signaling the importance of resprouting following disturbances such as fire in savanna systems. Fire is a frequent disturbance in savannas, and is considered a fundamental factor that can either promote tree sapling establishment (Bond 2008), or reduce the survival of woody juveniles (Augustine and McNaughton 2004; Ward 2005; Higgins et al. 2007; Balfour and Midgley 2008; Bond 2008). While the increased frequency and intensity of fire may act to kill off woody plants (Wigley et al. 2009), fire may also promote tree sapling growth by removing grass biomass (Ward 2005) and may mobilize stored nonstructural carbohydrate root reserves (Vadigi and Ward 2012; Charles-Dominique et al. 2017). The outcome of a fire event is largely dependent on the fire strategy of the tree saplings. Trees may choose to invest heavily in post-fire stem growth (resulting in an increase in height) and subsequently protect the apical meristem from fires, thereby “escaping the fire trap” (Sabiiti and Wein 1987; Midgley and Bond 2001; Balfour and

Midgley 2006; Charles-Dominique et al. 2017). Alternatively, tree saplings may employ a diameter response whereby increased bark thickness serves as a buffer against scorching (Lawes et al. 2012; Charles-Dominique et al. 2017).

In this study we observed a typical post-fire response in stem diameter, whereby the ratio of relative growth rate in terms of height to diameter decreased (Fig. 3), indicating that these trees invest heavily in stem growth, increasing their root collar diameter as they resprout. This may be a strategy to develop resilience by increasing the thickness of the stem (Balfour & Midgley 2006), thereby increasing the bark area and thickness (Lawes et al. 2011). Under these conditions, fire may be an ineffective tool in reducing woody plant encroachment, particularly where constant fire pressure promotes bark thickness to a point where fire is no longer detrimental to the tree (Lawes et al. 2011; Charles-Dominique et al. 2017).

Allocation of carbon stocks after fire is determined by the total non-structural carbohydrates available to the plant. Schutz et al. (2009) investigated the carbon allocation and biomass partitioning patterns that enable a commonly occurring savanna tree, *Acacia karroo*, to survive frequent and repeated topkill. They found that the concentration of starch in the roots of the burnt plants was half that of the unburnt plants (Schutz et al. 2009). The total available carbon available for resprouting in turn is governed by the energetic constraints of nutrient acquisition and competition for resources (Friend et al. 1994). It is not surprising then that we only found differential investment between the above-ground and below-ground biomass after fire, when grasses were present (Fig. 3). Fire had no effect on the ratio of allocation to roots or shoots in the absence of grass competition (through elimination of grass by simulated grazing). This implies that grass competition places energetic constraints on surrounding trees, forcing differential allocation of carbon to roots or shoots after a disturbance such as fire. However, in the absence of grass competition, the trees we used in this experiment can invest similarly in root and shoot regeneration (Vilá 1997).

Violle and Jiang (2009) have shown that instantaneous measurements of certain plant traits can provide information on how plants perceive competitive environments. While there are many potential traits to consider (e.g. Cornelissen et al. 2003), specific leaf area (as an index of photosynthetic ability) and thorn length (as an index of defensive ability) (Plas et al. 2013) are the key traits we examined in this study. High SLA results in high mass-based photosynthetic rate (Cornelissen et al. 2003) but also low stress tolerance (Grime 1977; Reich et al. 2003). Consequently, species with high SLA are better able to withstand above-ground competition for light. However, when experiencing intense below-ground competition for soil resources, such plants may reduce their SLA as a tradeoff with below-ground competition. In this study, we found no obvious effects of treatments on thorn length. However, we found higher tree SLA where surrounding grass biomass was lower (Fig. 4), which again highlights the detrimental effects of grass competition on the performance of savanna tree saplings.

### **3.6 Conclusions**

We have shown that nitrogen addition reduces the establishment and growth of early-stage saplings. This reduction in growth is achieved indirectly by the promotion of grass biomass. Similarly, we found that fire promotes woody plant establishment, by reducing grass cover and hence the competitive pressure by grasses on trees. While fire also promoted shoot growth of the woody resprouter *Acacia sieberiana* (see also Grellier et al. 2012), this may not be true for woody plants that are incapable of resprouting (Bond and Midgley 2001; Clark et al. 2013). Additionally, more frequent and intense fire may reduce sapling survival through the depletion of below-ground carbon stocks. Overall, we advocate that when water availability is not a limiting factor, nutrient addition, fire and sufficient grass cover can be used to control woody plant encroachment.

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### 3.8 References

- Aerts R, Boot RGA, van der Aart PJM (1991) The relation between above- and belowground biomass allocation patterns and competitive ability. *Oecologia* 87: 551-559.
- Augustine DJ, McNaughton SJ (2004) Regulation of shrub dynamics by native browsing ungulates on East African rangeland. *Journal of Applied Ecology* 41: 45-58.
- Aranda MJ, Tognetti PM, Mazía N (2015) Grass competition surpasses the effect of defoliation on a woody plant invader. *Acta Oecologica* 68:37-42.
- Balfour DA, Midgley JJ (2006) Fire-induced stem death in an African *Acacia* is not caused by canopy scorching. *Austral Ecology* 31: 892-896.
- Balfour DA, Midgley JJ (2008) A demographic perspective on bush encroachment by *Acacia karroo* in Hluhluwe-Imfolozi Park, South Africa. *African Journal of Range and Forage Science* 25: 147-151.
- Barbosa ER, Tomlinson KW, Carvalheiro LG, Kirkman K, de Bie S, Prins HH, van Langevelde F (2014) Short-term effect of nutrient availability and rainfall distribution on biomass production and leaf nutrient content of savanna tree species. *PLoS ONE* 9: e92619. [doi: 10.1371/journal.pone.0092619].
- Bond WJ (2008) What limits trees in C<sub>4</sub> grasslands and savannas? *Annual Review of Ecology, Evolution, and Systematics* 39: 641-659.
- Bond WJ, Keeley JE (2005) Fire as a global 'herbivore': the ecology and evolution of flammable ecosystems. *Trends in Ecology and Evolution* 20: 387-394.
- Bond WJ, Midgley GF (2000) A proposed CO<sub>2</sub> mechanism of woody plant invasion in grasslands and savannas. *Global Change Biology* 6: 865-869.
- Bond WJ, Midgley JJ (2001) Ecology of sprouting in woody plants: the persistence niche. *Trends in Ecology and Evolution* 16:45–51.
- Bond WJ, Staver AC, Cramer MD, Wakeling JL, Midgley JJ, Balfour DA (2017) Demographic bottlenecks and savanna tree abundance. In: Cromsigt J.P.G.M, Archibald S. and Owen-Smith N. (ed), *Conserving Africa's Mega-Diversity in the Anthropocene: The Hluhluwe-Imfolozi Park story*. Cambridge University Press. pp 161-188.
- Busso CA, Briske DD, Olalde-Portugal V (2001) Root traits associated with nutrient exploitation following defoliation in three coexisting perennial grasses in a semi-arid savanna. *Oikos* 93: 332-342.

- Charles-Dominique T, Midgley GF, Bond WJ (2017) Fire frequency filters species by bark traits in a savanna–forest mosaic. *Journal of Vegetation Science* 28: 728-735.
- Chesson P (2000) General theory of competitive coexistence in spatially-varying environments. *Theoretical Population Biology* 58: 211-237.
- Clarke PJ, Lawes MJ, Midgley JJ, Lamont BB, Ojeda F, Burrows GE, Enright NJ, Knox KJE (2013) Resprouting as a key functional trait: how buds, protection and resources drive persistence after fire. *New Phytologist* 197:19-35.
- Coates-Palgrave M (2002) Keith Coates-Palgrave's trees of southern Africa. Struik, Cape Town, South Africa.
- Cornelissen JHC, Lavorel S, Garnier E, Diaz S, Buchmann N, Gurvich DE, Reich PB, Ter Steege H, Morgan HD, Van Der Heijden MGA, Pausas JG, Poorter H (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany* 51: 335-380.
- Cramer MD, Van Cauter A, Bond WJ (2010) Growth of N<sub>2</sub>-fixing African savanna *Acacia* species is constrained by below-ground competition with grass. *Journal of Ecology* 98: 156-167.
- February EC, Higgins SI, Bond WJ, Swemmer L (2013) Influence of competition and rainfall manipulation on the growth responses of savanna trees and grasses. *Ecology* 94: 1155-1164.
- Friend AL, Coleman MD, Isebrands JG (1994) Carbon allocation to root and shoot systems of woody plants. In: Davis TD, Haissig BE (eds.), *Biology of Adventitious Root Formation*. Plenum Publishing Corporation, New York, pp. 245-273.
- Goldberg DE, Fleetwood L (1987) Competitive effect and response in four annual plants. *Journal of Ecology* 75: 1131-1143.
- Goldberg DE, Keith L (1991) Competitive effect and response: hierarchies and correlated traits in the early stages of competition. *Journal of Ecology* 79: 1013-1030.
- Grellier S, Barot S, Janeau JL, Ward D (2012) Grass competition is more important for *Acacia* recruitment than seed ingestion by livestock. *Plant Ecology* 213: 899-908.
- Grellier S, Ward D, Janeau JL, Podwojewski P, Lorentz S, Abbadie L, Valentin C, Barot S (2013) Positive versus negative environmental impacts of tree encroachment in South Africa. *Acta Oecologica* 53: 1-10.
- Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist* 111: 1169-1194.



- Hean J, Ward D (2012) Fire and herbivory are not substitutable: evidence from regrowth patterns and changes in physical and chemical defences in *Acacia* seedlings. *Journal of Vegetation Science* 23: 13-23.
- Higgins SI, Bond WJ, February EC, Bronn A, Euston-Brown DIW, Enslin B, Govender N, Rademan L, O'Regan S, Potgieter ALF, Scheiter S, Sowry R, Trollope L, Trollope WSW (2007) Effects of four decades of fire manipulation on woody vegetation structure in savanna. *Ecology* 88: 1119–1125.
- Higgins SI, Bond WJ, Trollope WSW (2000) Fire, resprouting and variability: a recipe for grass-tree coexistence in savanna. *Journal of Ecology* 88: 213-229.
- Kambatuku J, Cramer M, Ward D (2013) Nitrogen fertilisation reduces grass-induced N<sub>2</sub> fixation of tree seedlings from semi-arid savannas. *Plant and Soil* 365: 307-320.
- Kgope BS, Bond WJ, Midgley GF (2010) Growth responses of African savanna trees implicate atmospheric [CO<sub>2</sub>] as a driver of past and current changes in savanna tree cover. *Austral Ecology* 35: 451-463.
- Kohi EM, de Boer FW, Slot M, van Wieren SE, Ferwerda JG, Grant RC, Heitkonig IMA, de Knecht HJ, van der Waal C, Prins HHT (2009) Effects of simulated browsing on growth and leaf chemical properties in *Colophospermum mopane* saplings. *African Journal of Ecology* 48: 190–196.
- Kraaij T, Ward D (2006) Effects of rain, nitrogen, fire and grazing on tree recruitment and early survival in bush-encroached savanna, South Africa. *Plant Ecology* 186: 235-246.
- Lawes MJ, Adie H, Russell-Smith J, Murphy B, Midgley JJ (2011) How do small savanna trees avoid stem mortality by fire? The roles of stem diameter, height and bark thickness. *Ecosphere* 2: art42. [doi: 10.1890/ES10-00204.1].
- Lawes MJ, Midgley JJ, Clarke PJ (2013) Costs and benefits of relative bark thickness in relation to fire damage: a savanna/forest contrast. *Journal of Ecology* 101: 517-524.
- Lessin LM, Dyer AR, Goldberg DE (2001) Using upper boundary constraints to quantify competitive response of desert annuals. *Oikos* 92: 153-159.
- McConnaughay KDM, Bazzaz FA (1991) Is physical space a soil resource? *Ecology* 72: 94-103.
- Meyer KM, Wiegand K, Ward D, Moustakas A (2007) SATCHMO: A spatial simulation model of growth, competition, and mortality in cycling savanna patches. *Ecological Modelling* 209: 377-391.
- Midgley JJ, Bond WJ (2001) A synthesis of the demography of African *Acacias*. *Journal of Tropical Ecology* 17: 871–886.

- Miyazaki K, Eskes HJ, Sudo K (2012) Global NO<sub>2</sub> emission estimates derived from an assimilation of OMI tropospheric NO<sub>2</sub> columns. *Atmospheric Chemistry and Physics* 12: 2263-2288.
- Phoenix GK, Hicks WK, Cinderby S, Kuylenstierna JC, Stock WD, Dentener FJ, Giller KE, Austin AT, Lefroy RD, Gimeno BS (2006) Atmospheric nitrogen deposition in world biodiversity hotspots: the need for a greater global perspective in assessing N deposition impacts. *Global Change Biology* 12: 470-476.
- Pillay T, Ward D (2014) Competitive effect and response of savanna tree seedlings: comparison of survival, growth and associated functional traits. *Journal of Vegetation Science* 25: 226-234.
- Plas F, Howison R, Reinders J, Fokkema W, Olf H (2013) Functional traits of trees on and off termite mounds: understanding the origin of biotically-driven heterogeneity in savannas. *Journal of Vegetation Science* 24: 227-238.
- Reich PB, Wright IJ, Cavender-Bares J, Craine JM, Oleksyn J, Westoby M, Walters MB (2003) The evolution of plant functional variation: traits, spectra, and strategies. *International Journal for Plant Sciences* 164: S143-S164.
- Sabiiti EN, Wein RW (1987) Fire and *Acacia* seeds: a hypothesis of colonization success. *Journal of Ecology* 74: 937-946.
- Sankaran M, Hanan NP, Scholes RJ, Ratnam J, Augustine DJ, Cade BS, Gignoux J, Higgins SI, Le Roux X, Ludwig F, Ardo J, Banyikwa F, Bronn A, Bucini G, Caylor KK, Coughenour MB, Diouf A, Ekaya W, Feral CJ, February EC, Frost PGH, Hiernaux P, Hrabar H, Metzger KL, Prins HHT, Ringrose S, Sea W, Tews J, Worden J, Zambatis N (2005) Determinants of woody cover in African savannas. *Nature* 438: 846-849.
- Sankaran M, Ratnam J, Hanan NP (2004) Tree–grass coexistence in savannas revisited – insights from an examination of assumptions and mechanisms invoked in existing models. *Ecology Letters* 7: 480-490.
- Schutz A, Cramer MD, Bond WJ (2009) Juggling carbon: allocation patterns of a dominant tree in a fire-prone savanna. *Oecologia* 160: 235-246.
- Stevens N, Lehmann CE, Murphy BP, Durigan G (2017) Savanna woody encroachment is widespread across three continents. *Global Change Biology* 23: 235-244.
- Suding KN, Goldberg DE, Hartmann (2003) Relationships among species traits: separating levels of response and identifying linkages to abundance. *Ecology* 84: 1-16.

- Tedder M, Kirkman K, Morris C, Fynn R (2014) Tree–grass competition along a catenal gradient in a mesic grassland, South Africa. *Grassland Science* 60: 1-8.
- Tjelele J, Ward D, Dziba L (2015) The effects of seed ingestion by livestock, dung fertilization, trampling, grass competition and fire on seedling establishment of two woody plant species. *PLoS ONE* 10: e0117788. [doi:10.1371/journal.pone.0117788].
- Vadigi S, Ward D (2012) Fire and nutrient gradient effects on the sapling ecology of four *Acacia* species in the presence of grass competition. *Plant Ecology* 213: 1793-1802.
- Vilá M (1997) Effect of root competition and shading on resprouting dynamics of *Erica multiflora* L. *Journal of Vegetation Science* 8: 71-80.
- Violle C, Jiang L (2009) Towards a trait-based quantification of species niche. *Journal of Plant Ecology* 2: 87-93.
- Wakeling J, Bond W, Ghai M, February E (2015) Grass competition and the savanna-grassland ‘treeline’: a question of root gaps? *South African Journal of Botany* 101: 91-97.
- Walker BH, Ludwig D, Holling CS, Peterman RM (1981) Stability of semi-arid savanna grazing systems. *Journal of Ecology* 69: 473-498.
- Walter H (1971) *Ecology of tropical and subtropical vegetation*. Oliver and Boyd, Edinburgh, UK.
- Ward D (2005) Do we understand the causes of bush encroachment in African savannas? *African Journal of Range and Forage Science* 22: 101-105.
- Ward D, Esler KJ (2011) What are the effects of substrate and grass removal on recruitment of *Acacia mellifera* seedlings in a semi-arid environment? *Plant Ecology* 212: 245-250.
- Ward D, Ngairorue BT, Karamata J, Kapofi I (2000) The effects of communal and commercial pastoralism on vegetation and soils in an arid and a semi-arid region of Namibia. In: White PS, Mucina L, Leps J, Van Der Maarel E (eds), *Vegetation Science in Retrospect and Perspective*. Opulus Press, Uppsala, Sweden, pp. 344-347.
- Wiegand K, Saltz D, Ward D (2006) A patch-dynamics approach to savanna dynamics and woody plant encroachment - insights from an arid savanna. *Perspectives in Plant Ecology, Evolution and Systematics* 7: 229-242.
- Wigley B, Cramer MD, Bond WJ (2009) Sapling survival in a frequently burnt savanna: mobilisation of carbon reserves in *Acacia karroo*. *Plant Ecology* 203: 1-11.

Table 3.1 Univariate statistics based on the significant MANOVA results, showing the effects of fertilizer, grazing, burning and their interactions on growth parameters of the tree species *Acacia sieberiana* and the grass *Eragrostis curvula*.

	RGR <sub>h</sub> :RGR <sub>d</sub>		Tree root: shoot biomass		Thorn length		Grass biomass	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Fertilizer	1.21	0.275	0.04	0.82	1.54	0.222	<b>14.59</b>	<b>0.001</b>
Grazing	1.26	0.268	0.57	0.45	1.33	0.255	-	-
Fire	<b>28.76</b>	<b>&lt;0.001</b>	<b>4.46</b>	<b>0.04</b>	1.46	0.082	3.49	0.070
Fertilizer * grazing	5.57	0.241	0.93	0.33	0.17	0.678	3.77	0.061
Fertilizer * Fire	1.74	0.196	0.07	0.78	2.35	0.135	0.52	0.473
Grazing * Fire	3.55	0.068	<b>5.097</b>	<b>0.03</b>	5.09	0.031	2.74	0.107
Error df	41		41		41		41	

Ratio of relative growth rate height (RGR<sub>height</sub>) to relative growth rate in stem basal diameter (RGR<sub>diam</sub>) was measured in mm.mm<sup>-1</sup>.week<sup>-1</sup>. Tree root: shoot biomass and grass biomass measured in g, and final log<sub>10</sub> transformed thorn length measured in mm were used for the analysis. The error df, F-value and significance are indicated per factor and factor interactions. Significant values are indicated in bold. No values are indicated for Grazing under Grass biomass because we were testing the effect of grass biomass.



Fig. 3.1 Experimental set-up in the greenhouse, showing placement of bins.

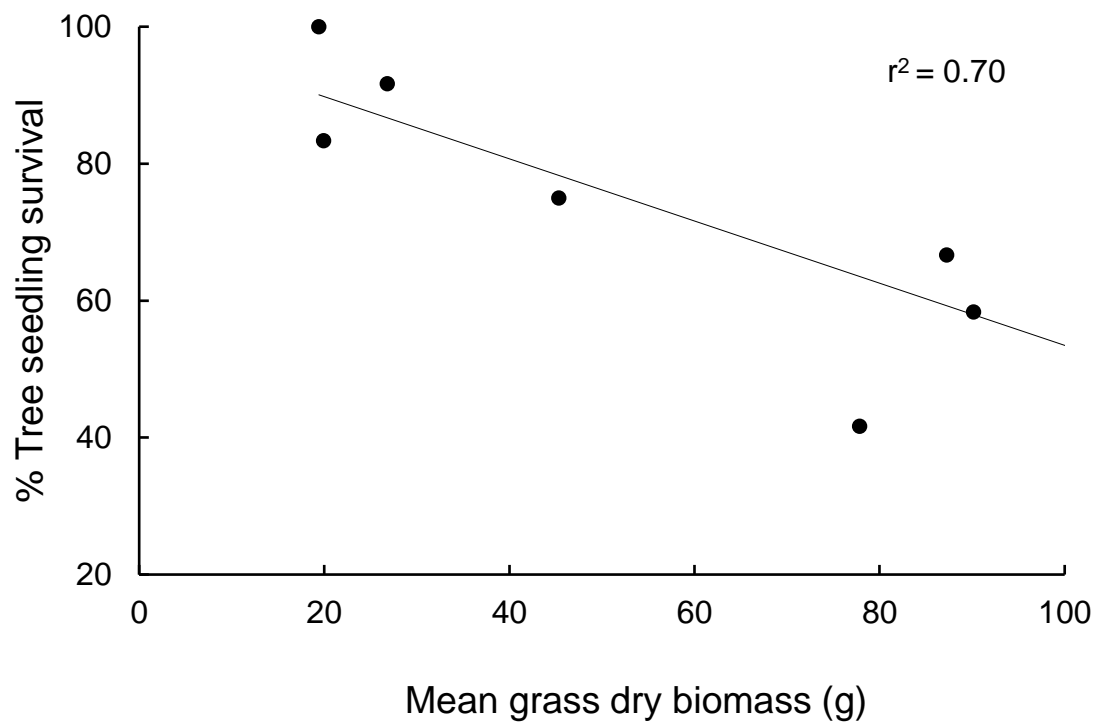


Fig. 3.2 Tree sapling survival (%) plotted against mean grass dry biomass. Closed circles (●) represent the mean % tree sapling survival within the respective grass biomass cluster.

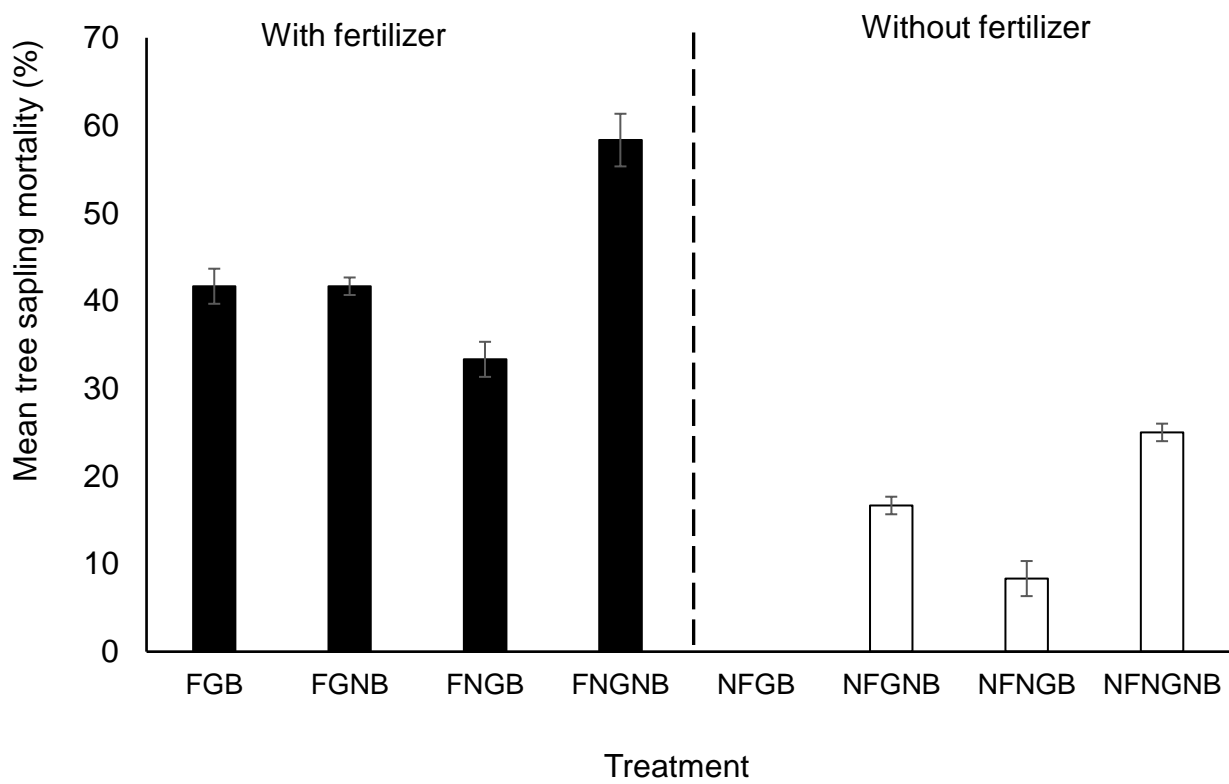


Fig. 3.3 Mean  $\pm$  SD tree sapling mortality (%) in each treatment group after 24 weeks. NF = no fertilizer, F = fertilizer, NG = no grazing, G = grazing, NB = no fire, B = fire.

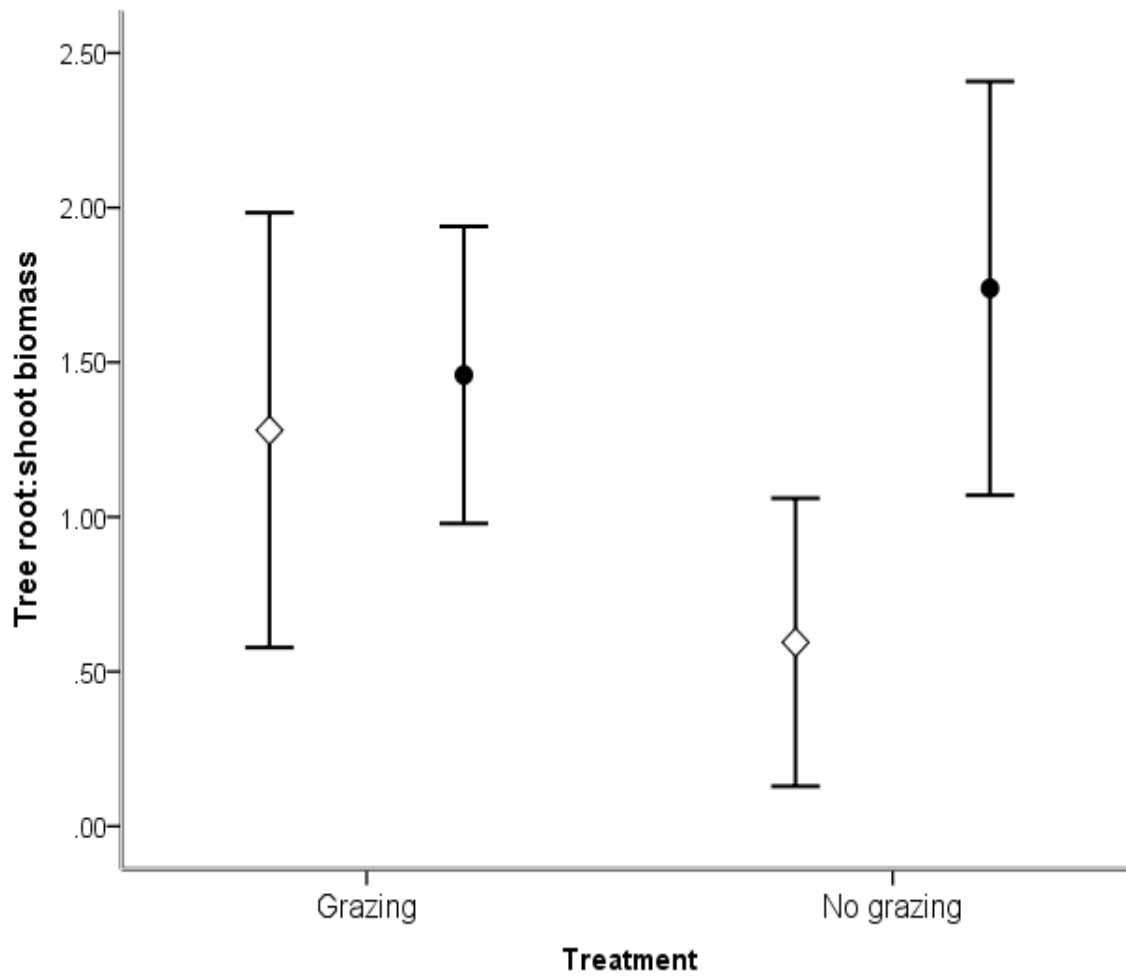


Fig. 3.4 Mean  $\pm$  95 % C.I tree root: shoot biomass with grazing and fire treatment. Open diamonds ( $\diamond$ ) indicate the “fire” treatment, and the closed circles ( $\bullet$ ) indicate the “no fire” (control) treatment.



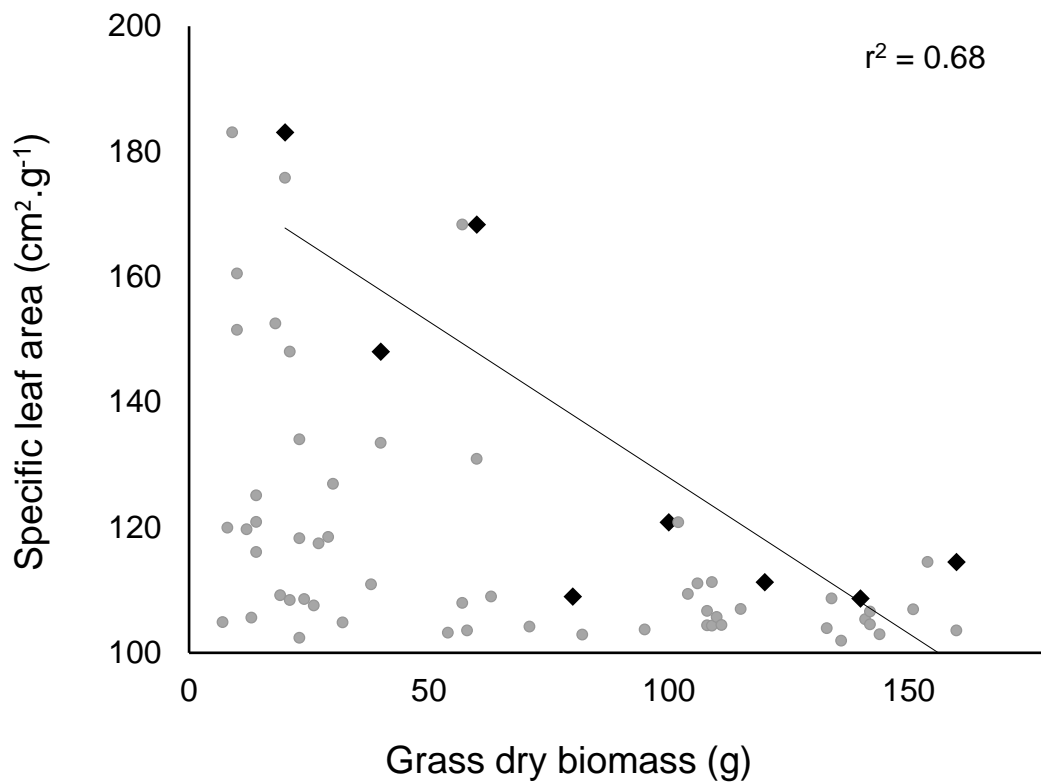


Fig. 3.5 Specific leaf area (cm<sup>2</sup>.g<sup>-1</sup>) plotted against grass dry biomass (g). Circles (●) indicate the data points of specific leaf area against grass biomass. Diamonds (◆) indicate the data points of the regression using the maximum specific leaf area plotted against grass biomass clustered into width classes of 20 g.

## **CHAPTER 4: Soil, litter and plant dynamics across a precipitation gradient: the effects of fertilization and woody plant encroachment**

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#### **4.1 Abstract**

Complex interactions between woody plant encroachment and nutrient availability may vary along precipitation gradients. With further predicted increases in N deposition due to increasing industrial emissions and changes in land use, potential increases in N storage and cycling are expected. We conducted a field experiment that tested whether nitrogen (N) and phosphorus (P) had independent effects on woody plant encroachment along a precipitation gradient, or whether there was co-limitation (additive effect) of N + P. We added these nutrients to adjacent encroached and open grasslands (ranging from 300 – 1,500 mean annual precipitation). We compared differential responses to fertilizer addition and encroachment in terms of soil N stocks and P, litter % N and C:N ratio (index of decomposition), plant productivity and species richness. In addition, we determined whether there were trends in responses to increased nutrient availability and encroachment across a precipitation gradient. Soil N stocks were on average 15 times higher in the higher precipitation sites regardless of nutrient addition or encroachment. Larger variation in soil P was noted at the high precipitation sites, with some evidence of P-limitation in the encroached area with the highest precipitation. We found significant positive effects of encroachment on litter % N (quality), C:N ratios and forb cover across the precipitation gradient. Fertilization increased grass biomass and reduced species richness in the high-precipitation sites only. The purportedly beneficial effects of encroachment on litter and plant species richness was more pronounced at the high-precipitation sites. Overall, increased nutrient availability was found to be of importance in high precipitation sites only, possibly due to water scarcity at the low-precipitation sites.

#### **Keywords:**

Nitrogen, Phosphorus, fertilization, encroachment, grassland, litter, species richness, *Acacia*, *Vachellia*.

## 4.2 Introduction

Woody plant encroachment (also known as bush or shrub encroachment) refers to the increasing prevalence of woody vegetation, which suppresses the palatable grass layer (Bond and Midgley 2012; Archer and others 2017). In southern Africa, tree and shrub densities have increased by 30-50% since the late 1800s (Hoffman and Ashwell 2001; Bond 2008; Russell and Ward 2014; Ward and others 2014). Changes in plant species composition caused by woody plant encroachment have been shown to increase the storage, turnover, and availability of nitrogen (N) in many ecosystems (Liao and others 2006; Yusuf and others 2015). With further predicted increases in N deposition due to increasing industrial emissions and changes in land use (Miyazaki and others 2012), potential increases in N storage and cycling are expected (Reich and others 2006; Vourlitis and others 2007).

Previous studies have described savannas as water- and N-limited (Scholes and Archer 1997; Kraaij and Ward 2006; Throop and others 2012), but little attention has been focused on potential phosphorus (P) limitations in these ecosystems (Ries and Shugart 2008). Furthermore, because many woody encroaching species are N<sub>2</sub>-fixing legumes, they usually have a high P requirement (Vitousek and others 2002), thereby increasing the availability of soil N relative to P (Hibbard and others 2001; Cech and others 2008), which may lead to P-limiting conditions over time (Blaser and others 2014). Sankaran and others (2008) found soil P to be the most important soil parameter influencing woody cover after mean annual precipitation (MAP) and fire. However, Craine and others (2008) have indicated that there may be co-limitation of N and P in South African grasslands. Indeed, Harpole and others (2011) have found that co-limitation with N and P are common in the world's grasslands.

Environmental conditions, such as increased nutrient availability and increased woody plant density, affect litter quality and decomposer activity, and ultimately have the potential to

substantially alter litter decomposition (Zhu and others 2016). Efficient high N reabsorption before senescence or low N requirements are often reflected in low N concentrations and high C:N ratios in leaf litter, which results in low rates of decomposition and N mineralization (Satti and others 2003). Consequently, the C:N ratio in litter has been utilized as an indicator of N cycling, with lower C:N ratios indicative of higher rates of decomposition (Manzoni and others 2010, Ward and others 2018).

Differences in litter quality and plant productivity linked to woody plant encroachment (Vitousek and others 1997; Phoenix and others 2006) may be explained by MAP. Studies that have examined the effects of woody plant encroachment along a precipitation gradient have revealed a number of important relationships between precipitation and changes in litter quality and plant productivity. For example, Knapp and others (2008) found that there was a linear relationship between precipitation and annual net primary production (ANPP) for grassland sites, but a non-linear relationship for shrub-encroached sites, with an asymptote at about 600 mm MAP. Furthermore, several studies report a hump in the species richness–biomass relationship (Crawley and others 2005; Dingaen and others 2017; Ward and others 2017). Because N is usually limiting across grasslands, particularly in southern Africa, N fertilization is often associated with increased productivity (Gough and others 2000; Ward and others 2017). Therefore, one may expect reduced species richness with nitrogen fertilization (Ward and others 2017).

We designed a two-year field experiment to explore the effects of fertilizer addition and woody plant encroachment on soil (N stocks and P), litter (N and C:N ratio) and plant productivity and richness along a precipitation gradient (MAP from 300 to 1,500 mm per year). Comparisons were made between four pairs of adjacent grasslands, in which one of each pair was invaded by woody species <100 years ago. We hypothesized that fertilizer effects on soil,

litter and plant dynamics would be evident in the high precipitation sites, while the effects of encroachment on these variables would be more pronounced at the low precipitation sites.

We predicted that:

1. Fertilizer addition will increase soil available N through increased biomass production, and litter inputs into the soil (Knapp and others 2008), with higher litter quality and lower C:N ratios (Cleveland and Liptzin 2007) in the higher precipitation encroached sites, as compared to the lower precipitation sites.
2. Soil P will be lower in the encroached plots at the higher precipitation sites, due to P limitation caused by N<sub>2</sub>-fixing legumes (Blaser 2014).
3. Fertilizer addition will increase productivity and reduce plant species richness at higher precipitation sites, due to interspecific competition (Ward and others 2017).
4. Similarly we expect a decline in species richness with encroachment. Woody encroachment may also also increase ANPP (Knapp and others 2008), which is often correlated with a decline in plant species diversity (Clark and others 2007).

### **4.3 Methods**

#### *Study sites*

This study was conducted at four sites along a precipitation gradient in South Africa (Table 1).

The spatial variation in precipitation in southern Africa displays an increasing gradient of precipitation from west to east, coinciding with the precipitation gradient of our study sites (Figure 1). Each site contained two distinct vegetation types, i.e. adjacent grassland and shrub/woodland communities, where management practices (intense grazing or absence of fire) may have contributed to woody plant expansion into native grasslands in the last 100 years (Bond and Midgley 2000; Ward 2010; Ward and others 2014).

### *Experimental design and sampling*

At each of the four study sites, 12 open grassland plots and 12 adjacent encroached plots (10 m x 10 m) were demarcated. Encroached plots were classified as having at least 40% woody cover rooted within the boundary of each plot (Wiegand and others 2006). We conducted a two-year-long fertilization field trial, where N (urea at 100 kg N/ha), P (superphosphate at 10 kg P/ha) and N + P were added to encroached and open grasslands. Fertilizers were hand-broadcast at the start of the rainy season (2014 and 2015) to promote penetration into the ground, and to minimize volatilization. Soil surface horizons are the most likely to be altered due to shifts in soil nutrient status (Gill and Burke 1999). We collected soil samples at the surface (0-10 cm) from each treatment (n = 12) and control (no fertilization; n = 12) plot at the start of the experiment (2013) and after two years (2015) within the encroached and open grassland plots at each site.

We measured soil % N (weight N per weight soil) and soil available P (mg kg<sup>-1</sup>). Soil N percentages were converted using soil bulk density, obtained by the core method (Blake and Hartage 1987) at each site, to organic N stocks (g m<sup>-2</sup>). For bulk-density calculations, five cores per plot were collected. Core samples were taken 1m from the four corners and the centre of each plot. It should be noted that soils at the four study sites were fine enough to allow for determination with cores, without the biases introduced by coarse fragments (Throop and others 2012). Representative surface-litter samples were also collected from each plot (n = 24, and litter % N and % C was measured. Litter C:N ratios were used as an index of decomposition (Manzoni and others 2010). Soil and litter samples were oven dried at 70 °C for 24 hours and then passed through a 1-mm sieve. For analysis of nitrogen and carbon, the samples were combusted in a Flash EA 1112 series elemental analyser (Thermo Finnigan, Milan, Italy). The gases were passed to a Delta Plus XP IRMS (isotope ratio mass spectrometer - IRMS) (Thermo Electron, Bremen, Germany), via a Conflo III gas control unit (Thermo Finnigan, Bremen, Germany). Only the % C

and N values from the IRMS data were used for this study, and not the stable isotope data. For the analysis of phosphorus we used an Ambic 1 extraction process (Thompson, 1995), followed by a modification of the Murphy and Riley (1962) molybdenum blue procedure (Hunter and others 1974).

### ***Grass nutrient concentration, grass biomass, proportion of forbs and species richness***

Two diagonal transects were set up in each plot. Plant species presence (grass, forb or woody plant) and grass biomass was recorded at every 50 cm interval along the transect. All of the grasses at each site are C<sub>4</sub> grasses. Grass biomass was measured non-destructively using a disc pasture meter (Bransby and Tainton 1977). The height above the ground at which the disc rests is proportional to grass biomass. We estimated biomass from disc height using equations derived from comparable grass swards in South Africa (Trollope and Potgieter 1986). We collected grass leaf samples from each treatment and control (no fertilization) plot at the start of the experiment and after two years, within the encroached and open grassland sites. Grass foliar N concentration was determined by the Dumas combustion method using the Elementar® Rapid N Cube analyser (Hanau, Germany). Plant species richness (sampled as the total number of species per plot) was recorded before the start of the experiment and after two years.

### ***Statistical analyses***

Species richness was surveyed as the total number of species in each plot. The response ratios (R) of species richness (Hedges and others 1999; Xu and others 2015) were calculated to quantify the impacts of fertilizer application and woody plant encroachment. This was done by:

- (a) comparing the species richness in the second year to that of the pre-fertilization species richness, and



(b) comparing the species richness in the encroached plots to that of the open grassland species richness.

We employed the response ratio so that the relative effect of treatments on species richness can be compared across sites (see Jost 2006). The response ratio was calculated as:

$$R = NA/NP \quad (1)$$

where NA is the species richness in each treatment plot (fertilizer addition or encroachment) and NP is the initial (year 0) species richness in the same plot (Jost 2006). To exclude the effects caused by other factors, such as inherent differences in soil type, we revised the response ratios by a correction factor (cf). The cf was calculated as:

$$cf = \text{Mean NP} / \text{Mean NA} \quad (2)$$

where Mean NP is the mean year 0 species richness in the control plot and Mean NA is the mean species richness in the treatment plot (fertilizer addition or encroachment).

To perform statistical analyses, we calculated the relative effects (E) by transforming the revised response ratios to the natural log, which was calculated as;

$$E = \ln (R \times cf) \quad (3)$$

where R is the response ratio and cf is the correction factor (Xu and others 2015).

We then calculated the relative effects of each treatment (fertilizer addition and encroachment) on species richness. The relative effects would be zero, positive or negative if no change, increased or decreased effects occurred with the fertilizer addition/ encroachment, respectively.

The effects of treatment variables (site, fertilizer (Control, N addition, P addition and N+P addition) and land cover type (encroached and open grassland)) on soil, litter (including C:N ratio, an index of decomposition; Ward et al. 2018), and plant parameters were analyzed using Multivariate Analysis of Variance (MANOVA) to control for Type I error caused by the simultaneous analysis of multiple dependent variables. The effects of treatment variables (fertilizer and land-cover type) on forb proportion, grass biomass and plant species richness were

analyzed using Analysis of Variance (ANOVA). All comparisons within groups were done using the Bonferonni *post hoc* test with a Holm correction (Holm 1979). All statistical analyses were conducted in SPSS (Version 18.0 for Windows).

## 4.4 Results

### *Soil N and P*

We found significant overall effects of fertilizer (MANOVA: Wilk's lambda = 0.812;  $p = 0.004$ ) and encroachment (MANOVA: Wilk's lambda = 0.119;  $p < 0.001$ ). Soil N stocks differed significantly among the four sites ( $F = 740.7$ ;  $p < 0.001$ ; Figure 1), with higher N stocks in the two high-precipitation sites (KwaMbonambi and Bergville) than in the low-precipitation sites (Middelburg and Pniel).

We found significant interaction effects of site and fertilizer addition on soil P ( $F = 2.75$ ;  $p = 0.004$ ; Figure 2). Soil P was significantly higher ( $p = 0.020$ ) in the N + P plots (mean =  $9.50 \text{ mg kg}^{-1} \pm 0.42 \text{ SE}$ ) than in the control plots (mean =  $8.83 \text{ mg kg}^{-1} \pm 0.49$ ), in the high precipitation site, Bergville (Figure 2). Furthermore, we found significantly higher soil P in the encroached sites than the open grasslands in Bergville ( $F = 43.29$ ;  $p < 0.001$ ; Figure 3). Contrastingly, we found higher soil P in the open grasslands than in the encroached sites at the highest precipitation site, KwaMbonambi (Figure 3).

### *Litter dynamics*

We found overall significant effects of site (MANOVA: Wilk's lambda = 0.512,  $p < 0.001$ ) and encroachment (MANOVA: Wilk's lambda = 0.778,  $p = 0.023$ ) and the interaction between site and encroachment (MANOVA: Wilk's lambda = 0.426,  $p < 0.001$ ). We found no overall significant effects of fertilizer (MANOVA: Wilk's lambda = 0.986,  $p = 0.692$ ).

We found significant differences in litter % N between the four sites ( $F = 2.83$ ;  $p = 0.004$ ; Figure 4). We found no significant effects of fertilizer ( $F = 1.070$ ;  $p = 0.069$ ) or encroachment ( $F = 0.702$ ;  $p = 0.406$ ). However, we did note significant interactions between site and land-cover type (encroached versus open grassland) ( $F = 21.24$ ;  $p < 0.001$ ), with increasing litter % N in the encroached sites across the gradient from low to high precipitation (Figure 4).

Sites differed significantly in litter C:N ratio ( $F = 7.19$ ;  $p < 0.001$ ), with overall higher C:N ratios in the low precipitation sites than at the higher-precipitation sites (Figure 5). We found no significant effects of fertilizer on litter C:N ratio ( $F = 0.633$ ;  $p = 0.597$ ). We did however find significant interaction effects ( $F = 9.57$ ;  $p < 0.001$ ) between site and land-cover type (encroached versus open grasslands). At the lowest precipitation site, Middelburg, we found a significantly higher litter C:N ratio in the encroached plots than in the open grasslands. Conversely, at the highest precipitation site (KwaMbonambi) litter C:N ratios were significantly higher in the open grasslands than in the encroached plots (Figure 5).

### ***Grass foliar nitrogen and biomass***

We found significant differences in grass foliar N concentration between the four sites (ANOVA:  $F = 6.99$ ;  $p < 0.001$ ). We found no significant effects of fertilizer (ANOVA:  $F = 0.373$ ;  $p = 0.772$ ) on grass foliar N concentration. Significant interactions between site and land-cover type (encroached versus open grassland) were however noted (ANOVA:  $F = 47.59$ ;  $p < 0.001$ ), with grasses in the encroached plots containing significantly higher foliar nitrogen (mean foliar N % =  $1.9 \pm 0.08$  SE) than grasses in the open grasslands (mean foliar N % =  $1.0 \pm 0.05$ ; Figure 6).

Grass biomass differed significantly among the four sites (ANOVA:  $F = 347.48$ ;  $p < 0.001$ ), with higher biomass in the two high-precipitation sites (KwaMbonambi:  $5118.3 \text{ kg h}^{-1} \pm 181.04$ ; Bergville:  $4753.1 \text{ kg h}^{-1} \pm 134.50$  SE) than in the low-precipitation sites (Middelburg:

555.5 kg h<sup>-1</sup> ± 48.52; Pniel: 280.3 kg h<sup>-1</sup> ± 4.25). Significant interaction effects of site and fertilizer treatment were only found in the high-precipitation sites (ANOVA: F = 4.64; p < 0.012). We found significantly higher grass biomass with N+P and P for the two high-precipitation sites (KwaMbonambi and Bergville; Figure 7).

### ***Forb proportion***

The percentage of forb cover per plot was not significantly different across sites (ANOVA: F = 0.31; p = 0.254). Fertilization addition did not have a significant effect on forb cover (ANOVA: F = 0.21; p = 0.085). We did, however, find significantly higher forb percentage cover (ANOVA: F = 13, 75; p = 0.001) in the encroached plots (mean = 34.1 % ± 4.34) than in the open grasslands (mean = 19.7 % ± 2.79; Figure 8).

### ***Relative effects of fertilizer and encroachment on species richness***

We found significant relative effects of fertilizer addition on species richness among the four sites (ANOVA: F = 3.42; p = 0.04; Figure 9), with greater negative responses in the high-precipitation sites than in the low-precipitation sites. We also found significant negative relative effects of fertilization on species richness (ANOVA: F = 2.85; p = 0.049) due to N and N+P addition at the two high-precipitation sites (KwaMbonambi and Bergville; Figure 9). The relative effects of encroachment on plant species richness were not significant (ANOVA: F = 0.02; p = 0.786).

## 4.5 Discussion

Semi-arid savannas in southern Africa are known to be limited by water and less by nutrient availability. This was demonstrated by Kraaij and Ward (2006), who showed that tree recruitment was constrained by both precipitation amount and frequency. Our study found low soil N concentration in the low-precipitation grasslands despite the addition of nutrients. This result may be explained by water limitation at these sites. Low mean annual precipitation (MAP) has negative effects on vegetative growth of plants, C input to soils and the soil nutrient status (Colman and Schimel 2013; Cragger and others 2014). A meta-analysis of African savanna field sites showed a strong positive relationship between woody plant cover and MAP (Sankaran and others 2005). Below 650 mm MAP, woody cover depends linearly on MAP (Meyer and others 2007). Consequently, changes in precipitation due to climate change may be more influential than fertilizer addition in driving encroachment (Kraaij and Ward 2006; Sankaran and Anderson 2009).

The limited effect of fertilizer addition on soil dynamics in the lower precipitation sites may be due to insufficient precipitation and volatilization. We acknowledge that volatilization of the fertilizer, especially nitrogen, may have occurred. Vitousek (2004) has found that two years is sufficient to show significant effects on plant growth patterns. However, Liu and others (2012) have reported no effects of fertilization on soil parameters even after 30 months of nutrient addition at a rate of application similar to that of this study.

Cech and others (2008) demonstrated that much greater amounts of N accumulate in the woodland than in the open grassland it replaces. This accumulation of available N may result in a negative feedback, with P-limitation under the canopies of encroaching trees (O'Halloran and others 2010), which are often capable of fixing N. We observed higher P in the open grassland plots than the encroached plots for the highest precipitation site, KwaMbonambi, suggesting that

a negative feedback results. At KwaMbonambi, the main encroaching species is *Dichrostachys cinerea*, a known N<sub>2</sub>-fixing legume. Contrastingly, this study showed no evidence of P limitation as a result of encroachment at Bergville, despite the high densities of a known N<sub>2</sub>-fixing woody plant, *Acacia sieberiana* (recently renamed *Vachellia sieberiana*). Although this study examined differences between open grasslands and encroached sites, P limitation may be better studied through the examination of woody gradients. For example, Blaser and others (2014) found that total P and extractable P pools increased along a shrub-cover gradient. Interestingly, in our study, the change in total soil P between encroached and open grasslands was minimal for low-precipitation sites, indicating that water availability may be the main driver of encroachment for these sites. However, the roles of grazing (Ward and Esler 2011), fire limitation (Throop and others 2017) and/or global climate change (especially increases in CO<sub>2</sub> concentrations) cannot be excluded (Ward and others 2014).

Both litter % N (used as an index of quality) and C:N (used as an index of decomposition) were expected to increase with fertilizer addition and woody plant encroachment. Zhang and others (2008) found that total nutrient concentration and the C:N ratio of the litter were the two factors that had the greatest effect on litter mass loss across a wide range of plant species and tissues. Interestingly, we found only marginal effects of fertilization addition on litter quality. Here again, for low-precipitation sites, water availability may have limited nutrient uptake into plant tissue. Lü and others (2014) found that water addition rather than N addition benefitted plant species in a semi-arid grassland. We did note increasing litter % N quality in encroached sites with increasing MAP. This result implies that increased litter quality by encroaching woody plants is only beneficial to surrounding vegetation when precipitation is not limiting.

Lower C:N ratios in encroached areas have been reported by several studies, possibly due to the presence of N<sub>2</sub>-fixing legumes (Hudak and others 2003; Baer and others 2006; Zhu and

others 2016). For example, Sugihara and others (2014) found that forest species which were able to fix N effectively contributed to the low C:N ratio of the forest soil. Both the wetter sites studied here, KwaMbonambi and Bergville, are dominated by N<sub>2</sub>-fixing legumes. Litter C:N ratios were on average higher in the low-precipitation sites, indicating lower decomposition. Hudak and others (2003) found that severely encroached areas had lower C:N ratios throughout the soil profile than less encroached areas. The C:N ratio of litter decreased across the precipitation gradient for the encroached sites, highlighting the positive effect of encroachment on litter decomposition when water is not limiting.

We found higher foliar N concentrations in grass leaves in the encroached plots, with only the lowest precipitation site, Middelburg, showing no positive effect of encroachment on grass foliar quality. While increases in soil N, and consequently foliar N, are sometimes observed beneath encroaching woody canopies even in the absence of N<sub>2</sub>-fixing species (Eldridge and others 2011), the woody plants in Middelburg are not N<sub>2</sub>-fixing (O'Connor and Roux 1995).

Although the term *woody plant encroachment* has become synonymous with desertification and declines in species diversity, Grellier and others (2013) have found positive effects of woody encroachers on plant diversity. Eldridge and Soliveres (2015) have suggested optimising landscape management to maximise the services provided by shrub-encroached areas. The purportedly positive effect of woody plant encroachment was elucidated by our study finding that forb cover increased in encroached plots. Barbosa da Silva and others (2016), also found that the encroachment of a woody invader was correlated to an increase in species richness and a decrease in important native forage grasses. Again, while not all woody encroachers are N<sub>2</sub>-fixing, plants growing at high densities create 'islands of fertility' (Hibbard and others 2001; Ravi and others 2010; Ward and others 2018), attributed to both abiotic and biotic processes such as local-scale variations in soil erosion and deposition, plant uptake of water and nutrients by lateral roots spreading into the surrounding soils, and activities of soil micro and macro-

organisms (Ravi and others 2010). Increased presence of forbs may also contribute to increased diversity, contrary to the widespread notion that encroachment is directly linked to reductions in diversity and ecological function (Eldridge and others 2011).

The effects of encroachment on net primary productivity are related to MAP, with reported decreases in ANPP in xeric sites, but dramatic increases at higher precipitation sites (Knapp and others 2008). Likewise, this study has shown that the lower precipitation sites exhibited minimal fluctuations in above-ground grass productivity, as compared to the high precipitation sites.

Several studies of fertilizer addition effects in South African grasslands have found that the addition of both nitrogen and phosphorus led to a higher yield in grass biomass than fertilization with one of these nutrients only (Fynn and others 2005; Craine and others 2008; Tsvuura and Kirkman 2013). Patterns of species richness in this study show that nutrient additions (N and N+P) resulted in declines in species richness, but for higher precipitation sites only. Plant diversity is generally shown to be positively related to precipitation (Adler and Levine 2007). Several N-addition experiments have shown a decline in species richness (Clark and Tilman 2008; Ward and others 2017), and similar trends have been reported for atmospherically deposited N (Stevens and others 2004; Dingaana and others 2017). A recent study by Ward and others (2017) compared the results of a long-term (65 years) experiment in a South African grassland with the world's longest-running ecological experiment, the Park Grass study at Rothamsted, U.K. The results showed remarkable similarities to those of the Park Grass study, particularly the seemingly universal overall inverse relationship between species richness and ANPP, as well as the negative impact of nitrogen addition on species richness. Improved nutrient availability may be advantageous to grasses in terms of productivity, assuming that grasses are superior competitors for soil resources such as water and nutrients because of access to precipitation in the upper soil layers (Walter 1939; Knoop and Walker 1985; Ward and others



2013) and their fine-root morphology (Aerts and others 1991). However, rapid N absorption by C<sub>4</sub> grasses could effectively allow a few resource-capturing grasses to dominate, thereby competitively excluding other species and decreasing overall species diversity (Busso 2014).

#### **4.6 Conclusions**

Total N stocks were higher in the high-precipitation sites regardless of increased nitrogen availability or encroachment, indicating N storage is water-limited in the low precipitation regions. Soil P indicated mixed trends across the precipitation gradient. Variation in soil P was greater in the high-precipitation sites, with the most humid site displaying P limitation in the encroached plots. Fertilizer addition improved grass productivity but reduced species richness, albeit for high-precipitation sites only. Low soil, litter and plant responses in semi-arid grasslands are possibly due to the strong effect of water limitation. However, the coefficient of variation in expected precipitation is high for semi-arid grasslands (Kraaij and Ward 2006). Hence, one may expect larger effects of nutrient addition and encroachment in high precipitation years. Overall, woody-plant encroachment had purportedly beneficial effects on litter quality, decomposition, grass foliar quality and forb cover in the high-precipitation sites. These positive effects must be taken into consideration, based on site specific precipitation, when implementing encroachment control.

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## 4.8 References

- Aerts R, Boot RGA, van der Aart PJM. 1991. The relation between above- and belowground biomass allocation patterns and competitive ability. *Oecologia* 87: 551-559.
- Archer SR, Andersen EM, Predick KI, Schwinning S, Steidl RJ, Woods SR. 2017. Woody plant encroachment: causes and consequences. Briske DD, Editors. *Rangeland Systems: processes, management and challenges*. Germany: Springer International Publishing, p25-84.
- Baer SG, Church JM, Williard KW, Groninger JW. 2006. Changes in intrasystem N cycling from N<sup>2</sup>-fixing shrub encroachment in grassland: multiple positive feedbacks. *Agriculture, Ecosystems and Environment* 115: 174-182.
- Baisden W, Amundson R, Cook A, Brenner D. 2002. Turnover and storage of C and N in five density fractions from California annual grassland surface soils. *Global Biogeochemical Cycles* 16: 64-61.
- Barbosa da Silva FH, Arieira J, Parolin P, Nunes da Cunha C, Junk WJ. 2016. Shrub encroachment influences herbaceous communities in flooded grasslands of a neotropical savanna wetland. *Applied Vegetation Science* 19:391-400.
- Blaser WJ, Shanungu GK, Edwards PJ, Olde Venterink H. 2014. Woody encroachment reduces nutrient limitation and promotes soil carbon sequestration. *Ecology and Evolution* 4: 1423-1438.
- Bond WJ, Midgley GF. 2000. A proposed CO<sub>2</sub> mechanism of woody plant invasion in grasslands and savannas. *Global Change Biology* 6: 865-869.
- Bond WJ, Midgley GF. 2012. Carbon dioxide and the uneasy interactions of trees and savannah grasses *Philosophical Transactions of the Royal Society Series B: Biological Sciences* 367: 601-612.
- Bond WJ. 2008. What limits trees in C<sub>4</sub> grasslands and savannas? *Annual Review of Ecology, Evolution and Systematics* 39: 641-659.
- Busso CA, Briske DD, Olalde-Portugal V. 2001. Root traits associated with nutrient exploitation following defoliation in three coexisting perennial grasses in a semi-arid savanna. *Oikos* 93: 332-342.
- Cech PG, Kuster T, Edwards PJ, Venterink HO. 2008. Effects of herbivory, fire and N<sub>2</sub>-fixation on nutrient limitation in a humid African savanna. *Ecosystems* 11: 991-1004.

- Clark CM, Cleland EE, Collins SL, Fargione JE, Gough L, Gross KL, Pennings SC, Suding KN, Grace JB. 2007. Environmental and plant community determinants of species loss following nitrogen enrichment. *Ecology Letters* 10: 596-607.
- Cleveland C, Liptzin D. 2007. C:N:P stoichiometry in soil: is there a “Redfield ratio” for the microbial biomass? *Biogeochemistry* 85: 235-252.
- Colman BP, Schimel DS. 2013. Drivers of microbial respiration and net N mineralization at the continental scale. *Soil Biology and Biochemistry* 60: 65-76.
- Craine JM, Morrow C, Stock WD. 2008. Nutrient concentration ratios and co-limitation in South African grasslands. *New Phytologist* 179: 829–836.
- Crawley MJ, Johnston AE, Silvertown J, Dodd M, de Mazancourt C, Heard MS, Henman DF, Edwards GR. 2005. Determinants of species richness in the park grass experiment. *American Naturalist* 165: 179-192.
- Cregger MA, McDowell NG, Pangle RE, Pockman WT, Classen AT. 2014. The impact of precipitation change on nitrogen cycling in a semi-arid ecosystem. *Functional Ecology* 28:1534-1544.
- Dingaen MNV, Tsubo M, Walker S, Newby T. 2017. Soil chemical properties and plant species diversity along a rainfall gradient in semi-arid grassland of South Africa. *Plant Ecology and Evolution* 150: 35-44.
- Eldridge DJ, Soliveres S. 2015. Are shrubs really a sign of declining ecosystem function? Disentangling the myths and truths of woody encroachment in Australia. *Australian Journal of Botany* 62: 594-608.
- Facelli JM, Pickett ST. 1991. Plant litter: its dynamics and effects on plant community structure. *Botanical Review* 57: 1-32.
- Fynn RWS, Morris CD, Kirkman KP. 2005. Plant strategies and trait trade-offs influence trends in competitive ability gradients of soil fertility and disturbance. *Journal of Ecology* 93: 384-394.
- Gill RA, Burke IC. 1999. Ecosystem consequences of plant life form changes at three sites in the semi-arid United States. *Oecologia* 121: 551-563.
- Gough L, Osenberg CW, Gross KL, Collins SL. 2000. Fertilization effects on species density and primary productivity in herbaceous plant communities. *Oikos* 89: 428-439.
- Grellier S, Ward D, Janeau J-L, Podwojewski P, Lorentz S, Abbadie L, Valentin C, Barot S. 2013. Positive versus negative environmental impacts of tree encroachment in South Africa. *Acta Oecologica* 53: 1-10.

- Harpole WS, Ngai JT, Cleland EE, Seabloom EW, Borer ET, Bracken MES, Elser JJ, Gruner DS, Hillebrand H, Shurin JB, Smith JE. 2011. Nutrient co-limitation of primary producer communities. *Ecology Letters* 14: 852-862.
- Hedges LV, Gurevitch J, Curtis PS. 1999. The meta-analysis of response ratios in experimental ecology. *Ecology* 80: 1150-1156.
- Hibbard KA, Archer S, Schimel DS, Valentine DW. 2001. Biogeochemical changes accompanying woody plant encroachment in a subtropical savanna. *Ecology* 82: 1999-2011.
- Higgins SI, Keretsetse M, February EC. 2015. Feedback of trees on nitrogen mineralization to restrict the advance of trees in C<sub>4</sub> savannas. *Biology Letters* 11: 20150572. [doi: 10.1098/rsbl.2015.0572]
- Hoffman MT, Ashwell A. 2001. *Nature divided: land degradation in South Africa*. Cape Town, South Africa: University of Cape Town Press.
- Hudak A, Wessman C, Seastedt T. 2003. Woody overstorey effects on soil carbon and nitrogen pools in South African savanna. *Austral Ecology* 28: 173-181.
- Hunter A, Bomemisza E, Albarado A. 1974. New techniques and equipment for routine soil/plant analytical procedures. In: Borremiza E, Alvarado A, Eds. *Soil Management in Tropical Soils*, Raleigh, NC, U.S.A.: North Carolina State University Press. p. 455-466.
- Jackson RB, Banner JL, Jobbagy EG, Pockman WT, Wall DH. 2002. Ecosystem carbon loss with woody plant invasion of grasslands. *Nature* 418: 623-631.
- Knapp AK, Briggs JM, Collins SL, Archers SR, Bret-Harte MS, Ewers BE, Peters DP, Young DR, Shaver GR, Pendall E, Cleary MB. 2008. Shrub encroachment in North American grasslands: shifts in growth form dominance rapidly alters control of ecosystem carbon inputs. *Global Change Biology* 14: 614-623.
- Kraaij T, Ward D. 2006. Effects of rain, nitrogen, fire and grazing on tree recruitment and early survival in bush-encroached savanna, South Africa. *Plant Ecology* 186: 235-246.
- Liao J, Boutton T, Jastrow J. 2006. Storage and dynamics of carbon and nitrogen in soil physical fractions following woody plant invasion of grassland. *Soil Biology and Biochemistry* 38: 3184-3196.
- Liu L, Gundersen P, Zhang T, Mo J. 2012. Effects of phosphorus addition on soil microbial biomass and community composition in three forest types in tropical China. *Soil Biology and Biochemistry* 44: 31-38.

- Lü XT, Dijkstra FA, Kong DL, Wang ZW, Han XG. 2014. Plant nitrogen uptake drives responses of productivity to nitrogen and water addition in a grassland. *Scientific Reports* 4: 1-7. [doi:10.1038/srep04817].
- Manzoni S, Trofymow JA, Jackson RB, Porporato A. 2010. Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter. *Ecological Monographs* 80: 89–106.
- Meyer KM, Wiegand K, Ward D, Moustakas A. 2007. SATCHMO: A spatial simulation model of growth, competition, and mortality in cycling savanna patches. *Ecological Modelling* 209: 377-391.
- Miyazaki K, Eskes HJ, Sudo K. 2012. Global NO<sub>2</sub> emission estimates derived from an assimilation of OMI tropospheric NO<sub>2</sub> columns. *Atmospheric Chemistry and Physics* 12: 2263-2288.
- Mucina L, Rutherford MC editors. 2006. *The vegetation of South Africa, Lesotho and Swaziland*. Pretoria, South Africa: South African National Biodiversity Institute.
- Murphy J, Riley JP. 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* 27: 31-36.
- O'Halloran LR, Shugart HH, Wang L, Caylor KK, Ringrose S, Kgope B. 2010. Nutrient limitations on aboveground grass production in four savanna types along the Kalahari Transect. *Journal of Arid Environments* 74: 284–290.
- O'Connor TG, Roux PW. 1995. Vegetation changes (1949-71) in a semi-arid, grassy dwarf shrubland in the Karoo, South Africa: influence of rainfall variability and grazing by sheep. *Journal of Applied Ecology* 32: 612-626.
- Phoenix GK, Hicks WK, Cinderby S, Kuylenstierna JC, Stock WD, Dentener FJ, Giller KE, Austin AT, Lefroy RD, Gimeno BS. 2006. Atmospheric nitrogen deposition in world biodiversity hotspots: the need for a greater global perspective in assessing N deposition impacts. *Global Change Biology* 12: 470-476.
- Ravi S, D'Odorico P, Huxman TE, Collins SL. 2010. Interactions between soil erosion processes and fires: implications for the dynamics of fertility islands. *Rangeland Ecology & Management* 63: 267-274.
- Reich PB, Hobbie SE, Lee T, Ellsworth DS, West JB, Tilman D, Knops JMH, Naeem S, Trost J. 2006. Nitrogen limitation constrains sustainability of ecosystem response to CO<sub>2</sub>. *Nature* 440: 922-925.

- Ries LP, Shugart HH. 2008. Nutrient limitations on understory grass productivity and carbon assimilation in an African woodland savanna. *Journal of Arid Environments* 72: 1423-1430.
- Russell JM, Ward D. 2014. Remote sensing provides a progressive record of vegetation change in northern KwaZulu-Natal, South Africa, from 1944 to 2005. *International Journal of Remote Sensing* 35: 904-926.
- Sankaran M, Anderson TM. 2009. Management and restoration in African savannas: interactions and feedbacks. Hobbs R, Suding K, editors. *New Models for Ecosystem Dynamics and Restoration*. Island Press, Washington. pp. 136–155.
- Sankaran M, Hanan NP, Scholes RJ, Ratnam J, Augustine DJ, Cade BS, Gignoux J, Higgins SI, Le Roux X, Ludwig F, Ardo J, Banyikwa F, Bronn A, Bucini G, Caylor KK, Coughenour MB, Diouf A, Ekaya W, Feral CJ, February EC, Frost PGH, Hiernaux P, Hrabar H, Metzger KL, Prins HHT, Ringrose S, Sea W, Tews J, Worden J, Zambatis N. 2005. Determinants of woody cover in African savannas. *Nature* 438: 846-849.
- Sankaran M, Ratnam J, Hanan NP. 2008. Woody cover in African savannas: The role of resources, fire and herbivory. *Global Ecology and Biogeography* 17: 236-245.
- Satti P, Mazzarino MJ, Gobbi M, Funes F, Roselli L, Fernandez H. 2003. Soil N dynamics in relation to leaf litter quality and soil fertility in north-western Patagonian forests. *Journal of Ecology* 91: 173-181.
- Scholes RJ, Archer SR. 1997. Tree-grass interactions in savannas. *Annual Review of Ecology and Systematics* 28: 517-544.
- Sugihara S, Shibata M, Mvondo Ze AD, Araki S, Funakawa S. 2014. Effect of vegetation on soil C, N, P and other minerals in oxisols at the forest-savanna transition zone of central Africa. *Soil Science and Plant Nutrition* 60: 45-59.
- Thompson GR. 1995. A comparison of methods used for the extraction of K in soils of the Western Cape. *South African Journal of Plant and Soil* 12: 20-26.
- Throop HL, Abu Salem M, Whitford WG. 2017. Fire enhances litter decomposition and reduces vegetation cover influences on decomposition in a dry woodland. *Plant Ecology* 218: 799-811.
- Throop HL, Archer SR. 2007. Interrelationships among shrub encroachment, land management, and litter decomposition in a semi-desert grassland. *Ecological Applications* 17: 1809-1823.

- Throop H, Archer S, Monger HC, Waltman S. 2012. When bulk density methods matter: Implications for estimating soil organic carbon pools in rocky soils. *Journal of Arid Environments* 77: 66-71.
- Tsvuura Z, Kirkman KP. 2013. Yield and species composition of a mesic grassland savanna in South Africa are influenced by long-term nutrient addition. *Austral Ecology* 38: 959-970.
- Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman DG. 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications* 7: 737-750.
- Vitousek PM, Cassman K, Cleveland C, Crews T, Field CB, Grimm NB, Howarth RW, Marino R, Martinelli L, Rastetter EB. 2002. Towards an ecological understanding of biological nitrogen fixation. *Biogeochemistry* 57: 1-45.
- Vitousek PM, Howarth RW. 1991. Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry* 13: 87-115.
- Vitousek PM. 2004. *Nutrient Cycling and Limitation: Hawai'i as a Model System*. Princeton University Press, Princeton, NJ, U.S.A.
- Vourlitis GL, Zorba G, Pasquini SC, Mustard R. 2007. Chronic nitrogen deposition enhances nitrogen mineralization potential of semiarid shrubland soils. *Soil Science Society of America Journal* 71: 836-842.
- Ward D. 2010. A resource-ratio model of the effects of elevated CO<sub>2</sub> on woody plant invasion. *Plant Ecology* 209: 147-152.
- Ward D, Esler KJ. 2011. What are the effects of substrate and grass removal on recruitment of *Acacia mellifera* seedlings in a semi-arid environment? *Plant Ecology* 212: 245-250.
- Ward D, Hoffman MT, Collocott SJ. 2014. A century of woody plant encroachment in the dry Kimberley savanna of South Africa. *African Journal of Range and Forage Science* 31: 107-121.
- Ward D, Kirkman K, Tsvuura Z. 2017. An African grassland responds similarly to long-term fertilization to the Park Grass Experiment. *PLoS ONE* 12: e0177208. [doi: 10.1371/journal.pone.0177208].
- Ward D, Trinogga J, Wiegand K, du Toit J, Okubamichael D, Reinsch S, Schleicher J. 2018. Large shrubs increase soil nutrients in a semi-arid savanna. *Geoderma* 310: 153-162.
- Wiegand K, Saltz D, Ward D. 2006. A patch-dynamics approach to savanna dynamics and woody plant encroachment: insights from an arid savanna. *Perspectives in Plant Ecology, Evolution and Systematics* 7: 229-242.



- Ward, D., J. Trinogga, K. Wiegand, J. Du Toit, D. Okubamichael, S. Reinsch, and J. Schleicher. 2018. Large shrubs increase soil nutrients in a semi-arid savanna. *Geoderma* 310: 153-162.
- Xu X, Liu H, Song Z, Wang W, Hu G, Qi Z. 2015. Response of aboveground biomass and diversity to nitrogen addition along a degradation gradient in the Inner Mongolian steppe, China. *Scientific Reports* 5: 10284. [doi:10.1038/srep10284].
- Yusuf HM, Treydte AC, Sauerborn J. 2015. Managing semi-arid rangelands for carbon storage: grazing and woody encroachment effects on soil carbon and nitrogen. *PLoS ONE* 10: e0109063. [doi.org/10.1371/journal.pone.0109063].
- Zhang D, Hui D, Luo Y, Zhou G. 2008. Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. *Journal of Plant Ecology* 1: 85-93.
- Zhu W, Wang J, Zhang Z, Ren F, Chen L, He J-S. 2016. Changes in litter quality induced by nutrient addition alter litter decomposition in an alpine meadow on the Qinghai-Tibet Plateau. *Scientific Reports* 6: 34290. [doi:10.1038/srep34290].

Table 4.1 Description of the four study sites (Mucina and Rutherford, 2006).

Site	GPS coordinates	Annual Precipitation (mm)	Temperature (°C)		Biome	Soil Characteristics	Major Plant Species	Bulk density (kg / m <sup>3</sup> )	
			Min	Max				Encroached plots	Grassland plots
<b>Middelburg</b>	31° 25' 98.83"S 24° 58' 82.10"E	300	-7.2	36.1	Eastern Upper Karoo	Sandy to loam soils of the Hutton soil form	<i>Aristida</i> and <i>Eragrostis</i> (grasses); <i>Searsia erosa</i> , <i>S.</i> <i>burchellii</i> , <i>Diospyros</i> <i>lycioides</i> and <i>Eriocephalus</i> <i>ericoides</i> (shrubs)	1002	1282.2
<b>Pniel</b>	28° 34' 50.00"S 24° 30' 30.70"E	350	-4.1	37.5	Kimberley thornveld	Sandy to loam soils of the Hutton soil form	<i>Eragrostis curvula</i> , <i>Schmidtia pappophoroides</i> (grasses); <i>Acacia erioloba</i> , <i>A tortilis</i> , <i>A. karroo</i> , <i>A.</i> <i>mellifera</i> , <i>Tarchonanthus</i> <i>camphoratus</i> (trees)	1296	1123
<b>Bergville</b>	28° 79' 06.30" S 29° 38' 98.40"E	700	5.8	32.6	KwaZulu- Natal moist grasslands	Ordovician Natal group sandstone	<i>Themeda triandra</i> , <i>Hyparrhenia hirta</i> (grasses); <i>Acacia karroo</i> , <i>A sieberiana</i> (trees)	1028	1191
<b>KwaMbomambi</b>	28° 49' 60.61"S 32° 16' 96.92"E	1500	3.5	35	Maputaland wooded grasslands	Quaternary redistributed sands supporting yellowish redistributed sands of the Berea formation	<i>Sporobolus fimbriatus</i> , <i>Digitaria natalensis</i> (grasses); <i>Diospyros</i> <i>lycioides</i> (shrub); <i>Terminalia sericea</i> (tree)	1031	1124.3

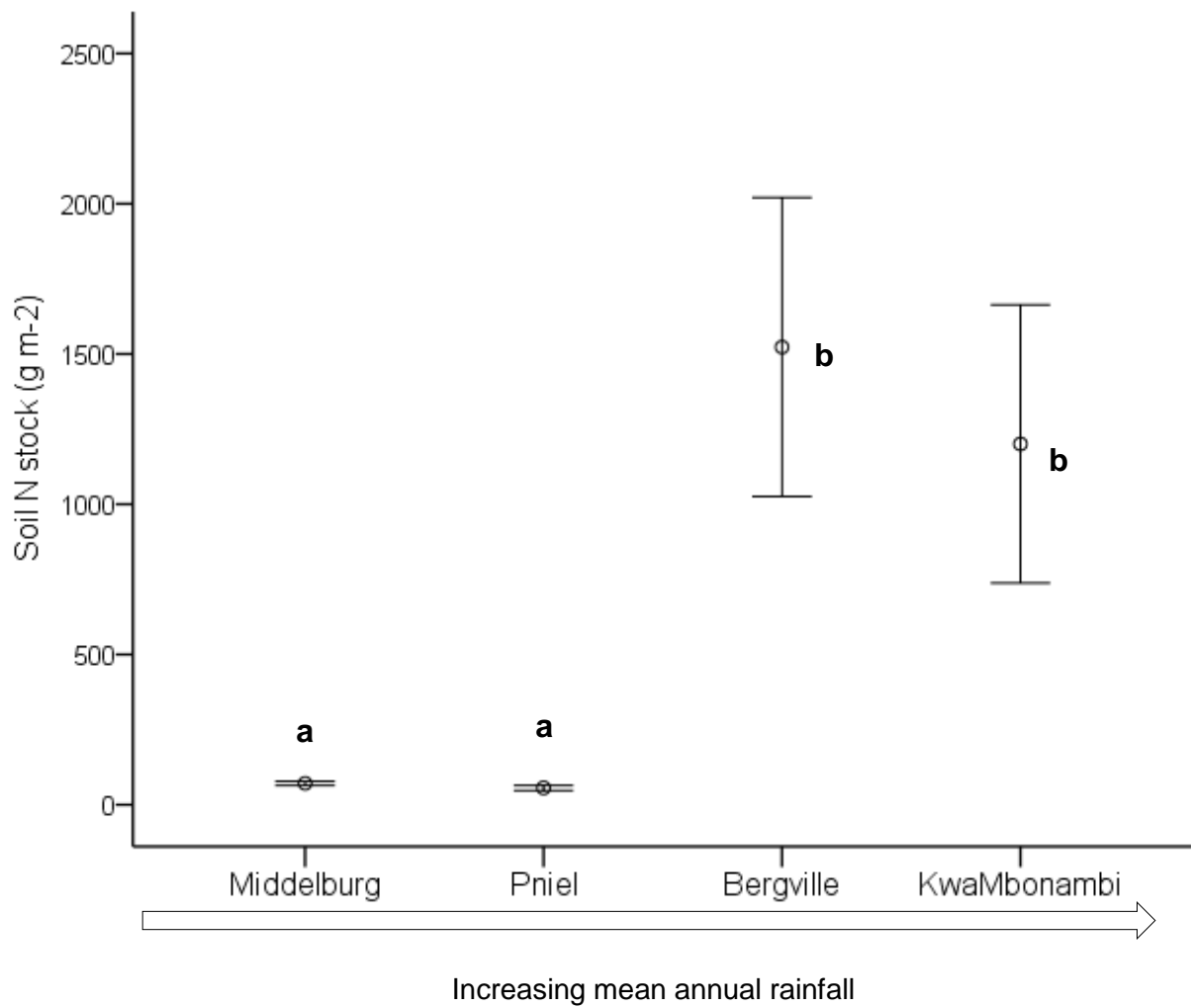


Figure 4.1 Mean  $\pm$  95 % C.I. soil N stocks ( $\text{g m}^{-2}$ ) for the four sites ranging from lowest precipitation (Middelburg, 300 mm MAP) to highest precipitation (KwaMbonambi, 1,500 mm MAP). Different letters indicate significant differences among sites.

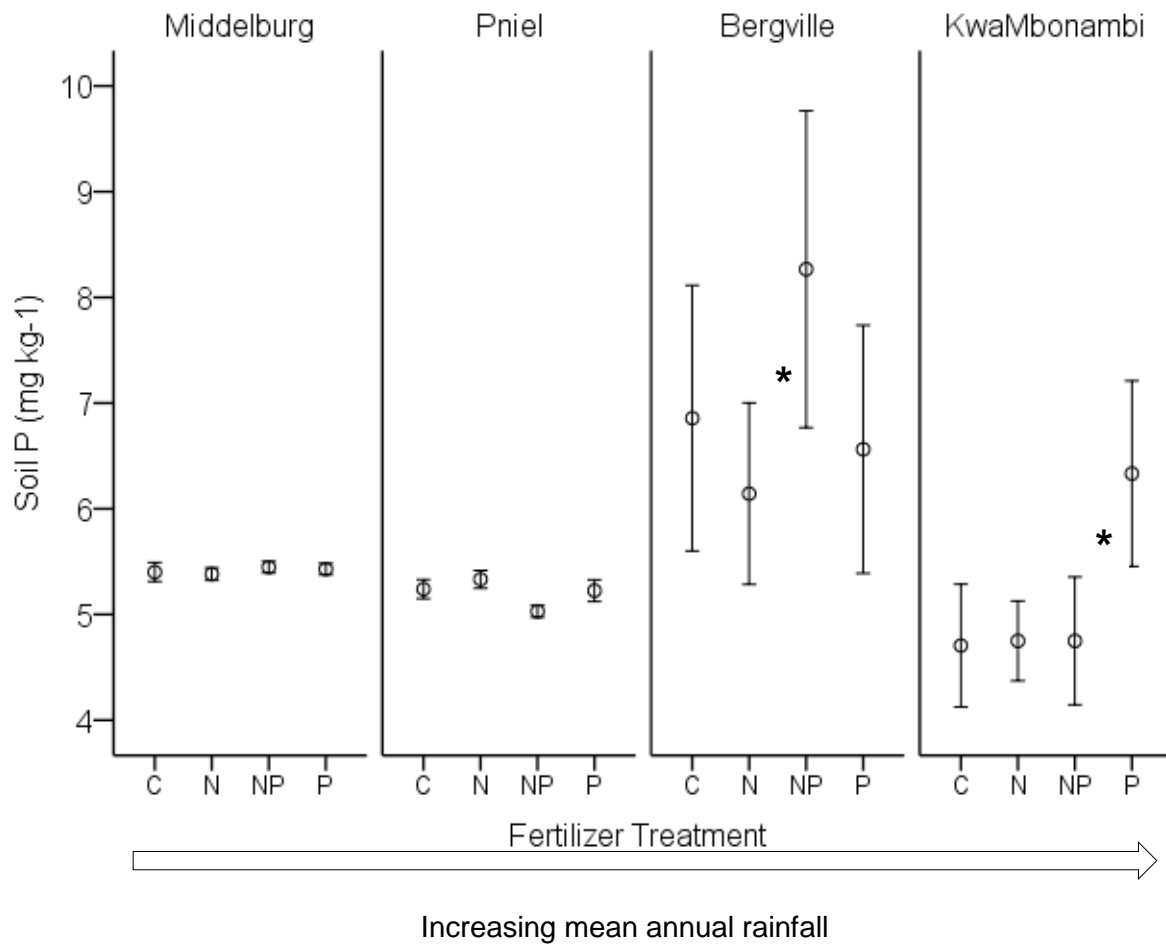


Figure 4.2 Mean  $\pm$  95 % C.I. soil P ( $\text{mg kg}^{-1}$ ) for the four sites ranging from lowest precipitation (Middelburg, 300 mm MAP) to highest precipitation (KwaMbonambi, 1,500 mm MAP). C = control, N = Nitrogen only, NP = Nitrogen and phosphorus, P = Phosphorus only. Asterisks (\*) indicate significant differences between fertilization treatments.

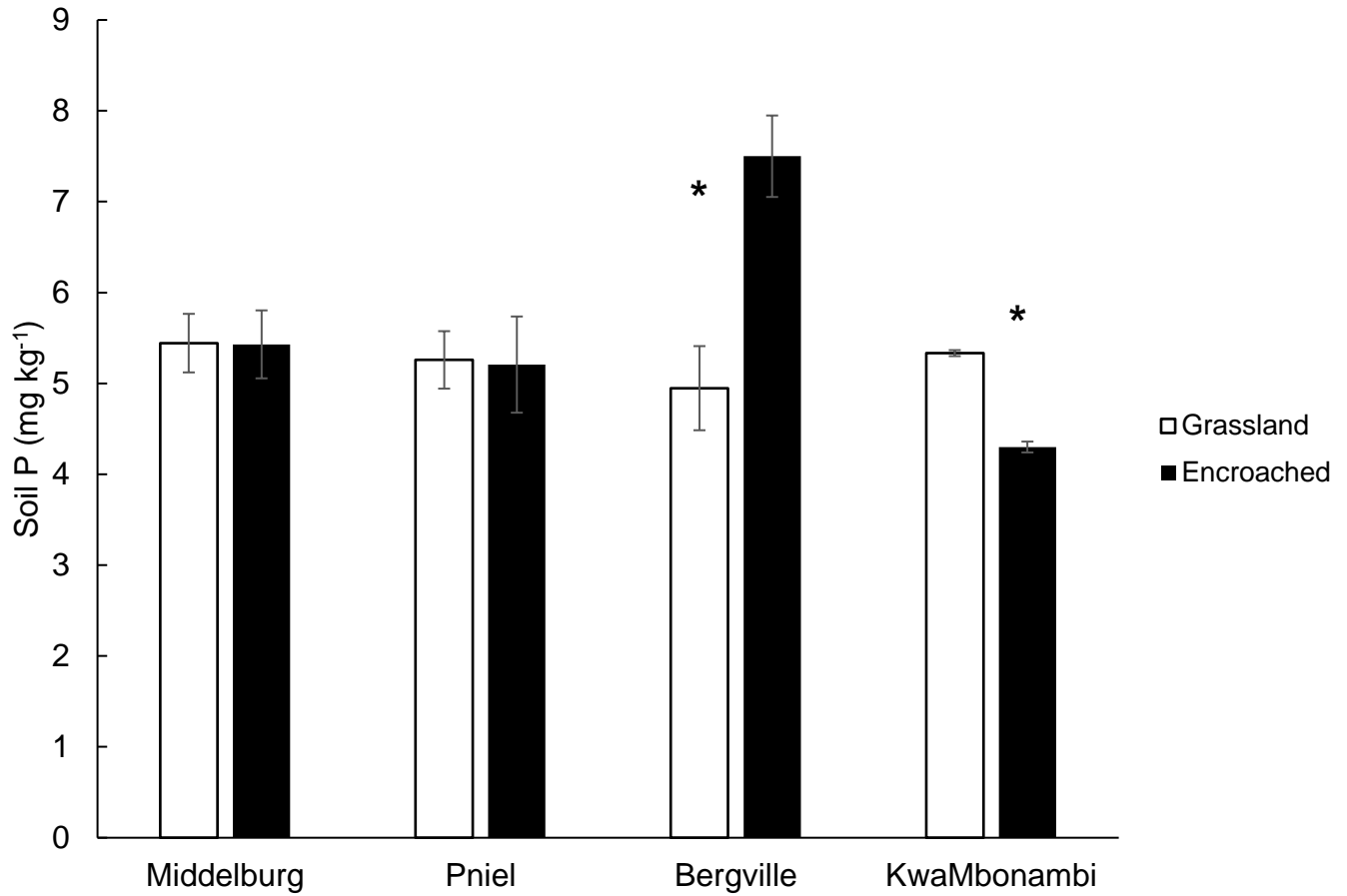


Figure 4.3 Mean  $\pm$  95 % C.I. soil P (mg kg<sup>-1</sup>) for the four sites ranging from lowest precipitation (Middelburg, 300 mm MAP) to highest precipitation (KwaMbonambi, 1,500 mm MAP). Asterisks (\*) indicate significant differences between encroached and open grasslands.

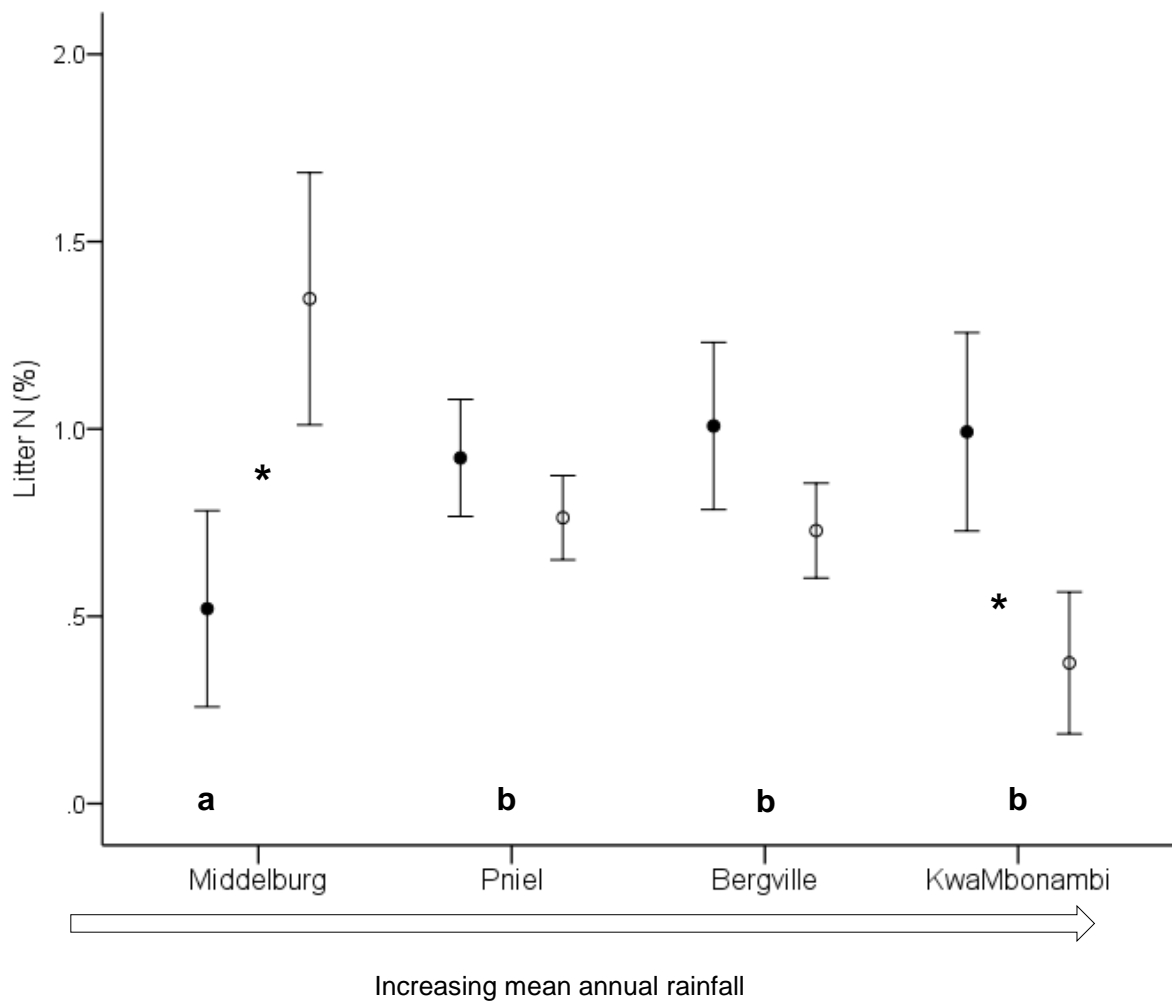


Figure 4.4 Mean  $\pm$  95 % C.I. surface litter N (%) for the four sites ranging from lowest precipitation (Middelburg, 300 mm MAP) to highest precipitation (KwaMbonambi, 1,500 mm MAP). Closed circles (●) represent encroached sites, open circles (○) represent open grasslands. Asterisks (\*) indicate significant differences between encroached and open grasslands. Different letters indicate significant differences among sites.

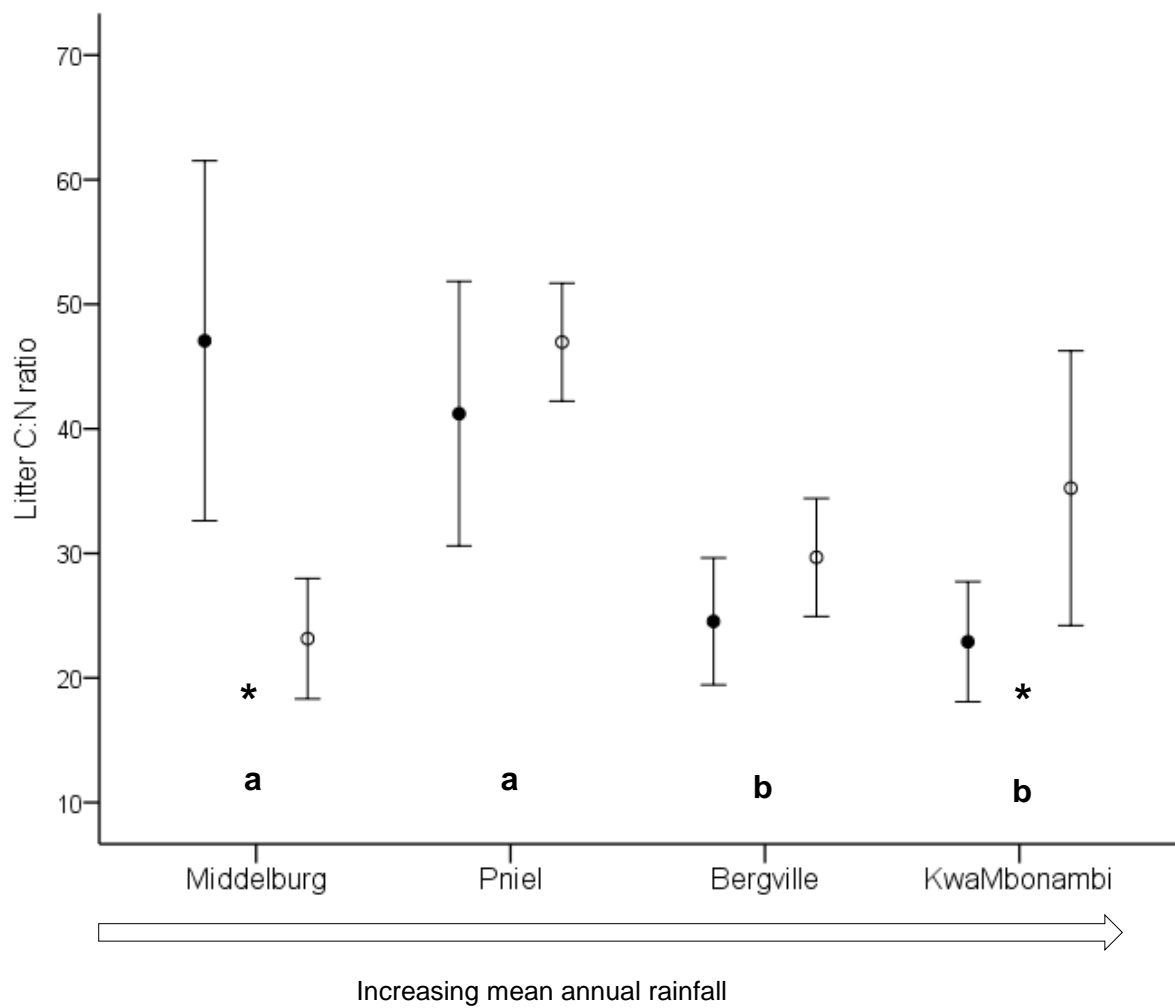


Figure 4.5 Mean  $\pm$  95 % C.I. litter C:N ratio for the four sites ranging from lowest precipitation (Middelburg, 300 mm MAP) to highest precipitation (KwaMbonambi, 1,500 mm MAP). Closed circles (●) represent encroached sites, open circles (○) represent open grasslands. Asterisks (\*) indicate significant differences between encroached and open grasslands. Different letters indicate significant differences among sites.

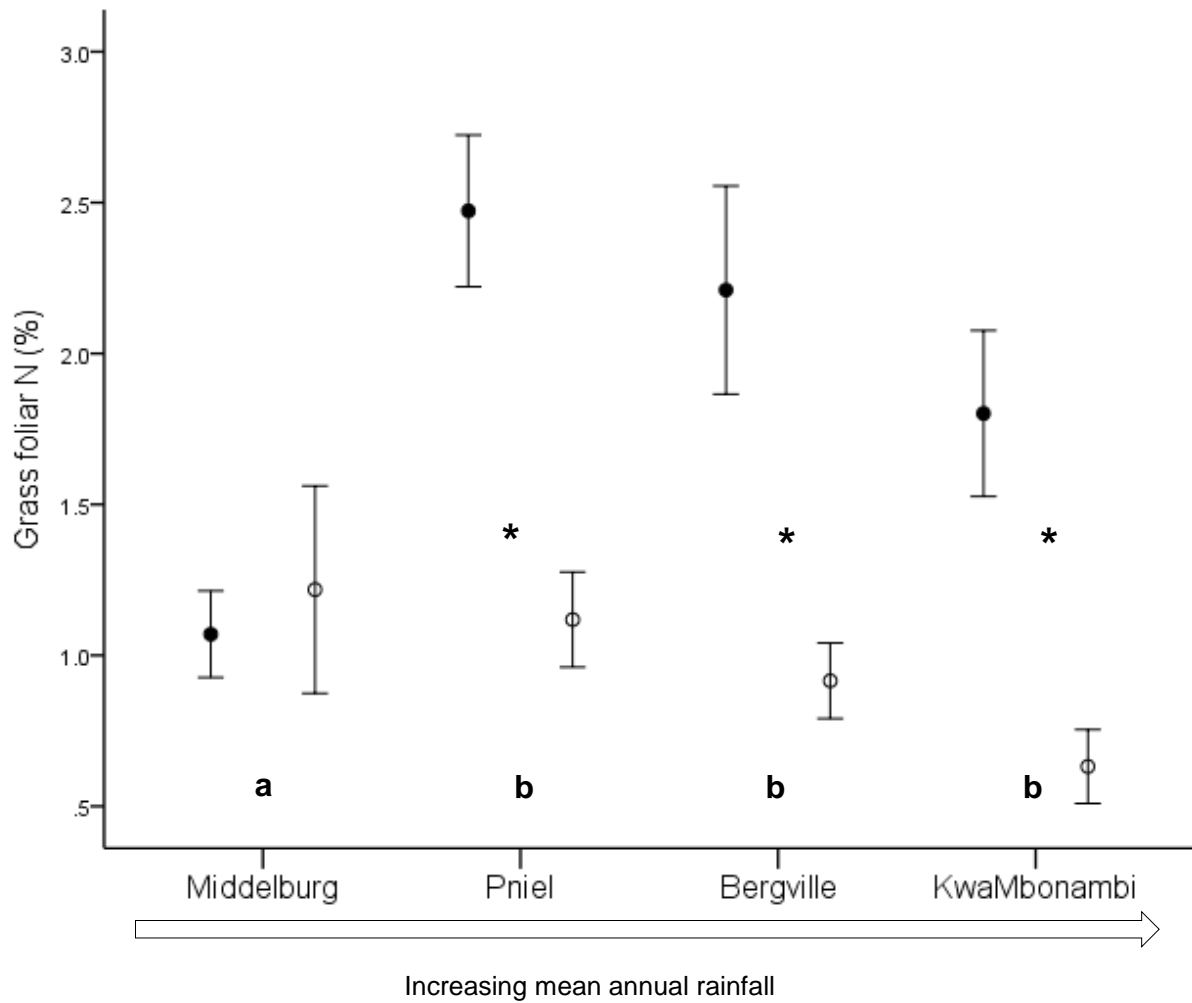


Figure 4.6 Mean  $\pm$  95 % C.I. grass foliar N for the four sites, ranging from lowest precipitation (Middelburg, 300 mm MAP) to highest precipitation (KwaMbonambi, 1,500 mm MAP). Closed circles (●) represent encroached sites, open circles (○) represent open grasslands. Asterisks (\*) indicate significant differences between encroached and open grasslands. Different letters indicate significant differences among sites.



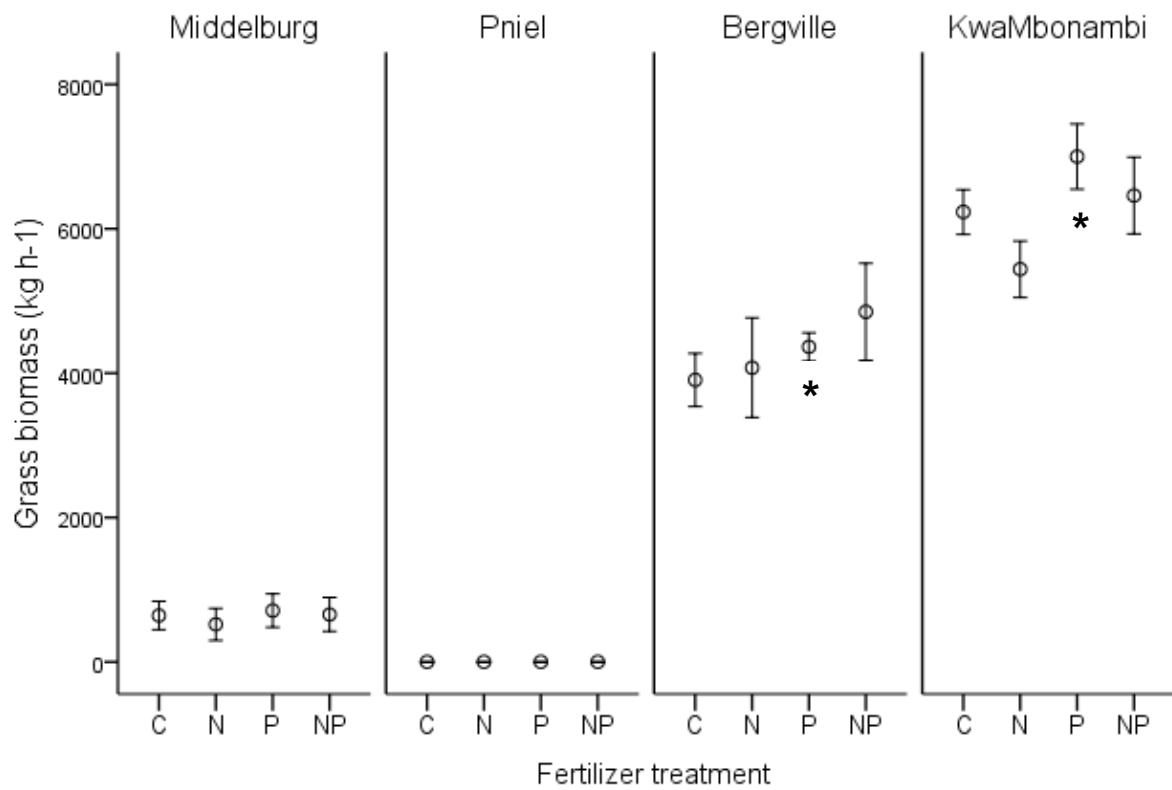


Figure 4.7 Mean  $\pm$  95 % C.I. grass biomass for the four sites, ranging from lowest precipitation (Middelburg, 300 mm MAP) to highest precipitation (KwaMbonambi, 1,500 mm MAP). C = control, N = Nitrogen only, NP = Nitrogen and phosphorus, P = Phosphorus only. Asterisks (\*) indicate significant differences among fertilization treatments.

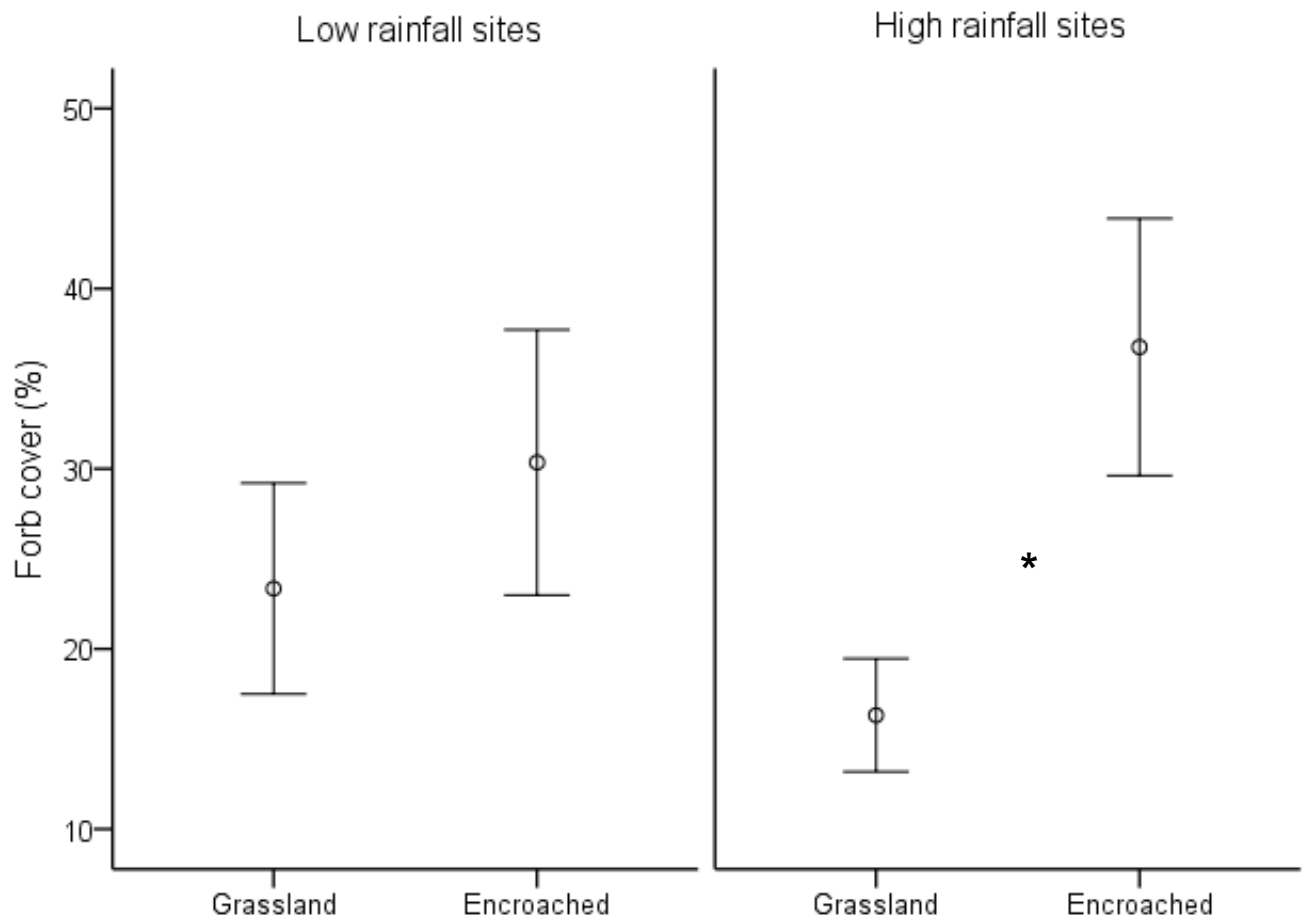


Figure 4.8 Mean  $\pm$  95 % C.I. forb cover (%) for the low and high precipitation sites. Asterisks (\*) indicate significant differences between encroached and open grasslands.

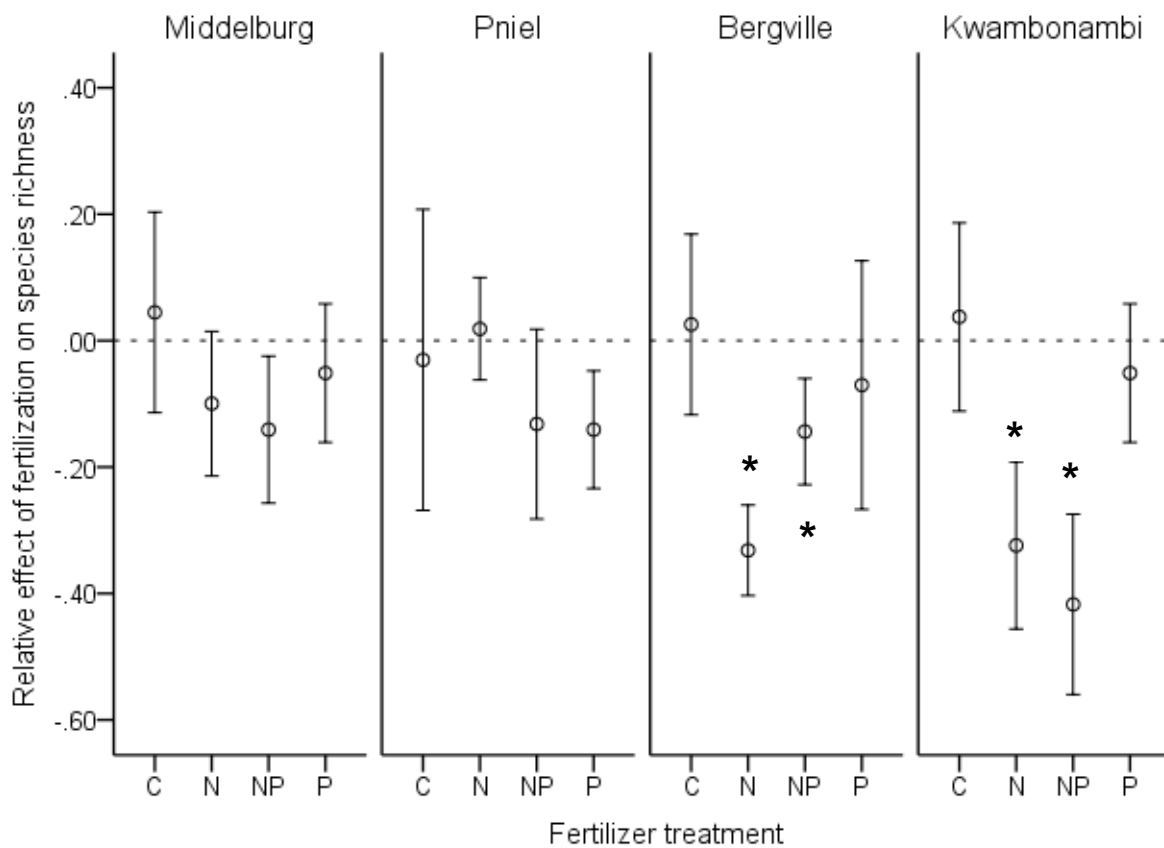


Figure 4.9 Mean  $\pm$  95 % C.I. relative effects of fertilization on species richness for the four sites, ranging from lowest precipitation (Middelburg, 300 mm MAP) to highest precipitation (KwaMbonambi, 1,500 mm MAP). C = control, N = Nitrogen only, NP = Nitrogen and phosphorus, P = Phosphorus only. Mean of zero indicates *neutral effects* of fertilizer addition on species richness; relative effects below zero indicate a *negative effect* of fertilizer addition on species richness; relative effects above zero indicate a *positive effect* of fertilizer addition on species richness. Asterisks (\*) indicate significant differences among fertilization treatments.

## **CHAPTER 5: Fertilizer and fire influence biological nitrogen fixation and soil respiration in *Acacia sieberiana***

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## 5.1 Abstract

**Aims** Biological nitrogen fixation (BNF) by woody legumes is likely to vary due to environmental factors such as nutrients, fire and grazing. BNF may also alter soil properties by increasing soil fertility, consequently affecting rates of soil respiration. Given the widespread increase in woody plant density (encroachment) in southern African savannas, we investigate the factors influencing BNF and soil respiration in a common encroaching woody legume, *Acacia sieberiana*.

**Methods** We conducted a pot experiment using a matrix of grass and saplings of *A. sieberiana*. We then assessed the effects of fertilizer addition, simulated fire and grazing on the rate of nitrogen (N) fixation, soil respiration, and the number and weight of root nodules.

**Results** We found a significant decrease in BNF with fertilizer addition, and increases in BNF after fire application. Soil respiration increased with fertilizer addition and decreased after fire application. Grazing had no independent effect on any of the response variables. However, decreased grass biomass resulted in increased BNF across all treatments. Furthermore, we found that larger woody saplings achieved a higher rate of BNF, with a positive correlation between the rate of BNF and both the number and weight of root nodules. There was a strong positive correlation between tree size and the number and weight of nodules.

**Conclusions** BNF in *A. sieberiana* is facultative, with differing responses to varying environmental factors. Fertilizer addition suppresses BNF because the presence of readily available N negates the costs of fixation. *A. sieberiana* saplings compensated for N lost by fire application by increasing BNF. Soil respiration was found to increase with fertilizer

addition, possibly due to higher carbon (C) inputs into the soil. Conversely, fire reduced soil respiration by removing biomass, and thus reduced C input into the soil.

**Key words:** *Acacia, fertilizer, fire, grazing, nitrogen fixation, soil respiration*

**Acronyms:** *Biological nitrogen fixation (BNF), nitrogen (N), carbon (C)*

## 5.2 Introduction

In savannas, the co-existence of woody vegetation and the herbaceous layer results in constant competition for limiting resources (Bond 2008; Cramer et al. 2010; Riginos 2009). While some degree of resource partitioning allows a balance between the two life forms, there has been a global increase in the prevalence of woody vegetation (termed woody plant encroachment, or bush/shrub encroachment). In southern African savannas, chronic cattle grazing, fire suppression and increased atmospheric carbon dioxide concentration are noted as important drivers of the large-scale shift toward woody plant dominance (Bond and Keeley 2005; Bond and Midgley 2012; Ward et al. 2014). Woody encroachment is environmentally important because an increase in tree density will have a marked effect upon ecosystem processes such as carbon (C) sequestration, soil and plant nutrient dynamics, and soil respiration (Bond 2008).

Tropical savanna ecosystems are considered ‘hot spots’ of biological nitrogen fixation (BNF), due to high densities of potentially fixing legumes (Vitousek et al. 2013). Species from the plant family Leguminosae are well known for forming a symbiotic relationship with nitrogen-fixing soil bacteria in the genera *Azorhizobium*, *Bradyrhizobium* and *Rhizobium* (Crews 1999). Through this association, the legume receives otherwise inaccessible biologically fixed nitrogen (N<sub>2</sub>) from the soil bacteria (Liu et al. 2010). In N-limited systems such as savannas, one would assume that the ability of leguminous trees to fix N<sub>2</sub> would increase their competitive effect over the grass component (Cramer et al. 2007; Kambatuku et al. 2013). The encroachment of woody legumes may then be attributed to their ability to fix nitrogen, especially during the tree seedling stage, where grasses are usually superior competitors (Van Der Waal et al. 2009). Under low soil nitrogen conditions, the nitrogen-fixing trees have a competitive advantage over other plants and, given enough precipitation,

may germinate *en masse* (Kraaij and Ward 2006). However, the tree-legume species in savanna systems do not always fix N<sub>2</sub> (Aranibar et al. 2003; Schulze et al. 1991).

Vitousek and Howarth (1991) showed that the persistence of legumes in a particular ecosystem is linked to (i) the energetic costs of N<sub>2</sub> fixation, (ii) limitation of N<sub>2</sub> fixation by other nutrients (e.g. P), and (iii) physical and ecological factors. While substantial research over the past decade has improved our knowledge of the wide array of species capable of nitrogen fixation, significantly less is known about the ecological factors involved in the regulation of BNF (see review by Vitousek et al. 2013). Furthermore, evidence suggesting that most tropical legumes are facultative N fixers implies that only certain environmental factors promote BNF (Kambatuku et al. 2013; Menge et al. 2009; Vitousek et al. 2013). The availability of resources (nutrients) and disturbances (fire and large mammalian herbivory) have been identified as interactive mechanisms that regulate savanna structure (Sankaran et al. 2005; Scholes and Archer 1997). The independent and interactive effects of these mechanisms may influence the regulation of BNF, such that certain factors increase the rate of nitrogen fixed by plants.

High soil-nutrient availability may reduce BNF due to the associated energetic costs of fixation: Vitousek and Field (1999) reported that nitrogen fixation has a high carbon (C) cost on nitrogen-fixers, such that when there are sufficient nitrogen levels in the soil, it is energetically advantageous to use the available soil nitrogen, rather than fixing nitrogen (Bohrer et al. 2003; Kambatuku et al. 2013). Furthermore, Cech et al. (2010) showed that a negative feedback through soil P depletion on N<sub>2</sub>-fixation in savannas does not prevent an open savanna from developing into dense woodland, because many woody legumes are able to exploit P from deeper soil layers. Assuming that southern African savannas do not become P limited with increased woody plant density (Blaser et al. 2014), one can expect that increased soil nitrogen alone will play a crucial role in the initiation of BNF.



Frequent fires effectively remove large quantities of N relative to P and other nutrients, which may explain the relatively high rates of BNF in savannas (Fynn et al. 2003). Although both grazing and frequent fires may remove N from the ecosystem (Aranibar et al. 2003; Fynn et al. 2003), nitrogen lost through fire or grazing may be replenished by biological nitrogen fixation, particularly by leguminous trees, which are capable of resprouting after fire. In mesic and humid savannas where fire is a frequent occurrence, plant performance in terms of N<sub>2</sub>-fixation on burnt and unburnt soils has not been investigated (Holdo 2013). Similarly, changes in soils accompanying long-term heavy grazing may shift the balance in favour of nitrogen fixing plants which are better adapted than grasses to nutrient-poor soils and warmer, drier microenvironments (Archer et al. 2001).

The high density of nitrogen-fixing legumes may alter soil properties by increasing soil fertility (Schleicher et al. 2011). Soil N status influences foliar plant N concentrations, which has a direct effect on plant growth, microbial activities, litter decomposition and root respiration. Thus, soil respiration is expected to change with changes in N (Zhang et al. 2014). Soil respiration (soil CO<sub>2</sub> emission) is the main pathway of carbon emission from soil to atmosphere in terrestrial ecosystems and an important source of atmospheric CO<sub>2</sub> (Thomas 2012). Therefore, knowledge of soil respiration dynamics and its controlling factors in different terrestrial ecosystems is essential to find proper management strategies to decrease soil CO<sub>2</sub> emissions. Tropical savannas contain a large proportion of the world's terrestrial C (Pacala et al. 2001; Resh, et al. 2002). Fires in tropical savannas are principal sources of CO<sub>2</sub> emission to the atmosphere (Bond and Keeley 2005). However, little is known about post-fire soil respiration in savanna systems (Raich and Potter 1995). Coupled with increased nitrogen deposition over southern Africa during the next few decades (Miyazaki et al. 2012), it is important to investigate the effect of varying savanna environmental pressures on soil respiration and the ability of woody legumes to biologically fix nitrogen.

Using a greenhouse study, we manipulated the presence of fertilizer, simulated fire and grazing, to examine their effects on the nodulation and N-fixation capabilities of the woody legume, *Acacia sieberiana*, as well as the associated effect on soil respiration. The following predictions were made:

1. We predicted a decrease in nitrogen fixation with fertilizer addition. Due to the high carbon cost of fixation, increased N availability in the soil should negate any benefit of further BNF. We expect that fertilizer addition will increase respiration through increased microbial activity under high soil N (Zhang 2014).
2. We predict nitrogen fixation to increase with fire, because of nutrient loss (particularly nitrogen) to the atmosphere by volatilization and via particulate matter formation during combustion and during subsequent wind-born transport (Holdo 2013). This depletion in available soil N can be compensated for by fixing nitrogen. Fire is predicted to decrease soil respiration by removing above-ground plant material and thereby reducing C inputs into the soil (Richards et al. 2012).
3. Simulated grazing should reduce the competitive effects of grass on *A. sieberiana* saplings, thereby decreasing N-limitation and subsequently lowering the overall level of nitrogen fixation (Kambatuku et al. 2013). For plants growing in highly competitive environments, growth is limited initially through exploitative competition, where resources are depleted by neighbouring plants, and possibly secondarily by interference competition where neighbouring plants make resources inaccessible (Aarssen and Keogh 2002). Consequently, we predict that decreased below-ground grass biomass will result in reduced competitive pressure between tree saplings and grasses, and will thereby indirectly decrease BNF. We predict that grazing will act similarly to fire, by removing above-ground plant material (Bond and Keeley 2005).

4. We expect that larger saplings will fix more nitrogen. In their natural habitat, N<sub>2</sub> fixation appears to be most important during early life stages of *Acacias* (Hansen and Pate 1987; Kambatuku et al. 2013) rather than in older populations.
5. We also predict a positive relationship between the rate of nitrogen fixation and both the number and weight of symbiotic root nodules. To determine whether BNF is linked to the energetic cost of producing and maintaining nodules, we tested the relationship between the rate of nitrogen fixation and the weight and number of nodules. Here we expect that tree saplings with a greater number and weight of root nodules would have higher rates of BNF.

### 5.3 Methods

#### *Experimental setup*

The experiment was carried out at the Botanical Gardens of the University of KwaZulu-Natal, Pietermaritzburg, South Africa. A tree-grass matrix was used to mimic a typical savanna microhabitat. To replicate the grass component of the savanna, we sowed *Eragrostis curvula*, (Schrad.) Nees, a widespread savanna grass species, into bins with a 90 L capacity (approximately 50 grass seeds per bin) containing Umgeni grit, an alluvial soil with a particle diameter of approximately 1.5 to 2mm. *Acacia sieberiana* DC. var. *woodii* (Burt Davy) Keay & Brenan (recently renamed *Vachellia sieberiana*; commonly referred to as the paperbark thorn), a tree known to encroach mountainous grasslands and savannas particularly in the mesic-humid areas of southern Africa (Grellier et al. 2013), was used to replicate the woody savanna component. At the beginning of the experiment, 200 seeds of *A. sieberiana* were chemically scarified for one hour by soaking in 1 M hydrochloric acid (HCl). Seeds were then rinsed repeatedly using distilled water, and soaked in boiling water overnight.

Seeds were then pre-treated with fungicide and germinated on agar plates. A single *Acacia sieberiana* sapling (height  $\pm$  10 cm) was placed at the centre of each bin.

We used 96 bins in a completely randomized design. Three treatments, in all combinations, were applied during the 24-week course of the experiment. Fertilizer was applied twice, as a standard NPK with an application rate of nitrogen of 100 kg.ha<sup>-1</sup>. Grasses were cut after 12 weeks to ground level using clippers, to simulate intense grazing. Fire treatments were applied once at 12 weeks, using a butane blowtorch. The aim of the fire treatment was to remove grass cover and “topkill” the saplings, in order to observe the post-fire response. One week prior to burning, the grass in the bins was cut and weighed. Thereafter, a mean volume of dry grass (60 g dry weight) was placed in each bin to standardize the fuel load and fire intensity (Hean and Ward 2012; Vadigi and Ward 2012). Soil temperature readings after fire were also taken, using the ThermoChron iButton® temperature logger, to ensure homogeneity of the fire treatment. Tree seedlings were scorched and sprouted from stems.

After 24 weeks, all tree saplings and grasses were harvested. Above- and below-ground material for trees and grasses were weighed and dried separately at 45 °C. Tree root mass was used as a proxy for tree size. *A. sieberiana* root systems were carefully detached, and the total root mass, number and weight of all nodules were recorded (Fig. 5). We then conducted an Acetylene Reduction Assay (ARA) to quantify the rate of nitrogen fixation (David, et al. 1980). While there is controversy regarding the use of the ARA method, ARA is a sensitive, cost effective method for estimating the rate of nitrogen fixation in trees (Danso et al. 1992). Results of the assay were analysed using a gas chromatograph–mass spectrometer (GC-MS model GCMS-QP2010SE). The GC-MS analysis revealed the amount of ethylene produced from the reduction of acetylene by the enzyme nitrogenase, which represents the rate of nitrogen fixation in *A. sieberiana*.

We used the Solvita<sup>®</sup> gel system for measuring soil respiration (Haney et al. 2008). CO<sub>2</sub> respiration from soil has been used as an indicator of the relative fertility of various soils and is considered to be a reliable method of assessing soil microbial activity (Haney et al. 2008). Soil samples were oven dried at 70 °C for 24 hours. Weighed samples (40 g) of soil were moistened to 50% water-filled pore space in 236 ml jars, triggering a flush or “burst” of carbon dioxide. The magnitude of the “burst” is measured after 24 h with a digital colour reader in ppm CO<sub>2</sub> (Haney et al. 2008).

### *Statistical analysis*

All statistical analyses were conducted in SPSS (Version 18.0 for Windows). Effects of treatment variables (fire, fertilizer and grazing) on several tree-grass growth parameters and soil respiration were analyzed using Multivariate Analysis of Variance (MANOVA), to control for Type I error caused by the analysis of multiple dependent variables.

An upper-boundary regression technique was used to determine if tree performance (as measured by rate of nitrogen fixation) was constrained by the biomass of neighbouring grasses (Pillay and Ward 2014). This was done to eliminate the greater variance encountered on one side of the x-axis when correlating ecological data (i.e. due to heterogeneity of residuals), so that competitive interactions are easily detectable (Goldberg 1991; Lessin et al. 2001). That is, there is a maximal (theoretical) relationship between a dependent variable such as percentage sapling survival and an independent variable such as grass biomass. However, a plethora of factors may skew this relationship upwards at low neighbour densities. No downward skew is likely to occur because there is a constraint placed by neighbouring grass biomass on percentage sapling survival below which additional survival cannot occur. Such a relationship violates the assumption of conventional least-squares regression that there is homogeneity of variance (by definition, an ‘envelope’ results in lower

variance at higher values of the independent variable, Ward et al. 2000). Grass biomass was clustered into 20 g width classes, and regressed against the maximum rate of nitrogen fixation per width class.

## 5.4 Results

### *Effects of fertilizer, fire and grazing on nitrogen fixation and soil respiration*

We found overall significant effects of fertilizer treatments (MANOVA: Wilk's lambda = 0.760,  $p = 0.032$ ), fire (MANOVA: Wilk's lambda = 0.789,  $p = 0.041$ ) and the interaction between fertilizer and fire (MANOVA: Wilk's lambda = 0.033,  $p < 0.001$ ). Overall, grazing was found to be non-significant (MANOVA: Wilk's lambda = 0.819,  $p = 0.109$ )

We found that fertilizer had a significant effect on nitrogen fixation ( $F_{1,47} = 9.826$ ,  $p = 0.003$ ; Table 1). With fertilizer application, nitrogen fixation was significantly lower (mean =  $0.078 \text{ mol ethylene plant}^{-1}\text{h}^{-1} \pm 0.006 \text{ SE}$ ), as compared to those saplings which did not receive fertilizer (mean =  $0.111 \text{ mol ethylene plant}^{-1}\text{h}^{-1} \pm 0.005 \text{ SE}$ ; Fig. 1). Fire also had a significant effect on nitrogen fixation ( $F_{1,47} = 4.308$ ,  $p = 0.044$ ; Table 1), with higher nitrogen fixation noted in tree saplings that were burnt (mean =  $0.109 \text{ mol ethylene plant}^{-1}\text{h}^{-1} \pm 0.005 \text{ SE}$ ), compared to those that did not receive the fire treatment (mean =  $0.092 \text{ mol ethylene plant}^{-1}\text{h}^{-1} \pm 0.007 \text{ SE}$ ; Fig 1).

We noted significant effects of fertilizer and burning on soil respiration (Table 1), with higher soil respiration in fertilized bins (mean =  $0.909 \text{ C} - \text{CO}_2 \text{ ppm} / 1.64 \text{ mg CO}_2 \text{ m}^{-3} \text{ day}^{-1} \pm 0.021$ ), and higher soil respiration in bins where fire was not applied (mean =  $0.925 \text{ C} - \text{CO}_2 \text{ ppm} / 1.66 \text{ mg CO}_2 \text{ m}^{-3} \text{ day}^{-1} \pm 0.022$ ). Grazing had no significant effect on any of the dependent variables.

### ***Nitrogen fixation and grass competition***

The rate of nitrogen fixation decreased with an increase in overall grass root biomass ( $r^2 = 0.63$ ,  $F_{1,7} = 7.024$ ,  $p = 0.047$ ; Fig. 2). When below-ground grass biomass was low ( $< 30$  g dry weight), the range of BNF was between 0.12–4.5 mol ethylene plant<sup>-1</sup>h<sup>-1</sup>. However, when below-ground grass biomass exceeded  $\pm 80$  g, BNF was constrained to below 0.5 mol ethylene plant<sup>-1</sup>h<sup>-1</sup>.

### ***Nitrogen fixation and tree size***

We found a significant positive relationship between rate of nitrogen fixation and tree root biomass ( $r^2 = 0.74$ ,  $F = 4.302$ ,  $p < 0.001$ ), with higher rates of fixation in saplings with greater root biomass (Fig. 3). Furthermore, we observed that the rate of nitrogen fixation was significantly correlated with both the total weight of root nodules (Fig. 4) and the total number of root nodules (Fig. 5).

## **5.5 Discussion**

### ***Effects of fertilizer on biological nitrogen fixation and soil respiration***

We found that biological nitrogen fixation (BNF) in *A. sieberiana* is influenced by fertilizer addition. That is, when fertilizer was applied, BNF was reduced (see also Kambatuku et al. 2013 for similar results in *A. mellifera*). Huss-Danell (1997) reported that nodule formation is reduced by high levels of nitrogen in the soil, thereby reducing BNF in legumes. High plant-available N inhibited BNF by reducing nodular biomass (Hellsten and Huss-Danell, 2000) and also retarded the rate at which N<sub>2</sub> was fixed (Svenning and MacDuff 1996). Even in agricultural pastures, increased nitrogen fertilization inhibited BNF; high-N livestock urine

resulted in a 50 % drop in BNF-derived N when added to plots of mixed ryegrass and white clover (Menner et al. 2003). Some of the explanations for this include:

1) A reduction in carbohydrates with an increase in nitrogen, such that no carbohydrates are excreted by the root hairs to attract nodule-forming bacteria (rhizobia), resulting in reduced nodulation and, hence, reduced BNF (Richardson et al. 1957).

2) It is more costly (energy-wise) for the host tree to maintain the symbiotic relationship when there are sufficient nitrogen levels in the soil (Crews 1999; Kambatuku et al. 2013). The trend of reduced BNF reliance with N supplementation suggests the facultative nature of N<sub>2</sub> fixation in *A. sieberiana* (see also Kambatuku et al. 2013).

This study also highlighted the effects of fertilization on soil respiration. Soil respiration is the main pathway of carbon emission from soil to atmosphere in terrestrial ecosystems and an important source of atmospheric CO<sub>2</sub> (Archer et al. 1995). Interactions between soil respiration and nitrogen will have great impacts on the carbon cycle and hence soil carbon emissions. Considering that increased global nitrogen deposition is coupled with efforts to control the encroachment of woody plants, attention must be paid to the indirect effects on soil respiration. Many studies on the effect of N additions on soil respiration have been conducted in forest ecosystems (Burton et al. 2012), but little information is available for grassland and savanna ecosystems (Zhang et al. 2014), particularly in southern Africa. Here we found that fertilization increased soil respiration. The stimulation of soil by N addition has been observed previously in grassland ecosystems (Xu and Wan 2008). Xu and Wan (2008) attributed this effect to N-induced increases in plant growth, ecosystem primary productivity, and amount of litter that together lead to increase in the supply of C substrates for root and soil microbial activities (Zhang et al. 2014).



### ***Effects of fire on biological nitrogen fixation and soil respiration***

The application of fire resulted in an increase in BNF by *A. sieberiana* saplings. Fire is known to cause large amounts of soil nutrient loss (particularly N loss) to the atmosphere through the process of volatilization (Aranibar et al. 2003). Data from long-term fire experiments in grasslands and savannas indicate that frequent fires over long periods lead to a reduction in total soil N pools (Coetsee et al. 2010). However, some studies found that nitrogen losses caused by fire could not be compensated for by nitrogen fixation (Coetsee et al. 2009). In this study, we found significant increases in BNF by *A. sieberiana* saplings under the fire treatment, suggesting a compensatory effect. López-Hernández et al. (2006) measured substantial rates of BNF in a Venezuelan savanna, especially in burned areas; in their study they found that most N fixation was carried out by microbial crusts and microbes associated with the roots of C<sub>4</sub> grasses.

We also found that soil respiration decreased with fire. In tropical savannas, it has been suggested that fire behaves as a “global herbivore”, removing above-ground C from other consumption pathways, such as the soil respiration pathway (Bond and Keeley 2005; Richards et al. 2012). Richards et al. (2012) observed a significant negative effect of fire on soil respiration, with 3-fold higher rates on unburnt plots, compared to burnt plots. In unburnt plots, all C in organic matter is returned to the atmosphere via decomposition pathways. However, in burnt plots, fire removes organic C in grassy fuels and leaf litter before it has a chance to enter decomposition pathways, reducing below-ground inputs (Bond and Keeley 2005; Richards, et al. 2012).

### ***Effects of grazing on nitrogen fixation and soil respiration***

We found no direct effect of grazing on BNF or soil respiration. While the treatment of simulated grazing (by removal of grass biomass) reduced competition for resources by woody saplings, other ecological processes could not easily be mimicked. These include nutrient redistribution and positive feedbacks of nitrogen through dung and urine deposition (Tjelele et al. 2015). Future efforts to ascertain the effects of grazing on BNF and soil respiration should be conducted in field experiments, using herbivores.

### ***Nitrogen fixation and grass competition***

The simulated grazing treatment removed grass biomass, which in turn relieved competitive pressure on the surrounding woody saplings. Grazing reduces the competitive ability of grasses on tree seedlings, by removing available grass biomass (Riginos 2009; Ward and Esler 2011). With grazing, grasses experience a reduction in water and nutrient uptake, which leads to an increase in available resources for the woody vegetation (Skarpe 1991).

Therefore, more resources are obtained by the trees, and as a consequence, this leads to the recruitment and encroachment of woody plants in savannas (Van Langevelde et al. 2003). It is expected that if competition from grasses is reduced, the rate of BNF would also decrease (Cramer et al. 2007; Kambatuku et al. 2013). We have shown here that increasing grass biomass limited the rate of BNF in *A. sieberiana* saplings. Our results are contrary to the findings of Kambatuku et al. (2013), who showed that BNF increased in the presence of grass competition for *A. mellifera* saplings. Cech et al. (2010) demonstrated that a dominant C<sub>4</sub> grass could outcompete an herbaceous legume for otherwise-available P (and N) in a tall-grass Tanzanian savanna ecosystem, thereby suppressing legume growth and N fixation. The energetic costs incurred by N<sub>2</sub> fixers, combined with the need to outcompete local non-fixing species, imply considerable selective pressure on individual fixation strategies (Cech et al.

2010). We postulate that the presence of intense interspecific competition from grasses forces *A. sieberiana* saplings to function as non-fixers as the cost of maintaining nodules and fixing nitrogen becomes limiting. This is related to the high carbon cost of BNF. For example, building nodules requires carbon for structure as well as the metabolic cost of fixing N. Furthermore, facultative N fixers may be paying a higher structural cost per unit of N fixed than obligate N fixers that keep their nodules for long periods (Menge et al. 2009). It appears that a superior strategy would be to fix N when N is in short supply, and to function as a non-fixer when N is abundant or (especially for an understory plant) when N fixation is too costly (Menge et al. 2009).

### ***Nitrogen fixation and tree size***

Lastly, we found that larger roots, which were on larger saplings of *A. sieberiana*, were capable of higher rates of BNF, across all treatments. This finding indicates that older stands of encroaching *A. sieberiana* may have greater effects on local soil properties, such as total available N. Azad et al. (2013) and Azad and Sumon (2016) found that nodule formation increase over time at the early stages of development in the agroforestry species, *Albizia saman*, *Leucaena leucocephala* and *Acacia auriculiformis*. We also found that both the number and weight of root nodules were significantly positively correlated with the rate of BNF. Because the creation/destruction of nodules or the activation/deactivation of symbionts carries a high carbon cost (Menge et al. 2009), we postulate that higher rates of BNF imply an energetic expense, which is further evidence that BNF in *A. sieberiana* is facultative. Our results are on the lower spectrum for *Acacia* rates of fixation, as ascertained using the ARA method. For example, Woldemeskel & Sinclair (1998) found high relatively high specific acetylene reduction rate of  $57.7 \mu\text{mol g}^{-1} \text{h}^{-1}$ . However, this could be attributed to plant size (i.e. pot experiments versus established plants in the field).

## 5.6 Conclusions

Land-management practices such as fertilizer addition and burning, as well as the encroachment of *A. sieberiana* into savanna ecosystems may affect soil processes such as BNF and soil respiration. BNF is inhibited when soil N is high, and also when competing grass biomass is so considerable that the energetic cost of fixation cannot be met. Higher nitrogen in the soil increases respiration, while burning reduces respiration. Larger trees are able to fix more nitrogen due to the increased abundance of root nodules. Overall, BNF in *A. sieberiana* appears to be facultative, and strongly influenced by environmental drivers.

## 5.7 References

- Aarssen LW, Keogh T (2002) Conundrums of competitive ability in plants: what to measure? *Oikos* 96: 531-542.
- Aranibar J, Macko S, Anderson I, Potgieter A, Sowry R, Shugart H (2003) Nutrient cycling responses to fire frequency in the Kruger National Park (South Africa) as indicated by stable isotope analysis. *Isotopes Environ Health* 39: 141-158.
- Archer S, Schimel DS, Holland EA (1995) Mechanisms of shrubland expansion: land use, climate or CO<sub>2</sub>? *Clim Change* 29: 91-99.
- Archer SR, Boutton TW, Hibbard KA (2001) Trees in grasslands: biogeochemical consequences of woody plant expansion. In: E-D Schulze, SP Harrison, M Heimann, EA Holland, J Lloyd, IC Prentice, D Schimel (eds) *Global Biochemical Cycles in the Climate System*. Academic Press, San Diego, CA, USA.
- Azad MS, Mondol S, Matin MA (2013) Functional relationships of nodulation response and biomass production at nursery stages of two fast-growing, leguminous multipurpose tree species in Bangladesh: *Albizia saman* and *Leucaena leucocephala*. *For Sci Pract* 15: 274-285.
- Azad MS, Sumon MMH (2016) Species specific responses to age on nodule formation, seedling growth, and biomass production of *Acacia auriculiformis* at nursery stage. *Journal of Botany* 2016: 8.
- Blaser WJ, Shanungu GK, Edwards PJ, Olde Venterink H (2014) Woody encroachment reduces nutrient limitation and promotes soil carbon sequestration. *Ecol Evol* 4: 1423-1438.
- Bohrer G, Kagan-Zur V, Roth-Bejerano N, Ward D, Beck G, Bonifacio E (2003) Effects of different Kalahari-desert VA mycorrhizal communities on mineral acquisition and depletion from the soil by host plants. *J Arid Environ* 55: 193-208.
- Bond WJ (2008) What limits trees in C<sub>4</sub> grasslands and savannas? *Annu Rev Ecol Syst* 39: 641-659.
- Bond WJ, Keeley JE (2005) Fire as a global 'herbivore': the ecology and evolution of flammable ecosystems. *Trends Ecol Evol* 20: 387-394.
- Bond WJ, Midgley GF (2012) Carbon dioxide and the uneasy interactions of trees and savannah grasses. *Philos Trans R Soc Lond B Biol Sci* 367: 601-612.

- Burton AJ, Jarvey JC, Jarvi MP, Zak DR, Pregitzer KS (2012) Chronic N deposition alters root respiration-tissue N relationship in northern hardwood forests. *Glob Chang Biol* 18: 258-266.
- Cech P, Olde Venterink H, Edwards P (2010) N and P cycling in Tanzanian humid savanna: Influence of herbivores, fire, and N<sub>2</sub>-fixation. *Ecosystems* 13: 1079-1096.
- Coetsee C, Bond WJ, February EC (2010) Frequent fire affects soil nitrogen and carbon in an African savanna by changing woody cover. *Oecologia* 162: 1027-1034.
- Coetsee C, February EC, Bond WJ (2009) Nitrogen availability is not affected by frequent fire in a South African savanna. *J Trop Ecol* 24: 647-654.
- Cramer MD, Chimpango SBM, Van Cauter A, Waldram MS, Bond WJ (2007) Grass competition induces N<sub>2</sub> fixation in some species of African *Acacia*. *J Ecol* 95: 1123-1133.
- Cramer MD, Van Cauter A, Bond WJ (2010) Growth of N<sub>2</sub>-fixing African savanna *Acacia* species is constrained by below-ground competition with grass. *J Ecol* 98: 156-167.
- Crews TE (1999) The presence of nitrogen fixing legumes in terrestrial communities: evolutionary vs ecological considerations. *Biogeochemistry* 46: 233-246.
- Danso SKA, Bowen GD, Sanginga N (1992) Biological nitrogen fixation in trees in agro-ecosystems. *Plant Soil* 141: 177-196.
- David KAV, Apte SK, Banerji A, Thomas J (1980) Acetylene reduction assay for nitrogenase activity: Gas chromatographic determination of ethylene per sample in less than one minute. *Appl Environ Microbiol* 39: 1078-1080.
- Fynn RWS, Haynes RJ, O'Connor TG (2003) Burning causes long-term changes in soil organic matter content of a South African grassland. *Soil Biol Biochem* 5: 677-687.
- Goldberg DE, Landa, K. (1991) Competitive effect and response: hierarchies and correlated traits in the early stages of competition. *J Ecol* 79: 1013 - 1030.
- Grellier S, Ward D, Janeau J-L, Podwojewski P, Lorentz S, Abbadie L, Valentin C, Barot S (2013) Positive versus negative environmental impacts of tree encroachment in South Africa. *Acta Oecol* 53: 1-10.
- Haney RL, Brinton WH, Evans E (2008) Estimating soil carbon, nitrogen, and phosphorus mineralization from short-term carbon dioxide respiration. *Commun Soil Sci Plant Anal* 39: 2706-2720.

- Hansen AP, Pate JS (1987) Comparative growth and symbiotic performance of seedlings of *Acacia* spp. in defined pot culture or as natural understorey components of a eucalypt forest ecosystem in S.W. Australia. *J Exp Bot* 38: 13-25
- Hean J, Ward D (2012) Fire and herbivory are not substitutable: evidence from regrowth patterns and changes in physical and chemical defences in *Acacia* seedlings. *J Veg Sci* 23: 13-23
- Hellsten A, Huss-Danell K (2000) Interaction effects of nitrogen and phosphorus on nodulation in red clover (*Trifolium pratense* L.). *Acta Agric Scand Sect B Soil Plant Sci* 50: 135-142.
- Holdo RM (2013) Effects of fire history and N and P fertilization on seedling biomass, specific leaf area, and root: shoot ratios in a South African savannah. *S Afr J Bot* 86: 5-8.
- Huss-Danell K (1997) Actinorhizal symbioses and their N<sub>2</sub> fixation. *New Phytol* 136: 375-405.
- Kambatuku J, Cramer M, Ward D (2013) Nitrogen fertilisation reduces grass-induced N<sub>2</sub> fixation of tree seedlings from semi-arid savannas. *Plant Soil* 365: 307-320.
- Kraaij T, Ward D (2006) Effects of rain, nitrogen, fire and grazing on tree recruitment and early survival in bush-encroached savanna, South Africa. *Plant Ecol* 186: 235-246.
- Lessin LM, Dyer AR, Goldberg DE (2001) Using upper boundary constraints to quantify competitive response of desert annuals. *Oikos* 92: 153-159.
- Liu Y, Wu L, Baddeley JA, Watson CA (2010) Models of biological nitrogen fixation of legumes. A review. *Agron Sustainable Dev* 31: 155-172.
- López-Hernández D, Santaella S, Chacón P (2006) Contribution of nitrogen-fixing organisms to the N budget in *Trachypogon* savannas. *Eur J Soil Biol* 42: 43-50.
- Menge DN, Levin SA, Hedin LO (2009) Facultative versus obligate nitrogen fixation strategies and their ecosystem consequences. *Am Nat* 174: 465-477.
- Menner JC, Ledgard S, McLay C, Silvester W (2003) The effect of a single application of cow urine on annual N<sub>2</sub> fixation under varying simulated grazing intensity, as measured by four <sup>15</sup>N isotope techniques. *Plant Soil* 254: 469-480.
- Miyazaki K, Eskes HJ, Sudo K (2012) Global NO<sub>2</sub> emission estimates derived from an assimilation of OMI tropospheric NO<sub>2</sub> columns. *Atmos Chem Phys* 12: 2263-2288.
- Pacala SW, Hurtt GC, Baker D, Peylin P, Houghton RA, Birdsey RA, Heath L, Sundquist E T, Stallard RF, Ciais P, Moorcroft P, Caspersen JP, Shevliakova E, Moore B,

- Kohlmaier G, Holland E, Gloor M, Harmon ME, Fan SM, Sarmiento JL, Goodale C L, Schimel D, Field C B (2001) Consistent land- and atmosphere-based U.S. carbon sink estimates. *Science* 292: 2316-2320.
- Pillay T, Ward D (2014) Competitive effect and response of savanna tree seedlings: comparison of survival, growth and associated functional traits. *J Veg Sci* 25: 226-234.
- Raich JW, Potter CS (1995) Global patterns of carbon dioxide emissions from soils. *Glob Biogeochem Cycles* 9: 23-36.
- Resh SC, Binkley D, Parrotta JA (2002) Greater soil carbon sequestration under nitrogen-fixing trees compared with *Eucalyptus* species. *Ecosystems* 5: 217-231.
- Richards AE, Dathe J, Cook GD (2012) Fire interacts with season to influence soil respiration in tropical savannas. *Soil Biol Biochem* 53: 90-98.
- Richardson D, Jordan D, Garrard E (1957) The influence of combined nitrogen on nodulation and nitrogen fixation by *Rhizobium meliloti* Dangeard. *Can J Plant Sci* 37: 205-214.
- Riginos C (2009) Grass competition suppresses savanna tree growth across multiple demographic stages. *Ecology* 90: 335–340.
- Sankaran M, Hanan NP, Scholes RJ, Ratnam J, Augustine DJ, Cade BS, Gignoux J, Higgins SI, Le Roux X, Ludwig F, Ardo J, Banyikwa F, Bronn A, Bucini G, Caylor KK, Coughenour MB, Diouf A, Ekaya W, Feral CJ, February EC, Frost PGH, Hiernaux P, Hrabar H, Metzger KL, Prins HHT, Ringrose S, Sea W, Tews J, Worden J, Zambatis N (2005) Determinants of woody cover in African savannas. *Nature* 438: 846-849.
- Schleicher J, Meyer KM, Wiegand K, Schurr FM and Ward D (2011) Disentangling facilitation and seed dispersal from environmental heterogeneity as mechanisms generating associations between savanna plants. *J Veg Sci* 22:1038-1048.
- Scholes RJ, Archer SR (1997) Tree-grass interactions in savannas. *Annu Rev Ecol Syst* 28: 517-544.
- Schulze ED, Gebauer G, Ziegler H, Lange OL (1991) Estimates of nitrogen fixation by trees on an aridity gradient in Namibia. *Oecologia* 88: 451-455.
- Skarpe C (1991) Spatial patterns and dynamics of woody vegetation in an arid savanna. *J Veg Sci* 2: 565-572.
- Svenning M, MacDuff J (1996) Low root temperature retardation of the mineral nitrogen induced decline in N<sub>2</sub> fixation by a northern ecotype of white clover. *Ann Bot* 77: 615-621.



- Thomas AD (2012) Impact of grazing intensity on seasonal variations in soil organic carbon and soil CO<sub>2</sub> efflux in two semiarid grasslands in southern Botswana. *Philos Trans R Soc Lond B Biol Sci* 367: 3076-3086.
- Tjelele J, Ward D, Dziba L (2015) The effects of seed ingestion by livestock, dung fertilization, trampling, grass competition and fire on seedling establishment of two woody plant species. *PLoS ONE* 10: e0117788. [doi: 10.1371/journal.pone.0117788].
- Vadigi S, Ward D (2012) Fire and nutrient gradient effects on the sapling ecology of four *Acacia* species in the presence of grass competition. *Plant Ecol* 213: 1793-1802.
- Van Der Waal C, De Kroon H, De Boer WF, Heitkönig IMA, Skidmore AK, De Knegt HJ, Van Langevelde F, Van Wieren SE, Grant RC, Page BR, Slotow R, Kohi EM, Mwakiwa E, Prins HHT (2009) Water and nutrients alter herbaceous competitive effects on tree seedlings in a semi-arid savanna. *J Ecol* 97: 430-439.
- Van Langevelde F, Van de Vijver C, Kumar L, Van de Koppel J, De Ridder N, Van Andel J, Skidmore A, Hearne J, Stroosnijder L, Bond WJ, Prins HHT, Rietkerk M (2003) Effects of fire and herbivory on the stability of savanna ecosystems. *Ecology* 84: 337-350.
- Vitousek PM, Field CB (1999) Ecosystem constraints to symbiotic nitrogen fixers: a simple model and its implications. *Biogeochemistry* 46: 179-202.
- Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry* 13: 87-115.
- Vitousek PM, Menge DNL, Reed SC, Cleveland CC (2013) Biological nitrogen fixation: rates, patterns and ecological controls in terrestrial ecosystems. *Philos Trans R Soc Lond B Biol Sci* 368: 20130119.
- Ward D, Esler KJ (2011) What are the effects of substrate and grass removal on recruitment of *Acacia mellifera* seedlings in a semi-arid environment? *Plant Ecol* 212: 245-250.
- Ward D, Hoffman MT, Collocott SJ (2014) A century of woody plant encroachment in the dry Kimberley savanna of South Africa. *Afr J Range Forage Sci* 31: 107-121.
- Woldemeskel E, Sinclair FL (1998) Variations in seedling growth, nodulation and nitrogen fixation of *Acacia nilotica* inoculated with eight rhizobial strains. *Forest Ecol Manag* 104: 239-247.
- Xu W, Wan S (2008) Water-and plant-mediated responses of soil respiration to topography, fire, and nitrogen fertilization in a semiarid grassland in northern China. *Soil Biol Biochem* 40: 679-687.

Zhang C, Niu D, Hall SJ, Wen H, Li X, Fu H, Wan C, Elser JJ (2014) Effects of simulated nitrogen deposition on soil respiration components and their temperature sensitivities in a semiarid grassland. *Soil Biol Biochem* 75: 113-123.

Table 5.1 Univariate statistics based on the significant MANOVA results, showing the effects of fertilizer, grazing, burning on soil respiration, number and weight of root nodules, as well as the rate of biological nitrogen fixation (mol ethylene plant<sup>-1</sup>h<sup>-1</sup>) of the tree species *Acacia sieberiana*. Bold text indicates significant F ratios and probabilities. Interaction effects were omitted because they were not significant for any of the dependent variables

Treatments	df	Number of nodules		Nodule mass (g)		Nitrogen fixation (mol ethylene plant <sup>-1</sup> h <sup>-1</sup> )		Soil respiration ( C – CO <sub>2</sub> ppm )	
		F	p	F	p	F	p	F	p
Fertilizer	1	0.564	0.457	0.174	0.697	<b>9.826</b>	<b>0.003</b>	<b>6.276</b>	<b>0.003</b>
Grazing	1	1.26	0.268	0.984	0.45	1.33	0.255	1.33	0.255
Burning	1	0.0001	0.972	0.187	0.668	<b>4.308</b>	<b>0.044</b>	<b>12.302</b>	<b>0.001</b>
Error df		47		47		47		79	

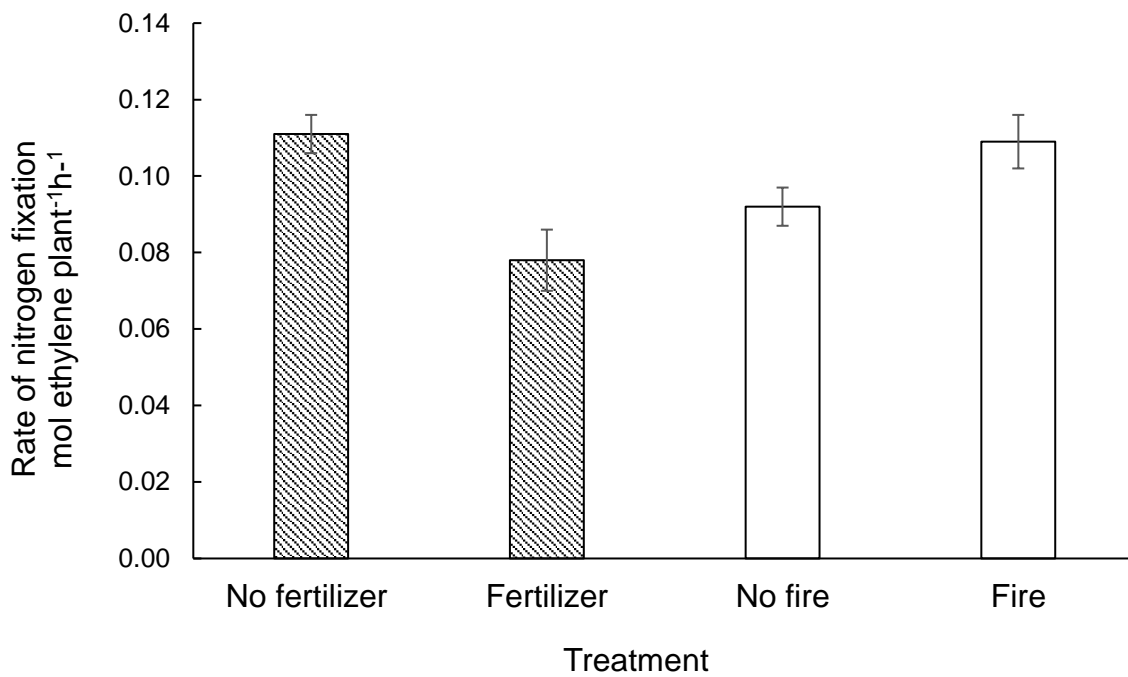


Fig. 5.1 Significantly lower rate of nitrogen fixation (mol ethylene plant<sup>-1</sup>h<sup>-1</sup>)  $\pm$  SE with the addition of fertilizer, and higher rates of nitrogen fixation (mol ethylene plant<sup>-1</sup>h<sup>-1</sup>)  $\pm$  SE with the application of fire.

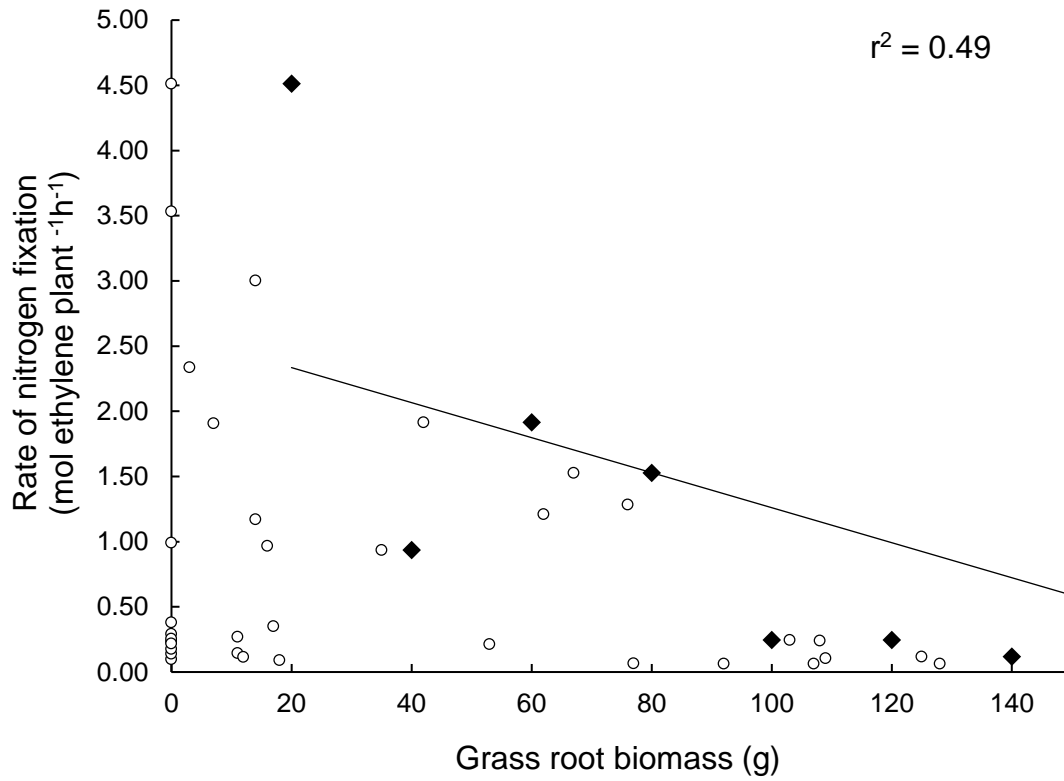


Fig. 5.2 The rate of nitrogen fixed (mol ethylene plant<sup>-1</sup>h<sup>-1</sup>) by *A. sieberiana* trees declined with increasing grass root biomass. Open circles (○) indicate the regression of rate of nitrogen fixation against unclustered grass biomass. Closed diamonds (◆) indicate the regression using the maximum rate of nitrogen fixation against grass biomass clustered into width classes of 20 g.

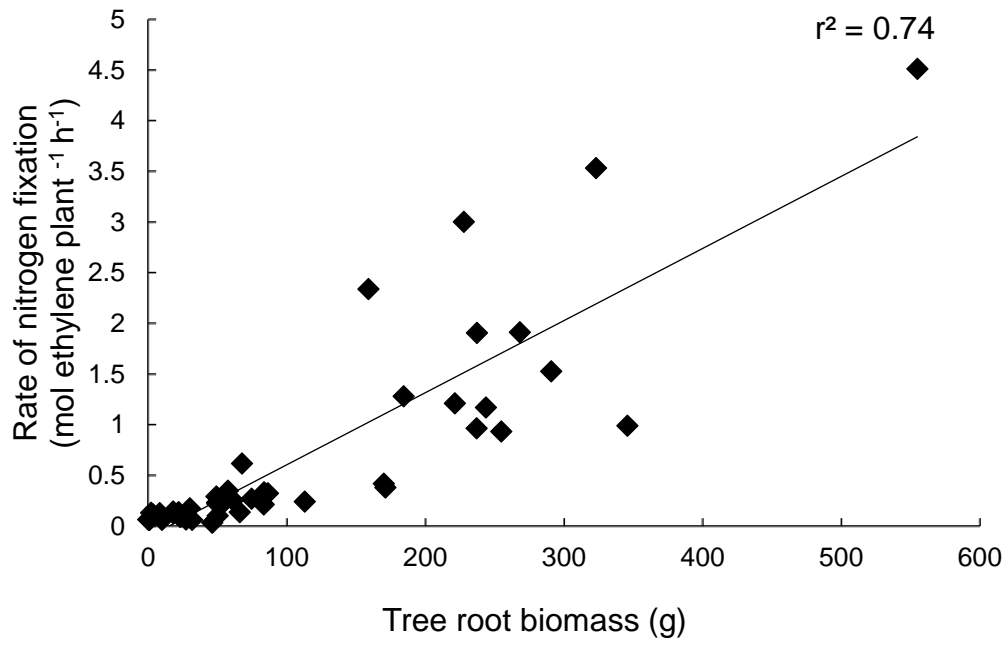


Fig. 5.3 Rate of nitrogen fixation in *A. sieberiana* (mol ethylene plant<sup>-1</sup>h<sup>-1</sup>) increased relative to tree root biomass. The rate of nitrogen fixation increased with an overall increase in tree root biomass.

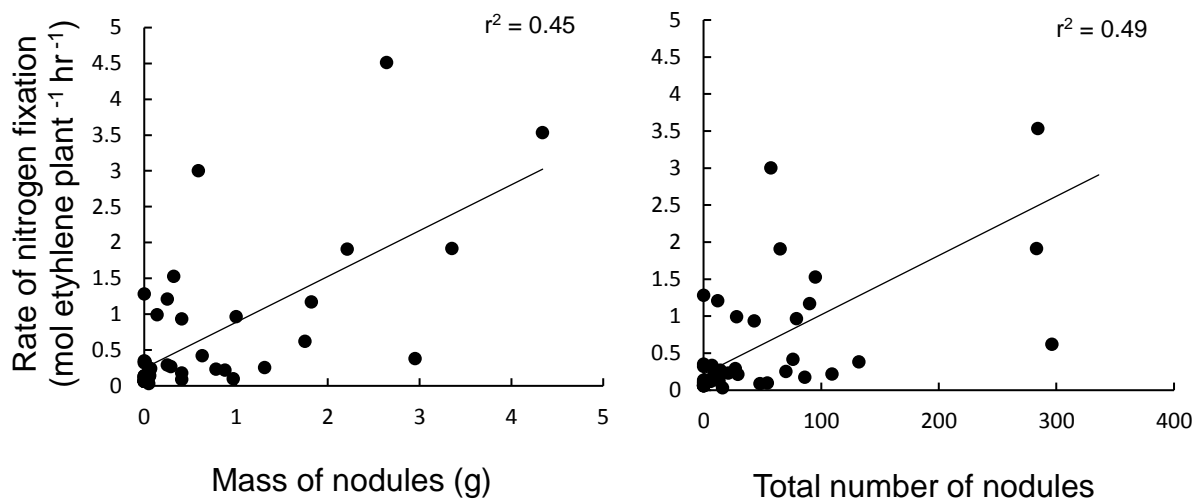


Fig. 5.4 There was a significant positive relationship between the rate of nitrogen fixation (mol ethylene plant<sup>-1</sup>h<sup>-1</sup>) and (a) the mass of root nodules, (b) the total number of root nodules.



Fig. 5.5 Root nodules produced by *A. sieberiana* woody saplings, attached to the root (left), and removed for weighing and counting (right).



## CHAPTER 6: Conclusions and Recommendations for Future Research

### 6.1 Introduction

Woody plant encroachment (often known as bush or shrub encroachment) has become an increasingly destructive force over the last fifty years, and is still a poorly understood phenomenon, lacking clear explanations and predictive tools (Ward 2005). Grassland and savanna regions worldwide are experiencing transformations due to agricultural intensification, over-grazing and changes in fire regimes (Bustamante *et al.* 2012). The potential short-term benefits of maximizing livestock units has resulted in severe over-grazing (Archer *et al.* 2017), reducing competition from grasses and allowing trees to proliferate. In addition, the removal of fire from historically fire-controlled landscapes has resulted in an increase in woody plant density (Archibald *et al.* 2012).

Further perturbations exist due to the effects of climate change, altered precipitation patterns and temperature ranges, as well as increased nitrogen deposition (Hibbard *et al.* 2001; Schlesinger 1999). The Intergovernmental Panel on Climate Change (IPCC) has predicted a reduction in precipitation over the south-western parts of South Africa by the end of the 21<sup>st</sup> century (IPCC 2014). Decadal analyses of temperatures also point strongly to an increased warming trend across the African continent over the last 50 to 100 years, with the mean annual temperature rise over Africa likely to exceed 2°C in by the end of this century (IPCC 2014). In addition, anthropogenic activities such as industrial air pollution, has led to a substantial increase in atmospheric nitrogen composition (Wenig 2003). Altered precipitation, climate warming, increased nitrogen deposition and changes in land use all contribute the

uncertainties of vegetation dynamics in ecosystems where woody plant encroachment has occurred, and also where it may potentially occur. This thesis aimed to disentangle the effects of varying climate and land-use on woody plant encroachment.

## **6.2 Summary and future directions**

The first chapter of this thesis examined the factors that limit woody plant establishment, under current and predicted climate-change scenarios. This study investigated the effects of temperature warming and altered precipitation on the germination and subsequent establishment of the common woody encroacher, *Acacia sieberiana* (**Chapter 2**). Climate models predict that precipitation regimes are likely to become more variable in the near future, including more extreme and prolonged droughts across seasons (IPCC 2014). Engelbrecht & Engelbrecht (2015) have also indicated possible intrusion of trees into the grasslands of southern Africa. We found that water availability was the main limiting factor for grass productivity while warming had no significant effect on grasses; the strong positive effect of water availability on grass biomass has also been demonstrated in **Chapter 3 and 4**. Volder *et al.* (2013) have shown that climate warming and drought alter tree establishment in savannas through differential responses of tree seedlings and grass to intermittent precipitation events (see also Wiegand *et al.* 2006). The positive effects of high precipitation on annual net primary productivity (ANPP) is well documented in the literature (O'Connor *et al.* 2001; Sankaran *et al.* 2005; Knapp *et al.* 2008; February *et al.* 2013; Bond *et al.* 2017), and need not be further explored. It is, however, crucial to understand the effects of timing and frequency of precipitation, which may result in *en masse* germination of woody plants following prolonged drought (February *et al.* 2013), especially in the semi-arid areas of southern Africa.

We established that warming had a large positive effect on tree growth and performance (measured as specific leaf area) when coupled with excess or normal water treatments (**Chapter 2**), elucidating the positive effect of increased temperature for *A. sieberiana* growth. The positive effects of warming on tree growth rates and functional traits, such as higher specific leaf area, may be attributed to increased metabolic rates under warmed conditions (Saxe *et al.* 2001; Way & Oren 2010; Pérez-Harguindeguy *et al.* 2013). This result highlights the likelihood of greater encroachment of woody plants in high-precipitation grasslands and savannas with future predicted climate warming. Violle & Jiang (2009) have shown that instantaneous measurements of plant traits can provide information on how plants perceive competitive environments. As environmental problems such as global climate and land use changes increase (IPCC 2014), the need for general predictive models in ecology becomes more crucial (Funk *et al.* 2017; Williams *et al.* 2008). The main challenge for future studies will be to identify which traits are likely to be most useful in predicting community and ecosystem dynamics. Funk *et al.* (2017) resolve that response and effect traits must be identified. Response traits, such as relative growth rate shown to be more prevalent in productive high-nutrient communities (Pillay & Ward 2014), suggesting that it provides some fitness advantage when resources are not limiting.

Intraspecific trait variability in natural populations may impact competitive interactions, ultimately shaping community composition and key ecosystem functions (Albert *et al.* (2010). For example, Madritch & Lindroth (2015) showed using carefully controlled conditions that condensed tannin concentrations varied among aspen genotypes and decreased with increasing nutrient availability. Shifts in community composition may also be favoured by switches in key plant functional traits, such as specific leaf area (Brantley & Young 2007; Pérez-Harguindeguy *et al.* 2013). A functional trait-based framework of assessing biotic interactions should therefore be the main focus of future studies (Cornelissen *et al.* 2003). For

example, Brantley & Young (2007) found that leaf area is an important structural variable in plant communities, and can be used as an indicator for a variety of ecosystem processes such as photosynthesis, transpiration, and nutrient cycling. Furthermore, leaf and photosynthetic traits are responsive to CO<sub>2</sub> and, therefore, may be key plant traits likely to result in increased fitness in the elevated CO<sub>2</sub> environments predicted for the future (Leakey & Lau 2012; Manea & Leishman 2014). It would, therefore, be interesting to determine which traits are favoured through predicted future climate change, in an attempt to predict possible encroaching species. For example, with the future drought scenarios predicted over much of sub-Saharan Africa, drought-resistant woody plants may be favoured. Drought resistance can be quantified using plant physiological indices such as leaf photosynthetic performance, leaf water loss, rehydration rate and leaf water use efficiency (Liu & Guan 2012; Ansley *et al.* 2014).

#### *Effects of land use changes - Fertilizer, fire and grazing*

We conducted a greenhouse experiment to investigate the effects of varying land-use practices (fertilization, grazing and fire) on the early stages of growth, survival and functional traits of an encroaching tree species, *A. sieberiana* (**Chapter 3**). We found that fertilization (increased nutrient availability) had a negative effect on tree seedling growth and survival, due to strong interspecific competition with grasses. This indicates that at the recruitment stages of tree growth, supplementary nutrients (either through use of fertilizers or increased nitrogen deposition; Stevens *et al.* 2017) will favour grass growth only (see also Kraaij & Ward, 2006), and possibly promote tree seedling mortality, as was shown in this study (**Chapter 2, 3 and 4**).

Strong positive effects of fertilization on grass productivity were found in both **Chapters 3 and 4**. However, this study did not find a hump-shaped relationship between

ANPP and species richness. This is likely due to the relatively short duration of the fertilization trial. Some studies have revealed significant effects of nutrient addition in a single year (Vourlitis *et al.* 2007). Other long-term studies such as the Park Grass experiment in England (Crawley *et al.* 2005), the global Nutrient Network experiments (Adler *et al.* 2011) and the Ukulinga experiments in South Africa (Ward *et al.* 2017) demonstrate that multiple factors, such as pH as well as the type and amount of fertilizer, affect the relationship between ANPP and species richness. It will thus be useful to determine the average time for fertilization to have significant effects on woody encroachers. However, the overall insight of a large majority of studies, including this thesis (**Chapter 4**), is that nutrient addition reduces species richness. Grassland biodiversity is therefore likely to decline in light of global eutrophication by N and P pollution, often associated with increased acidity from atmospheric inputs.

Fire was found to be ineffective at reducing tree seedling performance (**Chapter 3**). Rather, we found that the resprouting capability of the common invader *A. sieberiana* was sufficient to prevent seedling mortality. Many savanna trees are classified as resprouters, based on their post-fire responses (Higgins *et al.* 2000; Clarke *et al.* 2013; Smit *et al.* 2016). Carbohydrate reserves are essential for resprouting following a fire, and largely determine the capacity to survive fire, as well as maintain regrowth rate (Hoffmann *et al.* 2009). Kgope *et al.* (2010) found that after simulated fire, *A. karroo* plants showed a greater response in total stem length, total stem diameter and shoot dry weight, signalling the importance of resprouting following disturbances such as fire in savanna systems (see also Wakeling *et al.* 2007). Tree traits, such as the rate of bark production ( $\text{increment} \cdot \text{yr}^{-1}$ ) are also critical for determining how quickly a developing sapling would be protected or bark could regenerate between two fires (Charles-Dominique *et al.* 2017). We postulate that prescribed burning intensity and frequency is crucial for the control of woody encroachers, especially those that

have resprouting responses (Twidwell *et al.* 2016), as found in Chapter 3. Fire intensity determines tree dynamics in savannas (Hoffmann *et al.* 2009; Bond & Midgley 2012). However, the frequency of fire at a particular intensity will help determine the time taken to exhaust the non-structural carbon reserves for a given species (Clarke & Knox 2009; Accatino *et al.* 2016). The timing of fire is also of importance, and may have positive and negative effects (i.e. depending on whether the fire occurs before or after rains). For example, Kraaij & Ward (2006) found that post-fire tree germination was extremely low, probably due to below-average precipitation. Hence, future research should aim to quantify the fire intensity, frequency and timing that will induce mortality in encroaching seedlings (Smit *et al.* 2016). This will enable us to categorize species according to their resilience to fires and better predict landscape-level changes in species structure and composition.

Another interesting future direction is the use of pyric herbivory (also known as patch-burn grazing). This strategy has been adopted in North America as a form of mitigation against woody-plant encroachment, and incorporates the processes of fire and grazing to create structural heterogeneity and increase biodiversity (Fuhlendorf *et al.* 2009; Twidwell *et al.* 2013). The premise of this technique is the temporal interaction of fire and grazing, where positive and negative feedbacks promote a shifting pattern of disturbance across the landscape. Future studies in South Africa should focus on optimal patch burning and grazing regimes to limit woody plant encroachment, and to increase the palatable grass layer. This is particularly important for semi-arid rangelands in southern Africa, where pastoral production systems are of high economic value, relative to other forms of agriculture. For example, in semi-arid areas where pastoral production is crucial and ecosystem benefits of encroachment are relatively low, the efficacy of woody plant control must be thoroughly investigated (Archer 2010; Eldridge & Soliveres 2014). Conversely, in the mesic and humid areas of South Africa where eco-tourism and game ranching are viable, the exploitation of ecosystem

services offered by woody plants, such as increased carbon sequestration, improved soil fertility and hydraulic lift, must be further investigated.

We found significant positive effects of encroachment on litter quality, C:N ratios (indicator of decomposition) and forb cover (**Chapter 4**). The overall purportedly beneficial effects of encroachment on litter and plant species richness was more pronounced at the high-precipitation sites. Woody encroachment is generally associated with desertification and reductions in species diversity (Ratajczak *et al.* 2012). However, Eldridge & Soliveres (2015) have conducted a review of existing studies on the effects of encroachment. They noted several ecosystem services (biodiversity, soil C, hydrology, nutrient provision, grass growth and soil fertility) provided by woody encroachers. This study reinforces the positive effects of encroachment in high-precipitation areas. We propose future studies adopt a similar view to that of Eldridge & Soliveres (2015), whereby the costs of encroachment mitigation are weighed against the ecosystem benefits of encroachment, on a site-by-site basis.

The two-year fertilization experiment investigated the effects of woody plant encroachment and increased nutrient availability on soil, litter and plant parameters in the field (**Chapter 4**). We were particularly interested in the trends across a precipitation gradient (ranging from 300 – 1,500 mm mean annual precipitation). We found that soil nitrogen stocks were significantly lower in the semi-arid sites than in the high precipitation sites, regardless of nutrient addition. This result was possibly due to water scarcity. Semi-arid savannas in southern Africa are known to be limited by water and less by nutrient availability (Bond 2008). This has also been demonstrated by Kraaij & Ward (2006), who showed that tree recruitment was constrained by both precipitation amount and frequency. Our study found that nutrient addition increased grass biomass and reduced species richness in the high-precipitation sites only. Increases in nitrogen deposition due to rising industrial emissions are predicted across South Africa (Wenig *et al.* 2003; Phoenix *et al.* 2006). This may lead to

reduced species diversity in high-precipitation areas, due to the negative relationship between ANNP and plant species richness (Crawley *et al.* 2005; Ward *et al.* 2017). This is a particularly important finding as the IPCC (2014) have predicted increased precipitation for the eastern parts of southern Africa, which is the geographic location of the high precipitation sites used for this study.

We found considerable variation in soil P at the high-precipitation sites, with some evidence of P-limitation in the encroached site with highest precipitation (KwaMbonambi) (**Chapter 4**). This result is in agreement with Blaser *et al.* (2014), who also found that total P and extractable P pools increased along a shrub-cover gradient, due to a negative feedback from N-fixing woody shrubs. However, this trend was not consistent across the gradient, indicating site-specificity in soil P. Indeed, at the high-precipitation Bergville site, Grellier *et al.* (2014) have found variations in soil clay content linked to the spatial distribution of woody plants. Higher soil-clay content is linked to a higher diffusion coefficient of soil P (Olsen & Watanabe 1963), which may explain the unusually high soil P in the Bergville grassland sites. Importantly, not all woody encroachers are capable of N-fixation (e.g. *Larrea tridentata* in the U.S. southwestern deserts), and more importantly, N-fixation may not be obligate under certain environmental pressures, which prompts the need for further investigation into the factors which may promote N-fixation in encroaching species.

This led us to the final study, which examined the effects of fertilizer, simulated fire and grazing on the nodulation and N-fixation capabilities of the woody legume, *A. sieberiana*, as well as the associated effects on soil respiration (**Chapter 5**). We found that the ability of woody encroachers to fix nitrogen was constrained by nutrient addition. These effects were largely due to the positive effect of fertilizer on grass production, thereby increasing the competitive effect of grasses on neighbouring tree seedlings. This implies that grasses are superior competitors for soil resources (Knoop & Walker 1985), possibly due to their fine-



root morphology (Aerts *et al.* 1991; Wakeling *et al.* 2015). Several other studies have also shown that nitrogen has a strong positive effect on the competitive role played by grasses (Kraaij & Ward 2006; Cramer *et al.* 2010; Kambatuku *et al.* 2011). This result supports the findings of **Chapters 2 and 3**, which also showed that increased nutrient availability benefits grasses, which in turn suppresses tree growth. Therefore, we postulate that increased nitrogen deposition may limit woody plant encroachment, due to strong interspecific competition from grasses (Pillay & Ward 2014).

Furthermore, we found increased rates of biological nitrogen fixation in tree saplings exposed to fire (compared to no-fire controls), possibly as a compensatory response to post-fire soil nitrogen loss (**Chapter 5**). Coupled with the strong resprouting capability demonstrated in **Chapter 2**, low intensity or infrequent fires may actually promote woody plant encroachment by enhancing N-fixation. Hence, we again highlight the importance of prescribed burning intensity and frequency to control woody encroachers.

According to Leakey *et al.* (2012), most of the current research is based on temperate systems, with limited or no information available on the understanding of plant responses to elevated CO<sub>2</sub> concentrations, particularly in sub-Saharan Africa. Elevated CO<sub>2</sub> is argued to be a major potential cause of increased woody plant dominance in open savannas (Bond & Midgley 2012), owing to the greater resultant increases in the net photosynthetic rate of C<sub>3</sub> plants (which are usually trees) than C<sub>4</sub> plants. A possible area for future research is to examine the interactive effects of increased nutrient availability, precipitation and rising CO<sub>2</sub> concentrations on N-fixation. Gray *et al.* (2013) have found increased facultative N-fixation in response to higher CO<sub>2</sub> concentration, but interestingly only under drought conditions. Manea & Leishman (2014) found that woody plant seedling growth was reduced at elevated CO<sub>2</sub> levels compared to that at the ambient CO<sub>2</sub> level across all competition treatments. A reduction in precipitation coupled with increased CO<sub>2</sub> concentrations is likely over the south-

western parts of South Africa by the end of the 21st century (IPCC 2014). Therefore, nodulating encroachers occurring in the semi-arid regions of southern Africa, may have a distinct competitive advantage over other species.

### **6.3 Conclusions**

This thesis shows that changing climate and land-use will have a marked effect on woody plant encroachment, influencing plant germination, survival and performance. A large body of work points to key mechanisms that will be affected (Archer *et al.* 2017; Bond *et al.* 2017) however there is a long road ahead to understand the effects of changing climate and land use on woody plant encroachment with detail and certainty, in part because of the multi-factorial nature of these interactions. Furthermore, the effects of woody plant encroachment on grasslands and savannas are not uniform, with differences across the southern African precipitation gradient. These differences must be incorporated into mitigation measures for effective control of woody encroachment, considering that woody plant encroachment is the single most important factor hampering sustainable livestock production, particularly in the semi-arid regions of South Africa (Ward *et al.* 2014). Indeed, the gap in this research is the interactive effects of climate and land-use changes with rising CO<sub>2</sub> concentrations (Leahey *et al.* 2012) which must be addressed using multi-disciplinary field and experiments. However, the overall findings of our research indicate that, due to the strong influences of water availability, a site-specific method of assessment must be employed to determine the effects of woody plant encroachment, and furthermore the appropriate control measures.

## 6.4 References

- Accatino F., Wiegand K., Ward D. and De Michele C. 2016. Trees, grass, and fire in humid savannas - the importance of life history traits and spatial processes. *Ecological Modelling* 320: 135-144.
- Adler P.B., Seabloom, E.W., Borer E.T., Hillebrand, H, Hautier, Y., Hector, A., Harpole, W.S., O'Halloran, L.R., Grace, J.B., Anderson, T.B., Bakker, J.D., Biederman, L.A., Brown, C.S., Buckley, Y.M., Calabrese, L.B., Chu, C.J., Cleland, E.E., Collins, S.L., Cottingham, K.L., Crawley, M.J., Damschen, E.I., Davies, K.F., DeCrappeo, N.M., Fay, P.A., Firn, J., Frater, P., Gasarch, I., Gruner, D.S., Hagenah, N., HilleRisLambers, J., Humphries, H., Jin, V.L., Kay, A.D., Kirkman, K.P., Klein, J.A., Knops, J.M.H., La Pierre, K.J., Lambrinos, J.G., Li, W., MacDougall, A.S., McCulley, R.L., Melbourne, B.A., Mitchell, C.E., Moore, J.L., Morgan, J.W., Mortensen, B., Orrock, J.L., Prober, S.M., Pyke, D.A., Risch, C., Schuetz, M., Smith, M.D., Stevens, C.J., Sullivan, L.L., Wang, G., Wragg, P.D., Wright, J.P., and Yang, L.H. 2011. Productivity is a poor predictor of plant species richness. *Science* 333: 1750-1753.
- Aerts R., Boot R.G.A. and van der Aart P.J.M. 1991. The relation between above- and belowground biomass allocation patterns and competitive ability. *Oecologia* 87: 551-559.
- Albert C.H., Thuiller W., Yoccoz N.G., Soudant A., Boucher F., Saccone P. and Lavorel S. 2010. Intraspecific functional variability: extent, structure and sources of variation. *Journal of Ecology* 98: 604-613.
- Ansley R.J., Boutton T.W. and Jacoby P.W. 2014 Root biomass and distribution patterns in a semi-arid mesquite savanna: responses to long-term rainfall manipulation. *Rangeland Ecology and Management* 67: 206-218.
- Archer S.R. 2010. Rangeland conservation and shrub encroachment: new perspectives on an old problem. *In: Du Toit J.T., Kock R. and Deutsch J.C. (eds.), Wild Rangelands: Conserving Wildlife While Maintaining Livestock in Semi-Arid Ecosystems.* Blackwells, Oxford, U.K., pp. 53-97.
- Archer S.R., Andersen E.M., Predick K.I., Schwinning S., Steidl R.J. and Woods S.R. 2017. Woody plant encroachment: causes and consequences. *In: Briske D.D. (ed.), Rangeland Systems: Processes, Management and Challenges.* Springer International Publishing, New York, pp. 25-84.

- Archibald S., Staver A.C. and Levin S.A. 2012. The evolution of human-driven fire regimes in Africa. *Proceedings of the National Academy of Sciences, U.S.A.* 109: 847-852.
- Blaser W.J., Shanungu G.K., Edwards P.J. and Olde Venterink H. 2014. Woody encroachment reduces nutrient limitation and promotes soil carbon sequestration. *Ecology and Evolution* 4: 1423-1438.
- Bond W.J. 2008. What limits trees in C<sub>4</sub> grasslands and savannas? *Annual Review of Ecology, Evolution and Systematics* 39: 641-659.
- Bond W.J. and Midgley G.F. 2012. Carbon dioxide and the uneasy interactions of trees and savanna grasses. *Philosophical Transactions of the Royal Society Series B: Biological Sciences* 367: 601-612.
- Bond W.J., Staver A.C., Cramer M.D., Wakeling J.L., Midgley J.J. and Balfour D.A. 2017. Demographic bottlenecks and savanna tree abundance. *In: Conserving Africa's Mega-Diversity in the Anthropocene: The Hluhluwe-iMfolozi Park Story* (eds J.P.G.M. Cromsigt, S. Archibald & N. Owen-Smith), pp. 161-188. Cambridge University Press, Cambridge, U.K.
- Brantley S.T. and Young D.R. 2007. Leaf-area index and light attenuation in rapidly expanding shrub thickets. *Ecology* 88: 524-530.
- Bustamante M.C., de Brito D., Kozovits A., Luedemann G., de Mello T.B., de Siqueira Pinto A., Munhoz C.R. and Takahashi F.C. 2012. Effects of nutrient additions on plant biomass and diversity of the herbaceous-subshrub layer of a Brazilian savanna (Cerrado). *Plant Ecology* 213: 795-808.
- Case M.F. and Staver A.C. 2017. Fire prevents woody encroachment only at higher-than-historical frequencies in a South African savanna. *Journal of Applied Ecology* 54: 955-962.
- Charles-Dominique T., Midgley G.F. and Bond W.J. 2017. Fire frequency filters species by bark traits in a savanna-forest mosaic. *Journal of Vegetation Science* 28: 728-735.
- Clarke P.J. and Knox K.J.E. 2009. Trade-offs in resource allocation that favour resprouting affect the competitive ability of woody seedlings in grassy communities. *Journal of Ecology* 97: 1374-1382.
- Clarke P.J., Lawes M., Midgley J., Lamont B., Ojeda F., Burrows G., Enright N. and Knox K. 2013. Resprouting as a key functional trait: how buds, protection and resources drive persistence after fire. *New Phytologist* 197: 19-35.

- Cornelissen J.H.C., Cerabolini B., Castro-Díez P., Villar-Salvador P., Montserrat-Martí G., Puyravaud J.P., Maestro M., Werger M.J.A. and Aerts R. 2003. Functional traits of woody plants: correspondence of species rankings between field adults and laboratory-grown seedlings? *Journal of Vegetation Science* 14: 311-322.
- Cramer M.D., Van Cauwer A. and Bond W.J. 2010. Growth of N<sub>2</sub>-fixing African savanna *Acacia* species is constrained by below-ground competition with grass. *Journal of Ecology* 98: 156-167.
- Crawley M.J., Johnston A.E., Silvertown J., Dodd M., de Mazancourt C., Heard M.S., Henman D.F. and Edwards G.R. 2005. Determinants of species richness in the Park Grass experiment. *American Naturalist* 165: 179-192.
- Eldridge D.J. and Soliveres S. 2014. Are shrubs really a sign of declining ecosystem function? Disentangling the myths and truths of woody encroachment in Australia. *Australian Journal of Botany* 62: 594-608.
- Engelbrecht C.J. and Engelbrecht F.A. 2015. Shifts in Köppen-Geiger climate zones over southern Africa in relation to key global temperature goals. *Theoretical and Applied Climatology*: 1-15.
- February E.C., Higgins S.I., Bond W.J. and Swemmer L. 2013. Influence of competition and rainfall manipulation on the growth responses of savanna trees and grasses. *Ecology* 94: 1155-1164.
- Fuhlendorf S.D., Engle D.M., Kerby J.A.Y. and Hamilton R. 2009. Pyric herbivory: rewilding landscapes through the recoupling of fire and grazing. *Conservation Biology* 23: 588-598.
- Funk J.L., Larson J.E., Ames G.M., Butterfield B.J., Cavender-Bares J., Firn J., Laughlin D.C., Sutton-Grier A.E., Williams L. and Wright J. 2017. Revisiting the Holy Grail: using plant functional traits to understand ecological processes. *Biological Reviews* 92: 1156-1173.
- Gray S.B., Strellner R.S., Puthuval K.K., Ng C., Shulman R.E., Siebers M.H., Rogers A. and Leakey A.D. 2013. Minirhizotron imaging reveals that nodulation of field-grown soybean is enhanced by free-air CO<sub>2</sub> enrichment only when combined with drought stress. *Functional Plant Biology* 40: 137-147.
- Grellier S., Florsch N., Janeau J.L., Podwojewski P., Camerlynck C., Barot S., Ward D. and Lorentz S. 2014. Soil clay influences *Acacia* encroachment in a South African grassland. *Ecohydrology* 7: 1474-1484.

- Hibbard K.A., Archer S., Schimel D.S. and Valentine D.W. 2001. Biogeochemical changes accompanying woody plant encroachment in a subtropical savanna. *Ecology* 82: 1999-2011.
- Higgins S.I., Bond W.J. and Trollope W.S.W. 2000. Fire, resprouting and variability: a recipe for grass-tree coexistence in savanna. *Journal of Ecology* 88: 213-229.
- Hoffmann W.A., Adasme R., Haridasan M., De Carvalho M.T., Geiger E.L., Pereira M.A.B., Gotsch S.G. and Franco A.C. 2009. Tree topkill, not mortality, governs the dynamics of savanna–forest boundaries under frequent fire in central Brazil. *Ecology* 90: 1326-1337.
- Intergovernmental Panel on Climate Change. 2014. *Climate Change 2014 – Impacts, Adaptation and Vulnerability: Regional Aspects*. Cambridge, UK.: Cambridge University Press.
- Kambatuku J., Cramer M. and Ward D. 2011. Intraspecific competition between shrubs in a semi-arid savanna. *Plant Ecology* 212: 701-713.
- Kgope B.S., Bond W.J. and Midgley G.F. 2010. Growth responses of African savanna trees implicate atmospheric CO<sub>2</sub> as a driver of past and current changes in savanna tree cover. *Austral Ecology* 35: 451-463.
- Knapp A.K., Briggs J.M., Collins S.L., Archers S.R., Bret-Harte M.S., Ewers B.E., Peters D.P., Young D.R., Shaver G.R., Pendall E. and Cleary M.B. 2008. Shrub encroachment in North America grasslands: shifts in growth form dominance rapidly alters control of ecosystem carbon inputs. *Global Change Biology* 14: 614-623.
- Knoop W.T and Walker B.H. 1985. Interactions of woody and herbaceous vegetation in a southern African savanna. *Journal of Ecology* 73: 235-253.
- Kraaij T. and Ward D. 2006. Effects of rain, nitrogen, fire and grazing on tree recruitment and early survival in bush-encroached savanna, South Africa. *Plant Ecology* 186: 235 – 246.
- Leakey A.D.B., Bishop K.A. and Ainsworth E.A. 2012. A multi-biome gap in understanding of crop and ecosystem responses to elevated CO<sub>2</sub>. *Current Opinion in Plant Biology* 15: 1-9.
- Leakey, A.D. and Lau, J.A. 2012. Evolutionary context for understanding and manipulating plant responses to past, present and future atmospheric [CO<sub>2</sub>]. *Philosophical Transactions of the Royal Society Series B: Biological Sciences* 367: 613-629.

- Liu N. and Guan L. 2012. Linkages between woody plant proliferation dynamics and plant physiological traits in southwestern North America. *Journal of Plant Ecology* 5: 407-416.
- Madritch M.D. and Lindroth R.L. 2015. Condensed tannins increase nitrogen recovery by trees following insect defoliation. *New Phytologist* 208: 410-420.
- Manea A. and Leishman MR. 2014. Competitive interactions between established grasses and woody plant seedlings under elevated CO<sub>2</sub> levels are mediated by soil water availability. *Oecologia* 177: 499-506.
- O'Connor T.G., Haines L. and Snyman H.A. 2001. Influence of precipitation and species composition on phytomass of a semi-arid African grassland. *Journal of Ecology* 89: 850-860.
- Olsen S.R. and Watanabe F.S. 1963. Diffusion of phosphorus as related to soil texture and plant uptake. *Soil Science Society of America Journal* 27: 648-653.
- Pérez-Harguindeguy N., Díaz S., Garnier E., Lavorel S., Poorter H., Jaureguiberry P., Bret-Harte M.S., Cornwell W.K., Craine J.M., Gurvich D.E., Urcelay C., Veneklaas E.J., Reich P.B., Poorter L., Wright I.J., Ray P., Enrico L., Pausas J.G., De Vos A.C., Buchmann N., Funes G., Quétier F., Hodgson J.G., Thompson K., Morgan H.D., Ter Steege H., Van Der Heijden M.G.A., Sack L., Blonder B., Poschlod P., Vaieretti M.V., Conti G., Staver A.C., Aquino, S. and Cornelissen, J.H.C. 2013. New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany* 61: 167-234.
- Pillay T. and Ward D. 2014. Competitive effect and response of savanna tree seedlings: comparison of survival, growth and associated functional traits. *Journal of Vegetation Science* 25: 226–234.
- Ratajczak Z., Nippert J.B. and Collins SL. 2012. Woody encroachment decreases diversity across North American grasslands and savannas. *Ecology* 93: 697-703.
- Saxe H., Cannell M.G., Johnsen O., Ryan M.G. and Vourlitis G. 2001. Tree and forest functioning in response to global warming. *New Phytologist* 149: 369-399.
- Schlesinger W.H. 1999. Carbon sequestration in soils. *Science* 284: 2095-2097.
- Smit I.P., Asner G.P., Govender N., Vaughn N.R. and Van Wilgen B.W. 2016. An examination of the potential efficacy of high-intensity fires for reversing woody encroachment in savannas. *Journal of Applied Ecology* 53: 1623-1633.

- Stevens N., Lehmann, C.E.R., Murphy, B.P and Durigan, G. 2017. Savanna woody encroachment is widespread across three continents. *Global Change Biology* 23: 235–244.
- Twidwell D., Rogers W.E., Wonkka C.L., Taylor C.A. and Kreuter U.P. 2016. Extreme prescribed fire during drought reduces survival and density of woody resprouters. *Journal of Applied Ecology* 53: 1585-1596.
- Twidwell D., Rogers W.E., Fuhlendorf S.D., Wonkka C.L., Engle D.M., Weir J.R., Kreuter U.P. and Taylor C.A. 2013. The rising Great Plains fire campaign: citizens' response to woody plant encroachment. *Frontiers in Ecology and Environment* 11: 64-71.
- Violle C. and Jiang L. 2009. Towards a trait-based quantification of species niche. *Journal of Plant Ecology* 2: 87-93.
- Volder A., Briske D.D. and Tjoelker M.G. 2013. Climate warming and precipitation redistribution modify tree–grass interactions and tree species establishment in a warm-temperate savanna. *Global Change Biology* 19: 843-857.
- Vourlitis G.L., Zorba G., Pasquini S.C. and Mustard R. 2007. Chronic nitrogen deposition enhances nitrogen mineralization potential of semiarid shrubland soils. *Soil Science Society of America Journal* 71: 836-842.
- Wakeling J., Bond W., Ghauhi M. and February E. 2015. Grass competition and the savanna-grassland 'treeline': a question of root gaps? *South African Journal of Botany* 101: 91-97.
- Wakeling J.L. and Bond W.J. 2007. Disturbance and the frequency of root suckering in an invasive savanna shrub, *Dichrostachys cinerea*. *African Journal of Range and Forage Science* 24:73-76.
- Ward D. 2005. Do we understand the causes of bush encroachment in African savannas? *African Journal of Range & Forage Science* 22: 101-105.
- Ward D., Kirkman K. and Tsvuura Z. 2017. An African grassland responds similarly to long-term fertilization to the Park Grass experiment. *PLoS One* 12: e0177208. [doi: 10.1371/journal.pone.0177208].
- Ward D., Hoffman M.T. and Collocott S.J. 2014. A century of woody plant encroachment in the dry Kimberley savanna of South Africa. *African Journal of Range and Forage Science* 39: 107-121.



- Way D.A. and Oren R. 2010. Differential responses to changes in growth temperature between trees from different functional groups and biomes: a review and synthesis of data. *Tree Physiology* 30: 669-688.
- Wenig M., Spichtinger N., Stohl A., Held G., Beirle S., Wagner T., Jähne B. and Platt U. 2003. Intercontinental transport of nitrogen oxide pollution plumes. *Atmospheric Chemistry and Physics* 3: 387-393.
- Wiegand K., Saltz D. and Ward D. 2006. A patch-dynamics approach to savanna dynamics and woody plant encroachment - insights from an arid savanna. *Perspectives in Plant Ecology, Evolution and Systematics* 7: 229-242.
- Williams S.E., Shoo L.P., Isaac J.L., Hoffmann A.A. and Langham G. 2008. Towards an integrated framework for assessing the vulnerability of species to climate change. *PLoS Biology* 6: e325. [doi: 10.1371/journal.pbio.0060325].

