

Factors affecting savanna tree sapling recruitment

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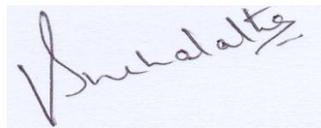


Preface

The research presented in this thesis was carried out at the School of Life Sciences (SLS) of the University of KwaZulu-Natal (UKZN) at Pietermaritzburg, South Africa, from 2009 to 2012, under the supervision of Professor David Ward.

The thesis, submitted in candidature for the Degree of Philosophy (Ph.D.) is the product of the candidate's unaided investigations and writings, with due acknowledgements and credit given and cited in appropriate sections of the text, and original in substance, not having been submitted in whole or in part to be examined for any degree, nor is it concurrently being submitted in candidature for any other degree.

The thesis is presented in a manuscript-based format. Except the Introduction (Chapter 1) and Conclusion (Chapter 5), each manuscript (or data chapter) follows the format of the journal where it has been submitted for publication. As a result, the thesis will not follow a consistent format. Tables and figures have been provided at the end of each paper (or data chapter) as they were submitted to the respective journal. Some overlaps in the introduction and method section of several chapters were unavoidable.



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I certify that above statements are true:



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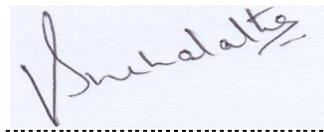
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Declaration 1: Plagiarism

I, the undersigned, hereby declare that the work contained in this dissertation is my original work and that it has not previously in its entirety or in part been submitted at any university for a degree

- The research reported in the thesis, except where otherwise indicated, is my original research
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Declaration 2: Publications

Details of contributions to publications that form part and/or include research presented in this thesis

Publication 1: Published in *Plant Ecology*

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. DOI: 10.1007/s11258-012-0134-1

Author contributions: SV conceived the paper with DW. SV carried out the experiment, collected and analysed the data as well and wrote the paper while DW contributed valuable comments to the manuscript.

Publication 2: Submitted to *Ecosphere*

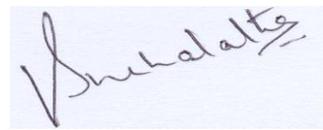
Vadigi, S & Ward, D, (2012) **Shade, nutrients and grass competition are important for tree sapling establishment in a humid savanna**

Author contributions: SV conceived the paper with DW. SV carried out the experiment, collected and analysed the data and wrote the paper while DW contributed valuable comments to the manuscript.

Publication 3: Submitted to *Functional Ecology*

Vadigi, S & Ward, D, (2012) **Herbivory effects on saplings are influenced by nutrients and grass competition in a humid South African savanna**

Author contributions: SV conceived the paper with DW. SV carried out the experiment, collected and analysed the data and wrote the paper while DW contributed valuable comments to the manuscript.



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Dedication

Father, my strength.

Abstract

Savannas are globally important ecosystems characterized by the coexistence of trees and grasses. Woody plants, which are slow-growing dominant life forms, influence the physiognomic structure and function of savanna ecosystems. Their density and distribution provides sustenance to a vast and unique savanna biodiversity, by forming a major source of food material to large mammalian herbivores, sheltering them and through their facilitation of diverse plant species.

Savanna tree existence is strongly affected by factors that determine their sapling recruitment. We defined 'sapling' as a young tree, in the first season of its growth, which does not depend on cotyledonary reserves (=seedling stage) and relies on external resources to grow further. Sapling recruitment may strictly be defined as the progression of a young plant from seedling to sapling stage. However, we believe that savanna tree saplings, present within the grass layer in the initial years of their growth, are equally vulnerable to environmental stresses. This study examines the factors affecting tree sapling establishment in a humid savanna (1250 mm mean annual precipitation). Additionally, the effects of fire were tested in a greenhouse experiment. Dominant species from humid savannas (> 1000 mm MAP), *Acacia karroo*, *Acacia sieberiana*, *Schotia brachypetala* and *Strychnos spinosa*, and mesic savannas (approx. 750 mm MAP), *Acacia nigrescens*, *Acacia tortilis*, *Colophospermum mopane* and *Combretum apiculatum*, were studied. In this thesis I examined the effects of resource availability (water, nutrients and light), disturbances (fire and herbivory) and competition (grass) on the sapling ecology of these species. Sapling recruitment and growth were assessed in terms of survival and aboveground growth responses, i.e. total biomass, stem growth rates (used as proxy measures for

assessing persistence) and leaf biomass proportion (important for producing root reserves necessary to resprout).

I studied the effects of fire and a nutrient gradient on survival and growth of four *Acacia* species in the presence of grass competition, in a controlled greenhouse experiment. Generally, Acacias invest in defenses after herbivory. I also determined their physical and chemical defense investments in this experiment. Sapling survival was not influenced by nutrients but highly varied among the species due to fire, indicating that fires may have a differential effect on species composition at a landscape scale. Intermediate levels of nutrients were found to be beneficial for sapling growth than high and low levels. This may be due to an increase in grass competition at higher levels of nutrients. Fires did not have a positive influence on sapling defence investment.

To evaluate the relative importance of resource availability on sapling tree recruitment and its interactions with grass competition, I tested the effects of water (frequent irrigation vs. rainfall), shade (presence vs. absence), nutrients (addition vs. no addition) and grass competition (presence vs. absence) on sapling survival and growth under controlled field conditions in a humid South African savanna. Treatments did not have an effect on sapling survival, indicating that mortality is not defined by resource availability and grass competition in humid savannas. Shade had the greatest negative effect on sapling growth, suppressing the beneficial effects of nutrients and absence of grass competition. Nutrient limitation and grass competition had a relatively small influence on savanna sapling growth. Frequency of water availability had no effect on sapling growth, perhaps owing to high rainfall experienced over the experimental

period. Therefore, canopy shade can be considered to be an important driver of tree dynamics in humid savannas with some degree of influence by nutrient availability and grass competition.

The effects of clipping (i.e. simulated herbivory of grass and tree saplings) as influenced by nutrient availability and grass competition were examined on sapling survival and growth of all study species in a humid savanna. None of the treatments had an effect on sapling survival. This signifies that herbivory alone cannot significantly decrease plant density in humid savannas. However, tree saplings grew taller with a reduction in diameter and overall biomass, implying that saplings may become more susceptible to fires after herbivory. Nutrient addition and grass competition in general had a positive and negative effect, respectively, on sapling growth. This response was prominent in the stem length growth rates of defoliated saplings of one humid and two mesic species. These results imply that clipping (or herbivory) is the major factor reducing sapling vigour to establish, but is affected by both grass competition and nutrient availability.

This study shows that fire has a differential effect on sapling survival of different species, particularly between humid savanna species. Light interception among all other resources limits the recruitment of saplings into adult size classes. Clipping, nutrient availability and grass competition had a relatively small direct effect, but may interact with other factors to alter sapling establishment dynamics. Wet-season droughts in humid savannas are not a hindrance to tree establishment because sapling survival was not dependent on frequency of rainfall. Thus, in humid savannas, fires can have a major impact on tree species density and composition while canopy shade has a very high potential to alter tree distribution.

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“The woods are lovely, dark and deep,

But I have promises to keep,

And miles to go before I sleep.”

– Robert Frost.

Chapter 1: Introduction

Savanna definition and distribution

Savanna ecosystems are characterized by a continuous layer of mostly C₄ grasses with a discontinuous spread of trees and distinct wet and dry seasons (Bourlière and Hadley 1970, Frost et al. 1986, van Langevelde et al. 2003). Savannas occupy about 20 % of the earth's terrestrial landscape (Scholes and Walker 1993, Shorrocks 2007). Geographically, savannas occur largely across the tropical (1600 M ha) and to a lesser extent in North American temperate regions (15 M ha) (Scholes and Archer 1997). The majority of the tropical savannas occur in Africa, followed by South America, Australia, India and Southeast Asia (Scholes and Archer 1997, Shorrocks 2007). In Africa, savanna constitutes approximately 40 % of the total land area and covers most sub-Saharan regions (Okigbo 1985, Scholes and Walker 1993). At a global scale, savannas occupy regions receiving mean annual rainfall ranging between 200-1500 mm, falling within a short period of a few months, with average temperatures ranging from 20-30 °C (Shorrocks 2007). Tropical savannas receive < 25 mm of rainfall during the driest period of the year (Nix 1983).

Climate and types of savanna

The physiognomic structure of savannas is generally modified by the density and organisation of savanna woody plant communities (Sarmiento 1984, Hoffmann 1996, Jeltsch et al. 2000). Walter (1971) proposed that the tree cover of savannas is mainly dependent on plant-available moisture (rainfall and soil water). The climate of savanna ecosystems varies from

humid to arid, mainly based on the quantity and seasonality of the rainfall (Johnson and Tothill 1985). Savannas form a broad transition zone between desert steppes and tropical woodland, with an increase in tree density across an increasing rainfall gradient (Bourlière and Hadley 1983, Accatino et al. 2010). However, in reality it is difficult to distinguish among savanna types depending on a single climatic variable (Menaut et al. 1985, Lehmann et al. 2009). Even within the rainfall boundary there is no precise demarcation of savanna types. Menaut et al. (1985) have distinguished three savanna zones, viz. humid (> 1000 mm rainfall and < 2 mo dry period), mesic (rainfall between 500-1200 mm and < 8 mo dry period) and arid (< 600 mm and > 8 mo dry period). While humid zones are clearly identified as areas receiving MAP > 1000 mm (Mordelet et al. 1997), there still is no consensus over the categorization of arid, semi-arid and mesic savannas. Sankaran et al. (2005) considered savannas receiving < 650 MAP as arid or semi-arid while Staver et al. (2011) and Buitenwerf et al. (2012) have considered that the savannas receiving approx. 500 MAP were semi-arid. Savannas with approx. 700 mm of rainfall annually were categorized as semi-arid by Higgins et al. (2010). However, corresponding to a classification done by Menaut et al. (1985), Bond and Midgley (2012) and Buitenwerf et al. (2012) described these savannas as mesic. In the present thesis, we considered humid savannas as areas receiving > 1000 mm MAP, mesic savannas receiving approx. 750 mm MAP and arid savannas as areas receiving < 500 mm MAP.

The structure of savannas is not only determined by the amount of rainfall received but also various other factors enabling tree-grass coexistence. Frost et al. (1986) have postulated the importance of plant-available moisture, plant-available nutrients, fire and herbivory in determining coexistence of dominant savanna growth forms. Savannas exist in zones that range from low rainfall but with relatively high nutrient levels to high rainfall with low nutrient levels.

In this respect, du Toit (1995) had explicitly classified savannas as moist-dystrophic (> 650 mm MAP with nutrient-poor leached soils) and arid-eutrophic (< 650 mm MAP with nutrient-rich soils). However, tree-grass coexistence in savannas is also strongly driven by the temporal effects of recurring disturbances, such as fire and herbivory (Jeltsch et al. 2000, van Langevelde et al. 2003). Hence, interactions of water and nutrient availability with fire and herbivory at a regional scale significantly determine savanna vegetation structure (Solbrig et al. 1996, Sankaran et al. 2005, van Langevelde et al. 2011).

Importance of tree cover in savannas

African savannas have high floristic species richness (1750 species) which is comparable to that of rainforests (2020 species) (Menaut 1983). Density and distribution of trees in savannas maintain ecosystem structure (Menaut et al. 1990, Hoffmann 1996). Ecologically, at a local scale, trees increase plant species richness and structural complexity by providing a distinct microclimate under sub-canopy zones with increased soil nutrients (Vetaas 1992, Roos and Allsopp 1997, Manning et al. 2006). Savannas of Africa sustain a wide variety of large mammalian herbivore populations, constituting grazers, browsers and mixed feeders (McNaughton and Georgiadis 1986, du Toit 2003). Although trees provide high quality forage to large mammalian herbivores, they also support a wide faunal diversity by providing shelter (Belsky and Canham 1994, Cumming et al. 1997, Fenton et al. 1998). Trees also provide food for a variety of invertebrate fauna. Evergreen tree species form a major source of reserve food material during the dry season to a range of wild ungulate browsers (Owen-Smith 1985). In addition to their ecological importance, many species of savanna trees also have high

commercial and nutritional value for humans (Kristensen and Mette Lykke 2003). Decline in tree cover reduces the capacity to retain a vast and unique savanna biodiversity. However, an increase in the abundance of woody plants or “bush encroachment” (also known as “shrub encroachment” in North America) is considered a serious problem to the quality, productivity, long-term economic viability of rangelands and ecological integrity of savanna biomes (van Auken 2000, Roques et al. 2001, Ward 2005). Considering that African savannas form the basis for major industries such as agriculture, pastoralism and tourism (Lamprey 1983, Hadley 1985, Scholes and Walker 1993, Prins 2000), an understanding of the factors driving tree cover in savannas is crucial.

Determinants of tree cover in savannas – Sapling establishment

A young plant completes its seedling phase when it ceases to depend on food reserves stored in the cotyledons and starts relying on external resources (Hanley et al. 2004). We considered ‘saplings’ as juvenile plants, in the first season of their growth, relying on external resources, after shedding their cotyledons during the seedling phase. There is no clear demarcation for the end of sapling phase. We presume that saplings, in their initial years of growth, are similarly vulnerable to environmental stresses as seedlings. The sapling stage is the most fragile phase in the life history of savanna trees (McPherson 1997, Higgins et al. 2000, Bond 2008, Chidumayo 2008). Tree recruitment success in savannas is determined by sapling resilience to adversity (Vadigi and Ward 2012) and the conditions that allow saplings to establish (Midgley and Bond 2001, Chidumayo 2008). The survival and growth of tree seedlings, most often are suppressed by competition within the grass layer (Jurena and Archer 2003, Riginos and

Young 2007), and are mainly influenced by resource availability (water, light and nutrients) (O'Connor 1995, Kraaij and Ward 2006) and disturbances such as fire and herbivory (Sankaran et al. 2008, Midgley et al. 2010). These factors affect sapling recruitment either directly or by interacting with one another.

Factors affecting tree sapling establishment

Frequent rainfall / water availability

Water stress during the dry season of savannas has a major impact on the survival of woody seedlings (Scholes 1985, Gignoux et al. 2009). The length and severity of the dry season is also very important in determining tree establishment (Nix 1983, Kraaij and Ward 2006, Shorrocks 2007). Although tree seedlings rapidly develop an extensive root system to gain better access to water (Ward and Esler 2011), evenly distributed rainfall is still a requirement to compensate for the loss of water through transpiration (Menaut 1983). Studies in semi-arid (Harrington 1991, Kraaij and Ward 2006) and humid savannas (Hoffmann 1996, Gignoux et al. 2009) have shown that a drought during the wet growing season is detrimental to tree seedlings. Rainfall amount also alters the competitive equilibrium between grasses and tree saplings, as grasses can establish themselves even in the presence of low rainfall (Menaut 1983).

Light interception / shade

Solar energy is a common plant resource requirement that is variably received at global, regional and temporal scales. Plants in the humid tropics of Africa receive relatively low solar radiation due to its interception by cloud cover and suspended aerosols, whereas clear skies make arid zones more prone to high radiation and temperatures (Jagtap 1995, Shorrocks 2007). Within savanna systems, light reception contrasts with seasonal variation in cloud cover. Light intensities received on rainy days in arid savannas are equivalent to those of humid tropics whereas light intensities characteristic of arid zones are received during drought periods in humid savannas (Nix 1983). Shaded micro-sites created by established woody plants provide a local-scale spatial heterogeneity in the amount of light received at ground level (O'Connor 1995, Rolhauser et al. 2011). Plants commonly undergo stress due to high transpiration rates with increased radiation levels. Sub-canopy microsites provide structural complexity to savannas by increased soil moisture, as well as increased nutrients due to high litter decomposition and animal activity and alleviation of grass competition by shading (Vetaas 1992, Hoffmann 1996, Riginos et al. 2009, Salazar et al. 2012). Amelioration of solar radiation and stressful environments under canopy shade is considered favourable for savanna tree sapling recruitment (O'Connor 1995, Hoffmann 1996, Rolhauser et al. 2011, Salazar et al. 2012). Tree canopies may facilitate grass growth in arid savannas due to amelioration of radiation but suppress grass production in humid conditions (Dohn et al. 2012). Therefore, the competitive effect of grasses on tree saplings may be altered based on light availability.

Soil nutrient availability

The interaction between the coexisting trees and grasses in savannas is widely understood to be controlled by light and water availability (House et al. 2003). However, nutrients may directly affect the competitive and facilitative interactions between trees and grasses (Scholes and Hall 1996, House et al. 2003). Tree seedling establishment in savannas can be affected by nutrients after water availability is taken into account (Kraaij and Ward 2006, Cramer et al. 2012). Plant productivity of terrestrial ecosystems is commonly limited by available soil nitrogen under natural conditions (Miller and Cramer 2005). On a continental scale, humid savannas consist of leached and infertile soils whereas arid savannas have relatively fertile soils (Scholes and Walker 1993, du Toit 1995). Nutrients may have both direct positive effects and indirect negative effects on savanna tree sapling recruitment. Availability of nutrients may directly enhance seedling growth (Wakeling et al. 2010) or suppress seedling establishment by increasing grass competition (Cohn et al. 1989, Kraaij and Ward 2006, van der Waal et al. 2009).

Grass competition

Grass competition can strongly suppress growth at all life history stages of woody plants (Riginos 2009), although increases in tree density may have an intense negative effect on grasses (Riginos et al. 2009). Grass competition is a major impediment for tree seedling survival and establishment, capable of creating a bottleneck for the succession of trees in savannas (Higgins et al. 2000, Jurena and Archer 2003, Riginos and Young 2007, Bond 2008, Ward and Esler 2011, Grellier et al. 2012). Young tree seedlings share the same rooting niche with grasses (Menaut

1983, Cramer et al. 2010, Ward and Esler 2011) and compete directly for water, nutrients and light (van der Waal et al. 2009). Indirectly, grasses hinder sapling growth by increasing fire intensity and frequency through accumulation of combustible fuel loads (Bond 2008). Humid savannas are capable of supporting more fires than mesic areas because moisture is not limited for grass production (Trollope 1980, Sankaran et al. 2008). Grass biomass may also benefit from nitrogen fixation by tree seedlings (Kambatuku et al. 2013) which may in turn be detrimental to seedlings. However, grasses may provide moist microclimatic conditions (Grellier et al. 2012) and cover from browsers, facilitating tree sapling establishment (Riginos and Young 2007, Seymour 2008).

Fire

Fires are common disturbance phenomena regulating tree establishment in tropical savannas, albeit at a more local scale (Frost et al. 1986, Lehmann et al. 2008). Historically, lightning was the principal source of fires in savannas (Solbrig et al. 1996) although deliberate (anthropogenic) use of fires started very early in African savannas (Scholes and Archer 1997). Nevertheless, savanna trees are highly resistant to fires and probably evolved under the influence of fires (Helm et al. 2011). However, fires are capable of reducing tree density through sapling mortality and diversity due to species-specific responses of trees to fires (Silva 1996, Andersen et al. 2005, Zida et al. 2007, Gordijn et al. 2012). Intensity of fires determines the recruitment of trees into larger size classes (Govender et al. 2006). Very young saplings have the ability to resist fire by resprouting (Midgley and Bond 2001, Clarke et al. 2013). However, repetitive

disturbances such as intensive browsing after fire (Dublin et al. 1990; Midgley et al. 2010) and frequent fires (Wigley et al. 2009) may lead to mortality.

Herbivory

African savannas accommodate a highly diverse group of mammalian herbivore species (Jewell 1985, Holdo et al. 2009), especially compared to the savannas in Australia and South America (Bond 2008). Savanna herbivores broadly includes grazers, browsers and mixed feeders (Holdo et al. 2009), which constitutes native ungulates, domestic livestock, small rodents and an array of invertebrates (Shaw et al. 2002). Relatively, among all the other factors affecting tree populations, herbivory by mammals has a very small effect (Roques et al. 2001, Sankaran et al. 2005, Bond 2008). Browsing mammalian herbivores have the ability to alter the tree vegetation structure but rarely have a devastating effect (van de Vijver et al. 1999, Bond 2008). However, the effects of large mammalian browsers, such as elephants, (van de Vijver et al. 1999, Western and Maitumo 2004) and small mammalian browsers can have a significant negative effect on shrub dynamics (Augustine and McNaughton 2004), especially when large trees are represented in low densities (Dublin et al. 1990). Tree recruitment in savannas may also be affected by heavy sapling predation by invertebrates (Weltzin et al. 1997, Shaw et al. 2002). However, substantial landscape-level vegetation changes due to insect herbivory are found mainly in North America (Naiman 1988) while ungulates play a prominent role in African savannas (Andersen and Lonsdale 1990). An increase in grazing pressure has been noted as a main cause for reduced fire frequency, leading to mass tree recruitment or bush encroachment (van Auken 2000, Roques et al. 2001, van Langevelde et al. 2003, Briggs et al. 2005, Gordijn et al. 2012).

Aims and outline of the thesis

This study seeks to evaluate the relative effects of various factors influencing the recruitment of different humid and mesic savanna species saplings in a humid South African savanna. All these factors were tested in a field experimental study, except for a test of the effects of fire which was performed under controlled greenhouse conditions. The field experiment was replicated by another PhD student, Eduardo Barbosa, in a mesic savanna site near the Kruger national park in South Africa. These field studies were undertaken as a part of a global experiment on savanna tree saplings (GEST), which are being conducted in mesic and humid savannas in Australia, India, and South America.

Chapter 2 investigates the effects of fire, five different levels of nutrients and their interactions on saplings of four *Acacia* species. Survival, growth and defence responses were evaluated after six month study period under irrigated greenhouse conditions. This chapter was published in *Plant Ecology*.

Chapter 3 examines the effects of resource availability (frequent water supply, shading and nutrients) and grass competition on the sapling survival and growth of eight savanna tree species from both humid and mesic origins. This study was conducted in a controlled field experiment for one wet season under field conditions. This chapter has been submitted to *Ecosphere*.

Chapter 4 deals with the effects of clipping with respect to nutrient availability and grass competition on the sapling survival and growth of eight savanna tree species from humid and

mesic savannas. This study was conducted under irrigated conditions in the same field experiment described in Chapter 3. This chapter has been submitted to *Functional Ecology*.

Chapter 5 provides an overview of the study results in the context of tree establishment in humid savannas along with an outline of directions for future research.

I measured aboveground growth responses in terms of sapling investment in total biomass, stem growth rates (important to withstand fires) (Higgins et al. 2000, Clarke et al. 2013) and leaves (important to produce root reserves) (Sakai and Sakai 1998) which will enable successful recruitment. Many studies have indicated that herbivory induced defense investment in savanna trees (Gowda 1997, Rohner and Ward 1997, Zinn et al. 2007). However, investment in defences after fire has been less frequently investigated (see Gowda and Raffaele 2004). Hence, we studied sapling allocation to defences with fire treatment.

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**Chapter 2: Fire and nutrient gradient effects on the sapling ecology of four *Acacia* species
in the presence of grass competition***

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Abstract

The variation among tree species in their sapling responses to disturbances such as fire, herbivory and grass competition results in different probabilities of recruitment into savanna communities. A sapling's ability to persist under these disturbances depends on its investment in growth, defences and stored reserves, based on soil available nutrients. We examined the survival and investments in growth and defence of the saplings of four *Acacia* species, in the presence of competing grasses, under varying soil nutrient levels. We also have evaluated the effects of fire on these saplings. We performed the study in a greenhouse at the UKZN Botanical Garden, Pietermaritzburg, South Africa. Nutrients had no significant effect on the survival of tree saplings regardless of fire treatment. Only 22.5 % saplings survived after fire treatment compared with 84 % of control saplings. *Acacia sieberiana* showed the highest resprout survival, while *A. karroo* did not survive the fire. Growth in *A. nigrescens* increased only at intermediate levels (0.5N, 1N) of the fertiliser provided. Condensed tannins in *A. karroo* also decreased at an intermediate level (1N) of fertiliser. Resprouts grew faster in stem height than in diameter and did not invest in chemical defences. No treatment had an effect on the proportion of root and shoot dry biomass. In conclusion, African acacias are not dependent on soil nutrients for their survival and differ in their ability to resprout after fire. Intermediate levels of nutrient availability are beneficial to sapling development as opposed to zero and high nutrient levels, due to nutrient-grass dynamics. The benefits of post-fire grass removal and an increase in nutrients can be effectively utilised by only a few species of acacias.

Key-words Condensed tannins · Coppicing · Humid savanna · Mesic savanna · Relative growth rate · Resprouting · Sapling establishment

Introduction

In savannas, grass fires and herbivory are common disturbances limiting tree population density (Scholes and Archer 1997; Higgins et al. 2000; Sankaran et al. 2008; Staver et al. 2009; Midgley et al. 2010). Savanna trees have evolved under the influence of fire and herbivory (Helm et al. 2011). Many species are capable of resprouting after burning (Archibald and Bond 2003; Balfour and Midgley 2006; Wigley et al. 2009). Trees resist herbivory by investing in various chemical and mechanical defences (Rohner and Ward 1997; Ward and Young 2002; reviewed in Stamp 2003) as well as rapid stem growth (Palo et al. 1993). Tree resilience to disturbances is prominent mainly in adults while the saplings remain largely susceptible (Higgins et al. 2000; Augustine and McNaughton 2004; Staver et al. 2009; Wahungu et al. 2011), although young trees may defend themselves better than mature trees (Brooks and Owen-Smith 1994; Rohner and Ward 1997; Ward and Young 2002). Hence, sapling establishment and growth play a defining role in the demographic composition of savanna tree population structure (Prins and Van der Jeugd 1993; King et al. 1997; Hanley 1998; Staver et al. 2009; Goheen et al. 2010). Rare ‘triggering events’ favourable for mass tree recruitment can occur (Kraaij and Ward 2006; Meyer et al. 2009), but more often than not savanna tree seedlings struggle to establish in the presence of disturbances and competing grasses (Ward and Esler 2011; Kambatuku et al. 2013).

Fire damage activates plants to resprout by mobilizing the stored carbon from roots to aboveground growth, later replenishing their depleted carbohydrate reserves (Schutz et al. 2009; Wigley et al. 2009). However, if disturbances persist then depletion of sapling reserves may result (Wigley et al. 2009; Paula and Ojeda 2011). Repetitive disturbances usually involve

intensive browsing after fire (Dublin et al. 1990; Midgley et al. 2010) and frequent fires (Wigley et al. 2009).

Resprouting success is mainly determined by species-specific bud anatomy, bud protection and resource availability (Wigley et al. 2009; Lawes and Clarke 2011). Many savanna species resprout from stem basal buds that are well protected and tolerant of disturbances (Midgley et al. 2010; Lawes and Clarke 2011). Effective bud protection results in higher resistance to fire and is primarily governed by stem bark thickness (commonly proportional to stem diameter) and height (Gignoux et al. 1997; Hoffmann and Solbrig 2003; Balfour and Midgley 2006; Lawes et al. 2011). Physiologically it is important for savanna saplings to have high resource investment rates in overall stem growth, but the allocation to stem height may be gained at a cost of diameter and vice versa (Sumida et al. 1997). Trade-offs exist not only between stem height and diameter growth, but also in overall plant growth and defence investment (Hean and Ward 2012). Plant investment in growth and defences is primarily determined by soil nutrient availability. Although resprouting roots depend highly on starch nutrient reserves (Miyanishi and Kellman 1986), they also obtain other mineral nutrition from soil (Teixeira et al. 2002; Peguero and Espelta 2011). Plants grow less in nutrient-poor soils and continued assimilation of carbon, not utilised in growth, results in the formation of carbon-based defences. On the other hand, plants at high nutrient availability do not invest in defences as growth is not restricted (Bryant et al. 1983). Savanna tree sapling establishment may depend on species-specific abilities to resist fires (stem height and diameter growth), deter herbivores (defence investment) and store root starch determined by the availability of soil nutrients.

We conducted an experiment to understand the resprouting ability, growth and defence responses of four predominant African *Acacia* species' saplings to fire, five different nutrient levels and their combinations. This experiment was conducted in the presence of grass competition to mimic natural savanna conditions. We studied two humid savanna species (high rainfall of $>1,000$ mm year⁻¹ and low soil nutrients), viz. *Acacia karroo* (a subtropical coastal variety from Richards Bay, Ward 2011) and *A. sieberiana* var. *woodii* and two mesic savanna species (lower rainfall ~ 750 mm year⁻¹ and nutrient-rich soils), viz. *A. nigrescens* and *A. tortilis* (Du Toit 1995; Mordelet et al. 1997; Bond et al. 2003; Buitenwerf et al. 2012). We predicted the following:

1. Fire, irrespective of the nutrient treatment given, will not have an effect on sapling survival. Most savanna acacias are capable resprouters (Midgley and Bond 2001) even at the seedling stage (Hean and Ward 2012).
2. Soil nutrients will have no effect on sapling survival and will mainly be necessary for increased growth. Acacias have persistent cotyledonary reserves which provide prolonged support after seedling emergence (Dube et al. 2010). Established seedlings are hard to eliminate in the absence of disturbances and are capable of withstanding grass competition with increased nutrient availability (Cohn et al. 1989).
3. Investment in growth and defence for resprouts will increase simultaneously, particularly if competition with grass is removed. The growth rates of resprouting trees will be greater in terms of height than in diameter. Although immediate plant growth after fire is common for resprouters, induction of plant defences is also possible (Gowda and Raffaele 2004) and a quick attainment of minimum escape height from subsequent grass

fires is important for successful recruitment (Higgins et al. 2000). The trade-off between height growth to escape fires and diameter will result in tall shoots with small diameters and low shoot biomass. Conversely, replenishment of root carbohydrate reserves will increase root:shoot biomass ratios.

4. Stem growth investment will be high and allocation to defences should be low with an increase in soil nutrients, regardless of grass competition. Resource-rich environments favour fast-growing plants associated with low defences, while resource-poor areas promote slow-growing plants with high defences (Endara and Coley 2011). To attain a minimum escape height to avoid fires, savanna tree saplings should invest in stem height growth faster than diameter with an increase in nutrient availability. Root carbohydrate storage will decrease with the increase in available soil nutrients for sapling shoot growth. Thus root:shoot biomass will decrease with increasing nutrient concentrations.
5. Saplings provided with both fire and nutrients will invest more in regrowth than defences and their input in root storage will be slower than shoot growth, as soil nutrient levels increase. Therefore, root:shoot biomass ratios of resprouts will decrease as nutrient concentrations increase.

Materials and methods

Study design

We investigated the effects of five levels of fertilisation (control, 13, 26, 52 and 104 g – details given below) and two levels of fire (control and fire) on the survival, growth and defence responses of saplings of four *Acacia* species in a completely crossed randomized experiment

under greenhouse conditions. The temperature was controlled to mimic ambient conditions, which fluctuated between 15 and 30 °C. Bins with a 95 L capacity (top diameter 0.45 m and height 0.6 m) were filled with Umgeni sand aggregate (alluvial sand from the Umgeni river, near Pietermaritzburg, South Africa). Each bin, which contained two saplings of the same species, received one of the 10 treatment combinations (2 fire levels x 5 nutrient levels), which were replicated three times. This resulted in a total of 240 saplings (60 saplings x 4 species) growing in 120 bins. To replicate the grass component of the savanna, we sowed *Eragrostis curvula*, a widespread savanna grass species, in each bin. A handful (~12 g) of grass seeds was sprinkled in all the bins following the application of fertilizer, enabling them to establish prior to planting the tree saplings.

Sapling development and transplantation

The seeds were scarified after being soaked in 95 % concentrated sulphuric acid for 1h. They were then washed thoroughly with water to remove the traces of acid and then soaked in water for 12 h. Thereafter, one seed was planted in a 750-mL potting bag containing a mixture of soil, vermiculite and coconut coir (to increase porosity and water retention). After 2-3 months, the saplings were transplanted into the bins in early January 2010, except the *A. nigrescens* saplings that were transplanted a month later due to delayed germination. The mean \pm SE heights of the saplings at the time of transplantation were 284.31 ± 34.20 mm for *Acacia karroo*, 124.25 ± 13.34 for *A. nigrescens*, 226.29 ± 41.24 mm for *A. sieberiana*, 63.05 ± 18.21 mm for *A. tortilis*. Two saplings in each bin were positioned at least 0.30 m apart across the bin diameter to reduce the probability of intraspecific root competition. Only saplings with no foliar damage were transplanted with minimal or no disturbance to the roots. Saplings were allowed to

acclimate to the new conditions for 1 month and any saplings that died during this period were replaced (n = 3).

Nutrient treatment

The nutrient compound applied was an Osmocote[®] exact fertiliser, which was a water-soluble, granular, controlled-release fertilizer with 15 % of total nitrogen, 9 % phosphorus pentoxide, 11 % potassium oxide, 2.5 % magnesium oxide and other trace elements. This fertiliser was chosen for the steady and continuous release pattern over the study period. We took 26 g (1N) of Osmocote[®] fertilizer as a basic amount, for each 0.16 m² surface area of the bin, to compute the five levels of nutrient application: 0 g (0N); 13 g (0.5N); 26 g (1N); 52 g (2N) and 104 g (4N). Nitrogen among all other nutrients is the most important element in plant growth, according to Liebig's law (Tilman 1987). The basic amount of (26 g) fertiliser was calculated such that nitrogen supply rate will be at 24.3 g N m⁻² year⁻¹, which is close to the highest nitrogen application rate of 27.2 g N m⁻² year⁻¹ used by Tilman (1987). Several *Acacia* species nodulate (De Faria et al. 1989); nutrient addition in terms of nitrogen may seem redundant for nitrogen-fixing acacias. However, fixing nitrogen, like growth, is a major carbon investment for the plant (Chapin et al. 1987) and nodulation decreases as soil nutrients increase (Kambatuku et al. 2013). Hence, we assumed that despite being able to fix nitrogen, addition of nitrogen should still have an effect on sapling growth and our nutrient prediction will hold true. Each bin was fitted with a micro-jet irrigation system with the capacity to deliver 20 L h⁻¹ and operated for half an hour every day, to provide water to the saplings.

Fire treatment

One month after the saplings were established, fire was applied to half the bins at sufficient intensity (flame height ≤ 0.45 m) to burn all the above-ground plant material. One week prior to burning, the grass in the bins was cut and placed in the same bins to dry, which provided the fuel for burning. A butane blow torch was used to burn the grass and a metal shield, encircling the saplings about to be burnt, prevented the fire from spreading to other bins. Soil temperature readings (in °C) at a depth of 5 cm were recorded in each bin using a digital thermosensor probe before setting fire to the grass and immediately after the fire subsided. The difference in soil temperature gave an indication of temperature increase in the top soil. There was no significant difference across the nutrient levels in either grass dry biomass (used as fuel) at the time of burning (ANOVA, $F = 1.32$, error $df = 59$, $p = 0.274$) or soil temperature (ANOVA, $F = 1.86$, error $df = 57$, $p = 0.130$), ensuring homogeneity of fire treatment. The burned saplings were left to regrow for 4 weeks.

Growth variables

Measurements of all surviving saplings were taken in March 2010 and July 2010 (in April 2010 and July 2010 for *A. nigrescens*). Stem height (from base to tip of the plant in mm) and stem basal diameter (mean of two readings taken diagonally opposite to each other 1 cm above the soil in mm) were the physical growth attributes measured for each sapling. Height and diameter of the longest stem of each coppice was measured. Percentage survival of saplings was calculated as the number of live saplings at harvest relative to the number of live saplings before burning. Calculations for relative growth rate of sapling stem height (RGR_h) and relative growth rate of basal stem diameter (RGR_d) were based on the \ln stem height increment and \ln basal

diameter increment, respectively, per unit time (Kohi et al. 2009). All live saplings and grass were harvested at the end of July 2010 and oven dried at 60 °C for 48 h. Root and shoot dry biomasses were assessed to evaluate sapling resource allocation. Root biomass is proportional to its carbohydrate content (Schutz et al. 2009) and can act as an indirect quantification of root reserves. Oven-dried grass biomass from each bin was also recorded.

Defence variables

Mechanical and chemical defence measures are positively correlated (Ward and Young 2002) and may reflect an overall investment in sapling defence investment. All species in this study invest in condensed tannins (CT) and have either spines or prickles (Rooke et al. 2004; Van Wyk and Van Wyk 2007; Zinn et al. 2007; Mboumba and Ward 2008). We measured CT using a standard acid-butanol assay (Hagerman 2011), as CTs are the principal protein-precipitating tannins found in forage leaves, reducing the animal's digestive ability by directly binding to their gut proteins (Horigome et al. 1988), forming a direct measure of unpalatability to herbivores. We used 2 g of the dried and powdered leaf material per sapling to prepare the extracts for the assay. The absorbance was read at 550 nm with a spectrophotometer (Hagerman 2011). The CT concentration was calculated in mg mL⁻¹ quebracho equivalents (QE) because there is no absolute standard for CT (Hagerman 2011). Quebracho for the standard was obtained from A. Hagerman (Miami University, Oxford, Ohio, USA).

Mean lengths of four thorns were measured on each surviving sapling in the final month of the experiment. The main stem was divided into four equal regions from the apex to the base and the longest thorn in each region was measured. The linear length from base to tip of each thorn was taken, using vernier calipers.

Data analysis

All analyses were done using GenStat (Version 12.1, VSN International 2009). Mean values of the two saplings in each bin for each variable were used for analysis to avoid pseudoreplication. However, the binomial data for survival of each sapling were considered. Only the saplings that were not treated with fire were used to assess the effect of different nutrient levels on survival because there was high mortality among the fire-treated saplings. χ^2 tests were used to test for differences in sapling survival. Variables not conforming to ANOVA assumptions were transformed. Box-Cox estimation of the best-fit power-lambda value (-0.3) was used to transform thorn length data. Log_{10} values of root:shoot dry biomass ratio and CT were used. All variables including ratio of RGR_h to RGR_d were analysed for each species separately. In addition log_{10} values of root dry biomass and square root-transformed data of shoot biomass were also analysed. High sapling mortality caused unequal sample sizes of the data sets; therefore an unbalanced ANOVA (Shaw and Mitchell-Olds 1993) with fire and nutrients as fixed factors was performed. A two-way ANOVA was performed on the grass biomass data to check for fertiliser effect. Scheffe post hoc tests were done for multiple comparisons.

Results

Sapling survival

Fire had a high impact on sapling survival. Of the saplings treated with fire, only 22.5 % survived, compared with a survival of 84 % of the control saplings ($\chi^2 = 88.24$, $n = 240$, $p < 0.0001$). There was no significant effect of fire on sapling survival in *A. sieberiana* ($\chi^2 = 0.08$, $n = 60$, $p = 0.77$). Fire had a significant negative effect on *A. nigrescens* ($\chi^2 = 41.71$, $n = 60$, $p <$

0.0001) and *A. tortilis* ($\chi^2 = 21.55$, $n = 60$, $p < 0.0001$) and clearly on *A. karroo* (no survival, $n = 30$). The highest coppice survival was observed in *A. sieberiana* (70 %), followed by *A. nigrescens* (10 %) and *A. tortilis* (10 %), with no survival in *A. karroo* ($\chi^2 = 52.90$, $n = 120$, $p < 0.0001$). Among the saplings not treated with fire, humid species - *A. karroo* (100 %) and *A. sieberiana* (93 %) - had better survival than mesic species - *A. nigrescens* (73 %) and *A. tortilis* (69 %) ($\chi^2 = 15.10$, $n = 119$, $p < 0.01$).

There was no significant effect of different levels of nutrients on the survival of the unburned saplings (*A. karroo*, no test, 100 % survival; *A. nigrescens*, $\chi^2 = 8.57$, $n = 30$, $p = 0.07$; *A. sieberiana*, $\chi^2 = 4.43$, $n = 30$, $p = 0.35$; *A. tortilis*, $\chi^2 = 3.92$, $n = 30$, $p = 0.41$) and burned saplings (*A. karroo*, no test, 0 survival; *A. nigrescens*, $\chi^2 = 2.22$, $n = 30$, $p = 0.69$; *A. sieberiana*, $\chi^2 = 0.63$, $n = 30$, $p = 0.95$; *A. tortilis*, $\chi^2 = 5.92$, $n = 30$, $p = 0.20$).

Growth and defence responses

Fire had a significant effect on the sapling relative growth rate ratio of *A. nigrescens* and *A. sieberiana* (Table 1). RGR_h : RGR_d of the coppicing main stems of these species was higher than unburnt saplings (Fig. 1). No significant differences were found in the ratio between root and shoot dry biomass with any of the treatments (Table 1). Additionally, root ($F = 9.64$, error $df = 26$, $p = 0.006$; Fig. 2a) and shoot dry biomass ($F = 7.79$, error $df = 26$, $p = 0.013$; Fig. 2b) was significantly higher only in unburned *A. sieberiana* when compared to the burned saplings of the same species. Fire had no significant effect on the condensed tannin levels of all saplings (Table 1). The final thorn length of *A. nigrescens* was significantly affected by the fire treatment (Table 1). However, the sample size of the fire-treated *A. nigrescens* saplings with thorns was very small ($n = 2$) and consequently is not discussed further.

Fertiliser had a significant effect on grass biomass with and without fire ($F = 3.44$, error $df = 116$, $p < 0.05$). In both burned and unburned treatments, grass biomass increased with successive nutrient increments (Fig. 3). Nutrients had a significant effect on the relative growth rate ratio of *A. nigrescens* (Table 1). Growth rate ratio in *A. nigrescens* increased at lower levels of added fertiliser when compared to high levels (Fig. 4). Nutrients had a significant effect on tannins in unburned *A. karroo* only (Table 1). CT concentrations of *A. karroo* were lowest at the intermediate level of nutrients provided (Fig. 5). Nutrient treatments had no significant effect on the final thorn length of the four *Acacia* saplings (Table 1). Except with regard to the RGR_h : RGR_d ratio of *A. nigrescens*, fire \times nutrient interactions had no significant effect on the growth and defence responses of the studied *Acacia* saplings. However, the sample size of resprouts at different individual nutrient levels is very small ($n = 1$) and is not discussed further.

Discussion

General patterns in sapling investment strategies, based on the treatments applied and common to all four acacias, were not observed. In accordance with our prediction, nutrient level increase had no effect on the survival of intact *Acacia* saplings. Dube et al. (2010) have found that in the absence of disturbances, *Acacia* seedlings had 100 % survival rate due to their persistent cotyledons, which assist in establishment. Seedlings can survive even in the presence of very little photosynthetic plant material (Kitajima 2003). Hence, without disturbances, mortality is rare in established tree seedlings (Gignoux et al. 2009). We also found that nutrients had no effect on the survival of saplings treated with fire. This could be due to the overpowering effect of fire which killed many saplings. However, *A. sieberiana* had high survival after fire without any nutrient effect. Hence, soil nutrients do not appear to play a role in the survival of

Acacia saplings. High mortality after fire, excluding *A. sieberiana*, observed in our study is in contrary to our prediction. Although Hean and Ward (2012) recorded 100 % survival in multiple *Acacia* species following burning, they did not test *A. nigrescens*, *A. tortilis* and the sub-species of *A. karroo* which performed poorly with fire treatment in our study. Hence survival ability in *Acacia* saplings after fire may not be generalised for the entire genus.

Consistent with our prediction, fire improved the subsequent growth rates of stem height relative to diameter in *A. nigrescens* and *A. sieberiana*. Higgins et al. (2000) implied that plant growth in terms of height is an important strategy to escape fire. Contrastingly, stem diameter has also been shown to be critical for escape through fire for savanna plants (Hoffmann and Solbrig 2003; Balfour and Midgley 2006). Recently, Lawes et al. (2011) have suggested that stem height and diameter growth are different pathways to achieve bark thickness adequate enough to protect the cambium and buds. Gowda and Raffaele (2004) have found that burned plants of *Berberis* spp., a fire-adapted genus, invested in defence without overcompensating shoot growth. However, contrary to our prediction, investment in defences after fire is not fundamental for the *Acacia* species we studied.

Nutrient increase did not have an effect on the growth and defence investment of *Acacia* saplings as predicted. However, increased $RGR_h: RGR_d$ ratio of *A. nigrescens* (0.5N, 1N) and a decline in CT concentration of *A. karroo* at intermediate levels (1N) of fertiliser could indicate high utilisation of nutrients in sapling growth at these levels. This result partially supports our hypothesis that savanna saplings invest in growth and not in defences with increasing nutrient availability. High levels of supplemented nutrients (2N, 4N) would have resulted in an increase in grass competition, which might have reduced sapling stem growth rates and increased defence

investment. Cohn et al. (1989) had observed that stargrass (*Cynodon dactylon*) was a better competitor than *Acacia smallii* seedlings in the presence of nutrients. However, no significant change in grass biomass at intermediate nutrient levels (0.5N, 1N) might indicate a higher competitive ability of *Acacia* saplings than grasses.

We observed no difference in root to shoot biomass ratio under any given treatment. This indicates that the carbohydrate reserves from resprouting roots are replenished simultaneously as shoots develop and contrary to our prediction, shoot growth does not exceed root biomass with the increase in soil nutrient availability. Furthermore, though the resprouts in *A. sieberiana* are smaller than unburnt saplings in terms of root and shoot biomass, the proportion of investment in roots and shoots remains similar under any given treatment.

Conclusions

Sapling survival was not affected by soil nutrients. Mortality due to fire varies highly among *Acacia* species and cannot be generalised. Stem height growth of *Acacia* saplings increased rapidly after fire with no change in defence investment. Soil nutrient increase can be beneficial to *Acacia* sapling growth to a certain extent, with a reduction in defence investment and ability to withstand grass competition. The negative effects of increased grass competition on sapling growth (Cohn et al. 1989; Van der Waal et al. 2009) was limited only to high levels of nutrient availability. Post-fire resprouting shoots invest simultaneously in root and shoot biomass.

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Table 1 Species-wise ANOVA (unbalanced design) results, showing the effects of fire (two levels), nutrients (five levels) and their interactions on the growth and defence responses of *Acacia karroo*, *A. nigrescens*, *A. tortilis* and *A. sieberiana* saplings, after the burning treatment was applied

	Growth response parameters				Defence response parameters					
	df	RGR _h : RGR _d		Root : shoot biomass		df	CT conc.		Thorn length	
		F	p	F	p		F	p	F	p
<u><i>A. karroo</i></u>										
Fire		-	-	-	-	-	-	-	-	-
Nutrients	4	1.7	0.2260	1.06	0.4240	4	8.16	0.0050	0.13	0.9670
Fire × Nutrients		-	-	-	-	-	-	-	-	-
Error df		14		14		13		14		
<u><i>A. nigrescens</i></u>										
Fire	1	206.5	<0.0010	0.82	0.3860	1	1.07	0.3280	6.89	0.0280
Nutrients	4	13.18	<0.0010	0.8	0.5530	4	1.28	0.3480	1.15	0.3940
Fire × Nutrients	2	35.44	<0.0010	0.14	0.8680	1	3.6	0.0900	0.74	0.4110
Error df		17		17		15		15		
<u><i>A. sieberiana</i></u>										
Fire	1	42.6	<0.0010	0.08	0.7810	1	0.28	0.6020	4.22	0.0560
Nutrients	4	2.91	0.0640	0.52	0.7220	4	2.46	0.0910	0.33	0.8520
Fire × Nutrients	4	1.26	0.3360	1.77	0.1810	4	0.22	0.9250	0.37	0.8270
Error df		22		26		24		27		
<u><i>A. tortilis</i></u>										
Fire	1	5.6	0.0500	0.14	0.7170	1	0.2	0.6710	0.11	0.7440
Nutrients	4	0.73	0.6000	2.36	0.1510	4	0.99	0.4800	0.39	0.8110
Fire × Nutrients	1	0.36	0.5700	0.96	0.3600	1	1.65	0.2470	1.51	0.2540
Error df		13		13		12		14		

Ratio of final-initial relative growth rate of stem height (RGR_h) to final-initial relative growth rate in stem basal diameter (RGR_d) measured in mm mm⁻¹ week⁻¹, final log₁₀ transformed root/shoot dry biomass ratio measured in mg, final log₁₀ transformed leaf CT concentration (in mg mL⁻¹ QE) and final power (-0.3) transformed mean thorn length (in mm) were analysed. The degrees of freedom (df), error df, *F*-value and significance are indicated per factor and factor interactions. The values bold are significant (*p* < 0.05) and absent values are indicative of factors with *df* = 0

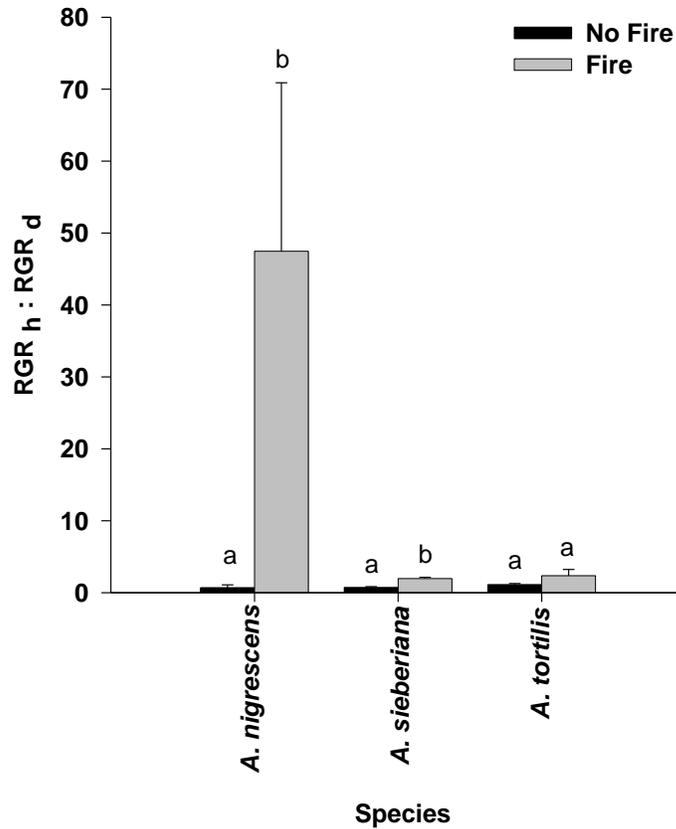


Fig. 1 Mean (+ SE) of the post-fire ratio of final-initial relative growth rate of stem height (RGR_h) in $\text{mm mm}^{-1} \text{ week}^{-1}$ to final-initial relative growth rate of stem basal diameter (RGR_d) in $\text{mm mm}^{-1} \text{ week}^{-1}$ of *Acacia* saplings with and without fire treatment. Initial readings were taken in March 2010 (except for *A. nigrescens* taken in April 2010) and the final readings were taken in July 2010. Significant differences ($p < 0.001$) within the species as given by an unbalanced ANOVA are indicated with dissimilar letters above the bars. *Acacia karroo* was excluded from this comparison because there was complete mortality of burned saplings.

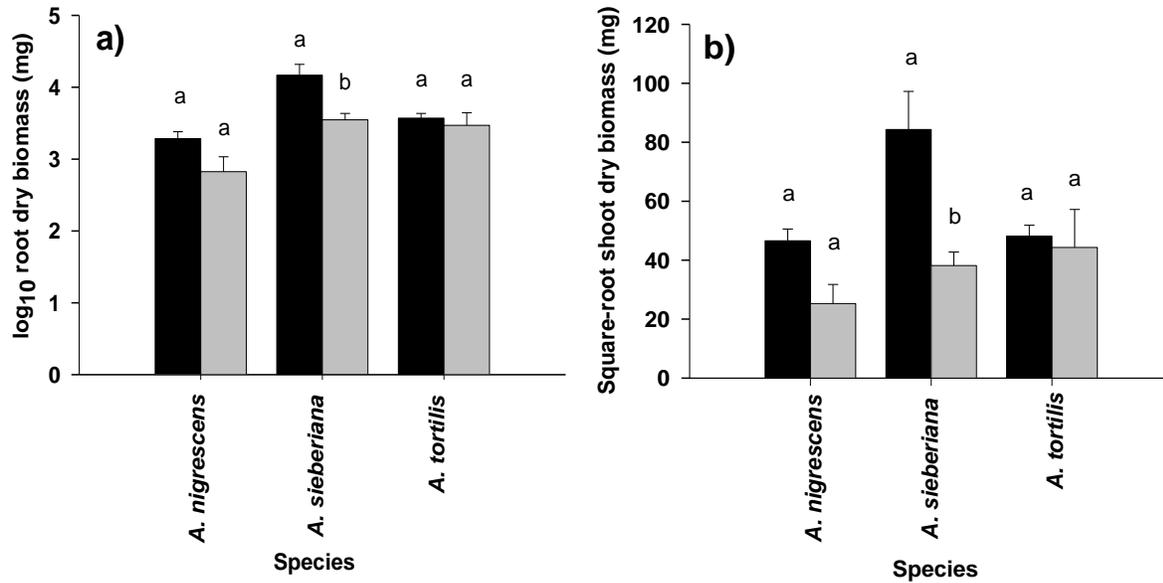


Fig. 2 Mean (+ SE) of the post-fire growth responses of *Acacia* saplings with (■) and without fire (□) treatment. Significant differences ($p < 0.05$) within species as given by an unbalanced ANOVA are indicated with dissimilar letters above the bars. **a** Final \log_{10} transformed root dry biomass in mg. **b** Final square root-transformed shoot dry biomass in mg. Readings were taken at the end of the experiment in July 2010. *Acacia karroo* was excluded from this comparison because there was complete mortality of burned saplings.

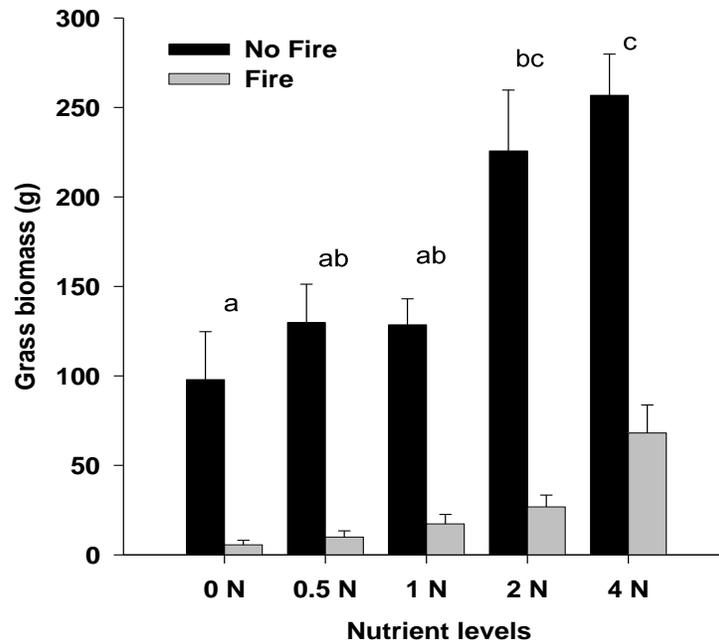


Fig. 3 Mean (+ SE) grass dry biomass in g, showing the effect of different levels of nutrients with and without fire treatment. Readings were taken at the end of the experiment in July 2010. Fire had a significant effect (ANOVA, $p < 0.001$). The dissimilar letters above the bars indicate significant differences ($p < 0.001$) in grass dry biomass between the nutrient levels as determined from Scheffe post hoc tests. N denotes the standard amount of fertiliser used: 0N = 0 g, 0.5N = 13 g, 1N = 26 g, 2N = 52 g, 4N = 104 g.

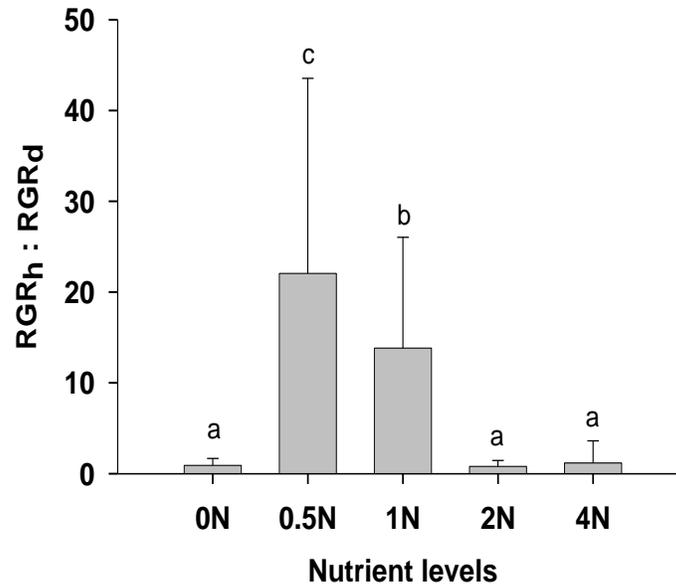


Fig. 4 Mean (+ SE) of the post-fire, ratio of final-initial relative growth rate of stem height (RGR_h) in $\text{mm mm}^{-1} \text{ week}^{-1}$ to final-initial relative growth rate of stem basal diameter (RGR_d) in $\text{mm mm}^{-1} \text{ week}^{-1}$ of *A. nigrescens*, showing the effect of different levels of nutrients (including the data from individuals treated with fire). Initial readings were taken in April 2010 and the final readings were taken in July 2010. The dissimilar letters above the bars indicate significant differences ($p < 0.01$) among different nutrient levels, as determined from Scheffe post hoc tests. N denotes the standard amount of fertilizer used: 0N = 0 g, 0.5N = 13 g, 1N = 26 g, 2N = 52 g, 4N = 104 g.

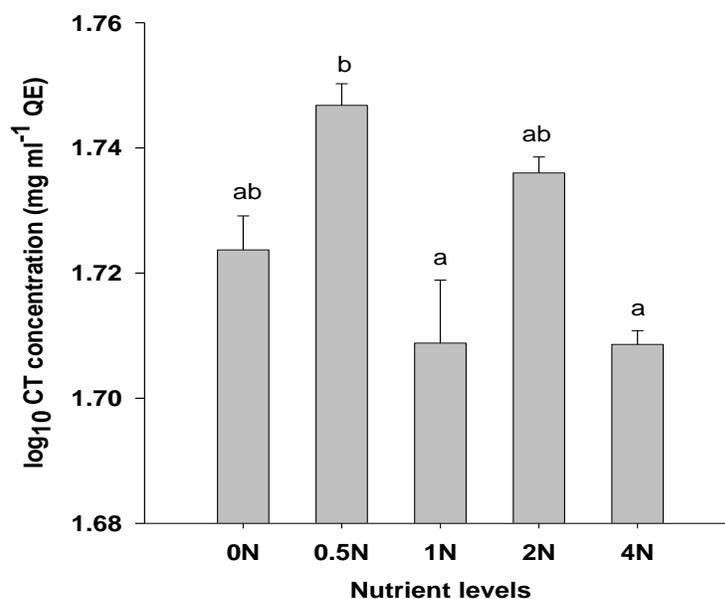


Fig. 5 Mean (+ SE) log₁₀ transformed leaf CT concentrations of *A. karroo*, showing the effect of different levels of nutrients without any fire treatment. The dissimilar letters above the bars indicate significant differences ($p < 0.01$) among different nutrient levels as determined from Scheffe post hoc tests. N denotes the standard amount of fertilizer used: 0N = 0 g, 0.5N = 13 g, 1N = 26 g, 2N = 52 g, 4N = 104 g.

Chapter 3: Shade, nutrients and grass competition are important for tree sapling establishment in a humid savanna

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Abstract. The structure and function of savannas is contingent upon tree sapling establishment into the ecosystem. Frequent rainfall, canopy shade and nutrient availability are the factors influencing recruitment of tree saplings. Grass competition has potential to change the tree establishment dynamics by interacting with these factors. The factors affecting tree sapling establishment have been well studied and tested in arid and semi-arid environments (approx. 750 mm MAP) compared to humid (> 1000 mm MAP) savanna ecosystems. We studied the effects of water (frequent irrigation vs. rainfall), shade (presence vs. absence), nutrient (addition vs. no addition) and grass competition (presence vs. absence) on sapling survival and growth of four humid savanna species and four mesic savanna species, under controlled field conditions in a humid South African savanna. None of the treatments had an effect on sapling survival. Growth of the saplings did not depend on the frequency of watering, probably due to high rainfall received in humid savannas. Shade had a strong negative effect on sapling growth, suppressing the beneficial effects of nutrient availability and absence of grass competition. Net positive effects of nutrient availability had less influence over sapling growth compared to the negative effects exerted by grass competition. Overall shoot growth increased with nutrients while investment in leaves drastically declined with grass competition. We conclude that canopy shade is a strong potential driver of structure and function of humid savanna ecosystems, although we recognize that nutrient limitation and grass competition additionally influence tree sapling establishment.

Key words: relative growth rate; sapling survival; grass competition; tree canopy; mesic savanna species; water availability; nutrients; Acacia.

INTRODUCTION

Savanna ecosystems are characterized by the co-existence of trees and grasses (Frost et al. 1986, Sankaran et al. 2004). Trees alter savanna ecosystem properties through interception of solar radiation, soil nutrient mediation and water availability (Vetaas 1992, Roos and Allsopp 1997, Shaw et al. 2002, Ludwig et al. 2003). Sapling recruitment is a critical life history stage of savanna woody plants which have long life spans (Scholes and Archer 1997, Chidumayo 2008). If the environmental conditions are not conducive, sapling establishment can be a major concern for tree recruitment into the ecosystem (Midgley and Bond 2001). Therefore, the factors that influence successful establishment of tree saplings into savannas control ecosystem functioning (Shaw et al. 2002). Sapling limitation by disturbances, fire and herbivory is often very intense (Midgley et al. 2010). Apart from disturbances, recruitment is also strongly limited by resource availability (water, light and nutrients) and grass competition (O'Connor 1995, Kraaij and Ward 2006, Sankaran et al. 2008, Riginos 2009, Ward and Esler 2011).

Woody cover is primarily determined by water availability in arid or semi-arid regions (< 650 mm mean annual precipitation) while other (disturbance-based) factors play an important role in tree cover dynamics in areas receiving > 650 mm MAP (Sankaran et al. 2005). Kraaij and Ward (2006) found that rainfall was the key factor in *Acacia mellifera* sapling establishment. In many savannas, seeds germinate with the onset of rainy season after being stimulated by dry season fires (Gashaw and Michelsen 2002). However, an early wet season drought after germination can cause heavy mortality of tree saplings (Harrington 1991, Hoffmann 1996, Gignoux et al. 2009). Therefore, frequent rainfall is more important than overall mean annual precipitation in estimating tree sapling establishment (Hoffmann 1996, Wilson and Witkowski

1998, Kraaij and Ward 2006). In their patch dynamics model, Meyer et al. (2007) have also explained the importance of recurring rainfall events for trees to establish.

Established woody plants often facilitate tree sapling recruitment by ameliorating stressful environments. Canopy microsite facilitation may enable the woody colonisers to form bush clumps (San José et al. 1991, O'Connor 1995, Rolhauser et al. 2011). Generally, canopy microsites enhance the recruitment and survival of woody saplings by intercepting light and thereby reducing high irradiance, increasing soil moisture, enhancing nutrients due to the decomposition of high litter cover and alleviating grass competition by shading grasses out (Hoffmann 1996, Salazar et al. 2012). Heightened animal activity under canopies also increases soil nutrient availability within the canopy zone (Vetaas 1992, Dean et al. 1999). However, other studies have shown that grasses increase in density in sub-canopy zones, eliminating any tree sapling establishment (Belsky et al. 1989, Scholes and Archer 1997, Abdallah and Chaieb 2012). Additionally, seedling response to canopy cover may be species-specific (see Hoffmann 1996). While shade may promote seedling establishment, some species such as *Colophospermum mopane* exhibit shade intolerance (Mlambo and Nyathi 2004).

Nutrients can be the second most important resource limitation for tree sapling establishment after rainfall (Kraaij and Ward 2006, Cramer et al. 2012). Nutrients have direct positive effects and indirect negative effects on savanna tree sapling recruitment. Savanna *Acacia* sapling growth rates were positively correlated with the available soil nutrients (Wakeling et al. 2010). On the other hand, nutrient supplementation may cause an increase in grass productivity which in turn suppresses tree sapling establishment (Cohn et al. 1989, Kraaij

and Ward 2006, Riginos 2009, van der Waal et al. 2011). However, the presence of nutrients may ameliorate the effects of grass competition (Cramer et al. 2012).

Competitive interactions between tree saplings and grasses may contribute significantly to the structure and function of savanna vegetation (Cramer and Bond 2013, Grellier et al. 2012). Grass competition affects savanna tree sapling establishment by mediating a number of abiotic stresses (see e.g. Cramer et al. 2010). Tree seedlings compete with grasses directly for light, mineral nutrients and soil moisture (Scholes and Archer 1997, van der Waal et al. 2009, Grellier et al. 2012). Grass competition can suppress growth at all life history stages of woody plants with a pronounced effect on sapling growth, survival and establishment (Riginos and Young 2007, Riginos 2009, Cramer et al. 2010, Kambatuku et al. 2011). However, grasses may also facilitate tree sapling establishment by providing moist microclimatic conditions and cover from browsers (Riginos and Young 2007, Seymour 2008, Grellier et al. 2012).

Many studies have drawn conclusions on various environmental factor effects based on semi-arid to mesic savanna systems. Hoffmann (1996), Gignoux et al. (2009), Rolhauser et al. (2011) and Bond et al. (2012) have made some key observations on tree sapling establishment in humid savannas. Very few studies conducted on savannas have concentrated on multiple species to make general interpretations of the seedling dynamics across environmental gradients (see Hoffmann and Franco 2003, Tomlinson et al. 2012). Differences in plant relative growth rates within savanna species can have important consequences for the structure of savannas. Stem survival ensures persistence of saplings in disturbance prone savannas and species-specific functional traits determines sapling regenerative capacity. Biomass allocation to stems (Clarke et al. 2013) and rapid stem growth rates in terms of height (Higgins et al. 2000) and diameter

(Gignoux et al. 1997) are the plant trait parameters found to be important in resisting fires. Savanna trees resprout by utilising stored root carbohydrate reserves (Schutz et al. 2009) substantially derived from leaf photosynthesis (Sakai and Sakai 1998). Hence, investment in leaves or leaf biomass provides an indirect measure of sapling success in savannas. We designed a controlled field experiment in a humid savanna to test the simultaneous effects of frequent water availability, shade, nutrient availability and grass competition on sapling survival and growth (in terms of shoot biomass, relative growth rates in stem length and diameter and leaf biomass proportion) of four dominant humid (> 1000 mm MAP) savanna species and four dominant mesic (approx. 750 mm MAP) savanna species. Based on the individual factor effects followed by the interactive effects on sapling survival and growth, we predicted that:

- 1) Regular watering increases the tree seedling survival (Kraaij and Ward 2006). Frequency of water availability has little effect on plant growth; however shrub seedlings subjected to severe water stress or drought increase their input into root biomass with a lower investment in shoots (Padilla et al. 2009). Hence, frequent watering will increase the survival of tree saplings with a high input in shoot growth.
- 2) Canopy shade mitigates stressful conditions and facilitates tree seedling survival (Salazar et al. 2012). Plants growing under shade have high allocation to stems and leaves at the expense of roots (Poorter et al. 2012). Hence, shade will have a positive effect on the survival and stem growth rates of all saplings with the exception of shade-intolerant species such as *Acacia tortilis* (Smith and Shackleton 1988) and *Colophospermum mopane* (Mlambo and Nyathi 2004).

- 3) Nutrients enhance the establishment success of tree seedlings (Siemann and Rogers 2003, Wakeling et al. 2010). It is well known that plants increase proportional allocation to roots with a decrease in shoot investment, particularly in leaves, at low nutrient levels (Poorter et al. 2012). Hence, nutrients will enhance the survival and growth of tree saplings.
- 4) Grass competition suppresses tree sapling establishment even under high nutrient availability (Grellier et al. 2012). Savanna tree seedlings increase their root biomass with a decrease in aboveground growth, in the presence of grass competition (Cramer et al. 2010, Kambatuku et al. 2011, Ward and Esler 2011). Hence, grass competition will negatively effect sapling survival and growth.
- 5) While grass in the presence of high resources (regular water supply, natural light, additional nutrients) will exert maximum negative effects on tree saplings, saplings growing in the absence of grasses and in the presence of high resource levels will have high survival and growth rates.

METHODS

Study site

The study was conducted on Mondi nursery grounds, KwaMbonambi which is situated in the north-eastern coastal region of KwaZulu-Natal, South Africa (28°35'59.20" S, 32°10'47.22" E). The long-term mean annual rainfall (1984 – 2010) of the study area is 1250 mm with a

maximum rainfall of 2233 mm in 1987 and a minimum of 563 mm recorded in 1992. Although precipitation is experienced throughout the year, rainfall in the area occurs predominantly in the summer months and on average peaks in November and February.

The terrain at KwaMbonambi is generally flat and is comprised of Recent sediments of Quaternary, clayey, alluvial sands of aeolian deposition overlying the Cretaceous conglomerates of the Berea formation or Maputaland group (King 1982, Mucina and Rutherford 2006). The high permeability of the soils allows rapid leaching of the nutrients due to heavy rainfall in these areas. Vegetation is classified as Maputaland wooded grassland, which consists of flat coastal plain supporting sandy grasslands rich in geoxylic suffrutices, dwarf shrubs and small trees (Mucina and Rutherford 2006).

The experimental site was fenced to exclude large herbivores (> 5 kg) from the area. Experimental blocks were laid out in an open, non-wooded and flat 70 m × 50 m area. The area was mown in the past and was not previously fertilised. Topsoil samples (up to 15 cm depth) were randomly collected from the experimental site and were sent to the University of KwaZulu-Natal laboratory, Pietermaritzburg, South Africa for soil N and P analysis. Nitrogen was analysed in a LECO Truspec Nitrogen Analyser (LECO corporation, Michigan) using the Dumas combustion method and phosphorous was analysed using a Technicon Autoanalyser II (Technicon Industrial Systems, Tarrytown, N.Y.). The topsoil in the study area contained a mean (\pm SE) of 0.1051 (\pm 0.013) % total N and 0.0236 (\pm 0.0021) % total P on a dry matter basis (n = 12 samples).

Experimental design and treatments

We studied the effects of regular water availability, shade, nutrient availability and grass competition on the sapling growth of eight savanna tree species. In order to test whether savanna tree species from different climate types have a similar or different growth patterns with respect to the treatments provided, we selected four humid savanna species, i.e. *Acacia karroo* Hayne (a subtropical coastal variety from Richards Bay (Ward 2011)), *Acacia sieberiana* Burt Davy, *Schotia brachypetala* Sond. and *Strychnos spinosa* Lam. and four species from mesic savannas, i.e. *Acacia nigrescens* Oliv., *Acacia tortilis* Hayne, *Combretum apiculatum* Sond. and *Colophospermum mopane* J.Kirk ex J. Léonard, as our study species. Tree species were selected based on dominance in their respective climate types. The most widespread grass species in South Africa, *Eragrostis curvula*, was used to study the effects of grass competition on the tree sapling growth.

The experimental site was cleared of all vegetation prior to the application of the treatments. We adopted a split-plot design to accommodate and test for all the treatment factors in a limited space. Water and shade treatments were applied at a whole plot level and at the split-plot level the treatment combinations of nutrients and grass were provided. The two whole-plot treatment factors of water (W) and shade (S) had two levels namely natural rain and even rainfall frequency and natural light and shade respectively. Within each whole plot treatment combination, two sub-plot treatment factors of nutrients or external fertilization (N) and grass (G), with two levels, *viz.* presence and absence were provided. One set of four whole plots, each containing four sub-plots was considered as one block unit. The whole plots in each block unit were spaced at least 2 m apart from one another. Each block unit was replicated five times, with

at least 3 m distance between block units. Randomisation of block units in the experimental area and whole plots within each block unit was done. Four saplings of each species were planted at least 15 cm apart in sub-squares within each sub-plot. Hence, the four saplings of each species were planted in $[2W \times 2S \times (2N \times 2G)] \times 5 = 80$ sub-plots.

Whole plot treatments were provided under experimental structures that were each 2 m high by 7 m \times 7 m ($n = 20$ whole-plots). Plastic-roofed structures, made of 200 μm -thick clear greenhouse plastic film, were used to prevent the entry of rain water in the irrigated whole-plots. Irrigation was provided such that each sub-plot, under even-water treated whole-plots, received an amount of water close to the long-term average annual rainfall at regular time intervals (for 90 min every 24 h) over a period of 24 weeks. Within the experimental period of 6 months each sub-plot in the open received about 1722 L or 431 mm (34 % of long-term MAP in this drought year) of rainfall and in the irrigated whole-plots received 5000 L or 1250 mm (100 % of long-term MAP) of water. Commercially available 80 % shade cloth was used for the whole-plot structures treated with shade, such that saplings are shaded at all times. We used a Sunfleck PAR Ceptometer model SF-80 (Decagon Devices Inc., Washington, DC, USA) and calculated the PAR transmission percentage based on Bauhus et al. (2004). On a cloudless sunny day we measured the photosynthetically active radiation (PAR) in $\mu\text{mol m}^{-2} \text{s}^{-1}$ within a range of 400 – 700 nm. We recorded an average percentage transmission at 1 m above the ground, approx. 10 % ($87.3 \mu\text{mol m}^{-2} \text{s}^{-1}$) and approx. 55 % ($504.1 \mu\text{mol m}^{-2} \text{s}^{-1}$) of PAR in the open ($905.9 \mu\text{mol m}^{-2} \text{s}^{-1}$) in the shaded plots and under the plastic roofed structures, respectively. The shade provided in our study was substantial enough to mimic the attenuation of natural light under savanna trees. For example, the canopies of *Acacia mearnsii* stands were reported by Bauhus et al. (2004) to

transmit 18.5 % of the open PAR. Salazar et al. (2012) have found that 32 % of natural sun light was sufficient for sapling emergence in various Neotropical woody savanna species.

Four sub-plots of 2 m × 2 m area were situated at the centre, leaving a peripheral width of 1.25 m, of the whole-plot. Sub-plots within the whole plots were spaced 0.5 m apart from one another. “Osmocote exact” was the nutrient compound applied to all sub-plots provided with nutrient treatment. It was a water soluble, granular, controlled-release fertilizer. The nutrient composition was 15 % N, 9 % P₂O₃, 11 % K₂O, 2.5 % MgO and other trace elements. This fertilizer was chosen for the steady and continuous release pattern over a 12 month period. Nitrogen is an important limiting factor for plant growth in tropical savannas (Bremen and de Wit 1983). The fertilizer was applied once-off in October 2009 at the rate of 4 g N m⁻² month⁻¹. This amount was approximately double the highest level of nitrogen (2.3 g m⁻² month⁻¹) provided by Tilman (1987) and 2.8 g N m⁻² month⁻¹ applied by Kraaij and Ward (2006) in their study. The fertilizer applied amounted to 320 g/ m². Ten days after the fertilizer was added, 300 g of grass seeds were sown in each grass treatment sub-plot. Within each grass sub-plot, the soil was lightly compacted after the grass seeds were added. Grasses were allowed to establish for two months before tree saplings were transplanted into the plots. However, grass was cut to 2 cm above ground prior to tree sapling transplantation to reduce grass competition and give some time for the tree saplings to establish. All plots were weeded every month to keep other herbaceous plants from interfering with the experiment. We observed that, under shade, grasses had established and grew very well for the first few months but, by the end of the experiment, the grasses thinned out. Therefore, in our analyses we have assumed that the tree saplings under the shade also endured adequate grass competition.

Sapling development and transplanting

The hard-coated seeds of *A. karroo*, *A. nigrescens*, *A. sieberiana*, *A. tortilis* and *S. brachypetala* were soaked in concentrated sulphuric acid and scarified for 1 h. They were then washed thoroughly with water to remove the traces of acid and then soaked in water for 12 h. The soft-coated seeds of *C. mopane*, *C. apiculatum*, and *S. spinosa* were not treated with acid but were soaked in 0.2 % HgCl₂ solution for 5 min to decontaminate the seeds and were then washed thoroughly and soaked in water for 12 h. Each seed was planted in 750 mL potting bags, were then allowed to grow in a mixture of soil, vermiculite and coconut coir. In order for the seeds to adapt to natural soil conditions, soil (up to 15 cm depth) from the experimental site was used. Vermiculite and coconut coir mixture was used to increase the porosity and water retention of the soil. After planting the seeds, potting bags were placed in mist beds under shade, ensuring moist conditions throughout the day. However, *C. mopane* seeds do not require a high water supply, as they are highly permeable to water and germinate easily (Mlambo et al. 2007) and therefore were watered once every week. Saplings were grown for 2 – 3 months and were transplanted into the treatment plots. All the saplings were transplanted after the first heavy rainfall for the season occurred; transplantation was completed by mid-January 2010. *Acacia nigrescens* were represented in only four replicate block units due to low germination and consequent lack of sapling availability. The potting bags were sprayed with a general preventative fungicide (Previcur[®]) once every week until the saplings were transplanted. Transplanted saplings in all plots were provided with water for a few days, allowing them to adapt to natural conditions and mitigate stress. Some replacement of dead saplings (n = 412) within a month after transplanting was done.

Data collection and compilation

Sapling survival status, as live or dead, was noted at the end of the wet season in June 2010 on all the saplings that were planted at the beginning of the experiment in February 2010. Sapling growth measurements were taken at the beginning and the end of the experiment within the wet season. Stem length (from the base to apex) and basal stem diameter (two perpendicular readings) were the pre-harvest measurements taken on each sapling. At the end of the experiment, in June 2010, aboveground plant material of half the number of live saplings were harvested, marked and placed in brown paper bags. The other half of the saplings was allowed to grow to the next season. Plant material collected was allowed to dry in a drying oven for 48 h at 60 °C. Harvested grass material was dried under the sun and weighed in g. Post-harvest dried leaf and stem material of each tree sapling were weighed separately in mg.

A quick attainment of overall sapling growth is essential to ensure its establishment in a savanna ecosystem (Vadigi and Ward 2012). Hence, we analysed sapling growth in terms of relative growth rate of the stem length (RGR_L), relative growth rate of the stem basal diameter (RGR_D), total aboveground dry weight, proportion of leaf dry biomass to the total aboveground plant dry weight (leaf mass proportion). The total aboveground dry biomass for each tree sapling was calculated as the sum of leaf and stem dry weight. Calculations for relative growth rate of sapling stem length (RGR_L) and relative growth rate of sapling basal stem diameter (RGR_D) were based on the increments in \ln (stem length) and \ln (basal diameter), respectively, per unit time (Kohi et al. 2009).

Statistical analysis

We used linear mixed modelling with multilevel regression and analysed our data using the R statistical program (R Development Core Team 2011). A multilevel model is not only capable of integrating predictors that vary across different spatio-temporal scales but also can incorporate non-predictor variables explaining the between-group variation (Qian et al. 2010). Furthermore, linear mixed models are capable of dealing better with missing values in the data than conventional ANOVA (Piepho et al. 2003; Schielzeth and Nakagawa 2012). The predictor factor effects on binomial survival data were estimated by using “glmer” (generalized linear mixed model) function and effects on sapling growth were analyzed by using ANOVA output of the “lme” (linear mixed model) function.

In our full model, we tested the fixed effects of species (Spp), water (W), shade (S), nutrients (N) and grass (G) treatments on the sapling survival and growth in order to understand species differences in response to treatment factors given. Species were considered as fixed effects because they were selected based on their dominance in either humid or mesic habitats. We excluded *A. nigrescens* from our full model analyses due to many missing values (unbalanced data), due to inadequate replication and complete sapling mortality which occurred under natural conditions in the presence of nutrients and grass. The outcomes measured at the sub-plot scale in the hierarchical structure are affected by factors measured at that scale and factors working at the whole-plot level within the randomized blocks (Qian et al. 2010; Schielzeth and Nakagawa 2012). A random factor was modelled to adjust variations in the data due to a complicated spatial pseudo-replication arising from the split-plot design (Piepho et al 2003, Crawley 2007). We considered a split-plot error term associated with the treatment factors,

representing a sub-plot level interaction of nutrient and grass within a whole-plot level of water and shade interaction within block (Blk) in our maximum likelihood models. In other words, block and the block \times treatment interaction were considered as random effects in the models. We analysed the sub-plot means for each measured growth response variable of each species to avoid pseudo-replication at the sapling level. Equation (1) shows an example of the ‘lme’ model used in R for the overall analysis:

$$\text{Overall RGR}_L \sim \text{Spp} * \text{W} * \text{S} * \text{N} * \text{G}, \text{ random} = \sim 1 | \text{Blk} / \text{W} : \text{S} / \text{N} : \text{G} \quad \text{Eqn (1)}$$

The above model represents predictor parameter effects on relative growth rate in sapling stem length (RGR_L) as a linear function of all species interacting with full factorial treatment factors viz. water (W), shade (S), nutrients (N), and grass (G) with a split-plot error. To test for the effects of treatments on individual species we modelled individual species growth as a linear function of the full factorial treatment effects, excluding the largest four-way interaction term for *A. nigrescens*, with split-plot error term in Eqn (2).

$$\text{Individual spp RGR}_L \sim \text{W} * \text{S} * \text{N} * \text{G}, \text{ random} = \sim 1 | \text{Blk} / \text{W} : \text{S} / \text{N} : \text{G} \quad \text{Eqn (2)}$$

To avoid Type I errors we only present individual species results where they are consistent with the overall analysis. However, the results for *A. nigrescens*, which were not included in our full model analyses, are presented separately. We also performed similar generalized linear mixed model (*glmer*) analyses on sapling *survival*, but observed no overall effects of the various treatments provided. Hence, the results of *survival analyses* are not presented. We observed 79 % overall sapling survival. All growth variables were checked for general linear model assumptions and the variable, sapling dry biomass, was \log_{10} transformed to

ensure normal distribution and homogeneity of variances. All main effects, interactions and treatment differences were considered to be significant at $\alpha = 0.01$, after Bonferroni corrections.

RESULTS

Relative growth rate of sapling stem length

There were significant differences among the species in all parameters measured (Table 1). Water did not have an overall effect on relative growth rate of sapling stem length (RGR_L) (Table 1). RGR_L differed significantly among species with the shade treatment (Table 1). There was an inconsistent pattern of investment in RGR_L between species of same savanna type with respect to shade treatment (Fig. 1a, b). Nutrients had an overall significant positive effect on sapling RGR_L (Table 1). When each species was tested separately, the nutrient fertilisation was positive and significant only for *C. mopane* saplings (Table A6; mean \pm 1SE, 0.007 ± 0.003 mm mm^{-1} week $^{-1}$ without nutrients and 0.020 ± 0.003 mm mm^{-1} week $^{-1}$ with nutrients). Shade and nutrient interactions had a significant effect on overall sapling RGR_L (Table 1). All species showed a positive effect of nutrients on RGR_L only in the presence of natural light (Fig. 2a, b). Overall, sapling RGR_L decreased significantly in the presence of grass competition (Table 1). When each species was tested separately, the results were significant for *S. brachypetala* only (Table A3; mean \pm 1SE, 0.027 ± 0.003 mm mm^{-1} week $^{-1}$ with no grass and 0.016 ± 0.002 mm mm^{-1} week $^{-1}$ with grass). Shade and grass had a significant interaction effect on the overall sapling RGR_L (Table 1). The negative effect of grass on RGR_L was found, under natural light, in five out of seven species (Fig. 3a, b).

Relative growth rate of sapling basal stem diameter

Water did not have an overall effect on relative growth rate of sapling basal stem diameter (RGR_D) (Table 1). All species had significantly higher RGR_D in the presence of natural light when compared to the shaded saplings, except in *S. spinosa* where shade was beneficial to sapling RGR_D (Table 1, Fig. 4a, b). Nutrients had an overall significant positive effect on sapling RGR_D (Table 1). When the individual species was tested separately, the nutrient effects on sapling RGR_D were significant in all species except *C. mopane* (mesic spp.) and *S. spinosa* (humid spp.) (Table A1 - A7). The shade by nutrient interaction was significant on overall sapling RGR_D (Table 1). The positive nutrient effect on sapling RGR_D was higher only in the presence of natural light in all species, except *S. spinosa* (Fig. 5a, b). Grass competition had an overall significant negative effect on the sapling RGR_D (Table 1). When each species was tested separately, the significant negative effect of grass competition on RGR_D was not observed for *A. sieberiana* only (humid sp.) (Table A1 - A7). There was a significant interactive effect of shade and grass on overall sapling RGR_D (Table 1). However, the negative effect of grass competition on sapling RGR_D of all species was observed only under natural light conditions (Table 1, Fig. 6a, b).

Sapling dry biomass

Water had no significant overall effect on (\log_{10}) sapling dry biomass (SDB) (Table 1). Shade had an overall significant negative effect on SDB (Table 1). The SDB of only *A. karroo* and *S. spinosa* (humid spp.) were found not to be affected by shade treatment (Fig. 7a, b). Nutrients had an overall significant effect on SDB. When considering the results from individual species analyses, a positive significant difference was not observed in *S. spinosa* only (Table A1-

A7). Shade and nutrient interaction had a significant effect on the overall SDB (Table 1). The positive effect of nutrient addition on SDB was observed only under natural light conditions in all species, except *S. spinosa* (Fig. 8a, b). Grass competition had an overall significant negative effect on SDB (Table 1). All species showed a significant decrease in their SDB with grass competition (Table A1-A7). However, there was a significant shade by grass interactive effect on overall SDB (Table 1). The negative effect of grass competition was observed only under natural light conditions (Fig. 9a, b).

Proportion leaf to sapling dry biomass

Water, shade and nutrients did not have an overall effect on the proportion of the leaf biomass (LP) of all species (Table 1). Grass competition had an overall significant negative effect on sapling LP (Table 1). Based on the individual species analyses, there was a significant decrease in the leaf biomass proportion of *A. sieberiana* and *S. brachypetala* (humid spp.) and *A. tortilis* and *C. mopane* (mesic spp.), in the presence of grass (Table A1-A7). There was a significant interactive effect of shade and grass on overall sapling LP (Table 1). The negative effect of grass competition on sapling LP was observed under natural light conditions only, in all species except *S. spinosa* (Fig. 10a, b).

Acacia nigrescens

No treatment had an effect on relative growth rate of stem length (RGR_L) of *A. nigrescens* saplings (Table 2). Grass competition had a significant negative effect on the relative growth rate in stem basal diameter (RGR_D) of *A. nigrescens* saplings (Table 2; mean \pm 1SE, 0.046 ± 0.003 mm mm⁻¹ week⁻¹ without grass and 0.031 ± 0.003 mm mm⁻¹ week⁻¹ with grass).

The main effects of nutrient and grass had a significant impact on (log₁₀) sapling dry biomass

(SDB) of *A. nigrescens* (Table 2). *Acacia nigrescens* saplings had higher SDB in the presence of nutrients (3.547 ± 0.116 mg) than without nutrients (3.152 ± 0.114 mg). The presence of grass competition reduced the SDB of *A. nigrescens* (mean \pm 1SE, 3.606 ± 0.101 mg without grass and 3.016 ± 0.113 mg with grass). There was a significant interactive effect of shade and grass on *A. nigrescens*' SDB (Table 2). A negative effect of grass competition on *A. nigrescens*' SDB was observed only in the presence of natural light conditions (Fig. 11). *Acacia nigrescens* saplings had a significantly higher proportion of leaf biomass (LP) in the presence of shade (0.338 ± 0.165 , mean \pm 1SE) than under natural light (0.245 ± 0.027 , mean \pm 1SE; Table 2). Water interacting with grass had a significant effect on the LP of *A. nigrescens* saplings (Table 2). *Acacia nigrescens* saplings had higher LP without grass competition and frequent watering (0.334 ± 0.019 , mean \pm 1SE) than in the presence of grass and under natural rainfall (0.256 ± 0.044 , mean \pm 1SE).

DISCUSSION

Sapling survival

No treatment had a significant effect on the *survival* of the savanna tree saplings we studied. Although there was high sapling mortality in open plots due to a short dry period immediately after transplanting, irrigation and shade did not have a significant effect on sapling survival, despite the fact that the even water treatment received approximately double the rainfall that was received in the open. Humid savannas may receive solar radiation equivalent to arid zones during the dry periods in between rainfall seasons (Jagtap 1995). Generally, dry spells

during the wet season are fatal to tree seedlings in arid/semi-arid savannas (Wilson and Witkowski 1998, Kraaij and Ward 2006) and may also be so in humid savannas (Hoffmann 1996). However, irradiance received in humid savanna zones in Africa is reduced due to the presence of cloud cover and suspended aerosols (Jagtap 1995) and may have promoted sapling survival against adversity in the open plots. Hence, shade and an increase in moisture availability under tree canopies (O'Connor 1995) may have no effect on tree sapling *survival* in humid savannas. Greater soil nutrient availability under tree canopies is also often cited as a plausible reason for an increase in tree sapling survival (Vetaas 1992, Hoffmann 1996, Salazar 2012). However, consistent with the findings of Vadigi and Ward (2012) for *Acacia* saplings, we found no nutrient effect on sapling survival in any species. Grass competition, identified as the greatest hurdle for sapling survival by Grellier et al. (2012) and Ward and Esler (2011), also did not have an effect on sapling survival. Perhaps, in humid savannas, other environmental factors such as temperature, fire and herbivory play a more influential role in savanna sapling survival (Shaw et al. 2002, Hoffmann and Solbrig 2003, Sharam et al. 2006, Chidumayo 2008, Gignoux et al. 2009).

Sapling growth

Water

We found that frequent water supply had no effect on any measured growth parameters of the savanna tree saplings, despite the fact that the even-water treatment received approximately double the rainfall that was received in the natural-water treatment in this drought year. Many studies have indicated the dependence of frequent rainfall in tree sapling establishment (Hoffmann 1996, Wilson and Witkowski 1998, Kraaij and Ward 2006, Gignoux et al. 2009, van

der Waal et al. 2009). Contrastingly, our results show that in humid savannas, sapling growth does not depend on rainfall frequency. This also indirectly supports the idea that trees growing in sites receiving heavy rainfall (> 1000 mm mean annual rainfall) are not constrained by water availability for their establishment (see also Sankaran et al. 2005). Nonetheless, we found that *A. nigrescens* had a higher proportion of leaf biomass (LP) with irrigation in the absence of grass competition when compared to *A. nigrescens* saplings growing in the presence of grass under natural conditions. Similarly, Kraaij and Ward (2006) found that watering and grass defoliation were necessary to trigger mass recruitment of *A. mellifera* saplings.

Shade

Shade had mixed effects, with more negative than positive effects, on sapling growth. Very few positive effects were observed, for example on the RGR_L of *S. brachypetala* and LP of *A. nigrescens*. There is a strong consensus that mitigation of environmental stress under canopy shade facilitates tree sapling growth (Hoffmann 1996, Siemann and Rogers 2003, Rolhauser et al. 2011, Salazar et al. 2012). Low irradiance, reduced temperature and enhanced moisture availability are the characteristics associated with shade benefiting sapling growth (Vetaas 1992, O'Connor 1995, Salazar et al. 2012). However, in our study, the more frequent negative effects show that, in humid savannas, shade inhibited tree sapling establishment regardless of water availability. Similarly, Milton (1982) has found that shade significantly reduced the growth rates of certain Australian *Acacia* species. Furthermore, Chirara (2001) established that low irradiance had a strong negative effect on *A. karroo* seedlings, even in the presence of high water availability. Many savanna woody plants especially leguminous trees can be shade intolerant (Kambatuku et al. 2011). As indicated earlier, the interception of solar radiation by cloud cover

and aerosols is higher in humid than arid savanna regions in Africa (Jagtap 1995) and more so during the wet season. Therefore, it could possibly mean that the solar radiation received in humid savannas is adequate for sapling growth and shading is detrimental. The positive growth investment in *S. brachypetala* and *A. nigrescens* under shade may have led to an increase in sapling efficiency of light reception. Due to low light availability under canopy shade, saplings invest heavily in shoot growth and not in root reserves, which makes them more vulnerable to drought (Barnes 2001). For this reason, fire is also a considerable threat, particularly as fire is a phenomenon frequent to humid savannas (Bond 2008, Gignoux et al. 2009). Hence, the benefits of shade on sapling growth in *S. brachypetala* and *A. nigrescens* may not apply to long-term survival and growth.

Many studies have cited nutrient increases in the sub-canopy soil zone due to high litter cover, animal droppings, nutrient re-distribution and N₂ fixation by leguminous trees (Bernhard-Reversat 1982, Belsky et al. 1989, Vetaas 1992, Belsky 1994, Scholes and Archer 1997, Abdallah and Chaieb 2012, Boyd and Davies 2012, Salazar et al. 2012). Some of them have noted an improvement in herbaceous production, as a result of an increase in sub-canopy soil fertility (Belsky et al. 1989, Scholes and Archer 1997, Abdallah and Chaieb 2012), and quality (Treydte et al. 2007). However, other authors have observed a decline in grass production under shade (Hoffmann 1996, Salazar et al. 2012), especially in humid savannas (Mordelet and Menaut 1995). Ludwig et al. (2001) have concluded that herbaceous density depends on the interactions between soil fertility, shade and water availability under tree canopies. Savanna tree saplings establish well on highly fertile soils with no grass competition in the sub-canopy zones (Rolhauser et al. 2011, Salazar et al. 2012). Conversely, we found that the majority of our study species grew better (except in proportion of leaf biomass (LP)) with nutrients, but only under

natural light. We also found that the negative effect of grass competition on sapling growth was suppressed under shade. In other words, except for a few species, the effect was significant only under full sunlight. Midgley and Bond (2001) have described shaded under-canopy sites as poor sites for *Acacia* sapling establishment. Similarly our results have indicated that shade exerts a strong negative effect on savanna tree sapling growth despite favourable conditions in terms of moisture and nutrient availability and decrease in grass competition. Hence, shade must be included in modelling studies as an important manipulator of savanna ecosystem properties.

Nutrients

In most cases, the main effects of nutrient addition on sapling growth were not significant. However, there were several instances of positive nutrient effects on sapling growth: RGR_L of *C. mopane*, RGR_D of all spp except *C. mopane*, *S. spinosa* and *A. nigrescens*, SDB of all species except *S. spinosa*. Tree species in savannas generally are associated with fertile soils and have high nutritional requirements (Ben-Shahar 1991). However, moist-dystrophic savannas may host increased woody vegetation of low nutritional quality (du Toit 1995). Additionally, *Acacia* sapling growth rates were higher in nutrient-rich soils when compared to growth in nutrient-poor grassland soils (Wakeling et al. 2010). In contrast, certain studies have found that abundant soil nutrients improve herbaceous productivity which in turn suppresses tree sapling establishment (Cohn et al. 1989, Kraaij and Ward 2006, van der Waal et al. 2011). Although we did not find any significant nutrient-grass interactive effect on sapling growth, the negative effects exerted by grasses were greater than the positive effects of nutrients (see also Kambatuku et al. 2013 for *Acacia mellifera*). However, some positive nutrient effects observed in our study could mean that nutrients partially ameliorate the effects of grass competition (see also Cramer et

al. 2012). We also found that most species invest in basal diameter and overall plant biomass when provided with nutrients. Investment in stems (height or diameter) for increased growth out of the “flame zone” is important for saplings (Lawes et al. 2011), especially in humid environments.

Grass competition

Grass competition had a negative effect on sapling growth (but not *survival* – see above). There was no effect of grass competition on RGR_L of all species excluding *A. nigrescens* and *S. brachypetala*, RGR_D of *A. sieberiana*, and LP of *S. spinosa* and *C. apiculatum*. It is widely understood that grass competition is a basic hindrance to savanna tree sapling establishment (Riginos and Young 2007, Kambatuku et al. 2011, Ward and Esler 2011, Grellier et al. 2012). Presence of grass competition reduces tree sapling growth even when conditions are conducive for tree establishment (Riginos 2009, Ward and Esler 2011). However, exclusion of grass alone might not be sufficient for tree sapling establishment (Seymour 2008). Alleviation of grass competition with the presence of high rainfall and soil nutrients is necessary for mass tree recruitment (Ward 2005, Kraaij and Ward 2006). With grass competition, saplings invested less in biomass and leaves. This could mean that grasses outcompeted tree saplings. It could also possibly mean that, in the presence of grass, saplings invested more in root biomass than in shoot biomass (Riginos 2009) to reach deeper layers of soil for access to water and perhaps to minimize competition. Kambatuku et al. (2011) have shown that the grasses competing with *A. mellifera* saplings invested more in root production. In our study, we found that grass competition had a considerable negative effect on sapling LP when compared to other treatments

provided. Therefore, proportional investment in leaves could be a good indicator of savanna tree sapling sensitivity to grass competition.

CONCLUSIONS

Among the factors tested, shade is an important factor with the potential to affect the ecosystem properties of a humid savanna. Shade exerted a strong negative effect on tree sapling growth, reducing the beneficial effects of nutrients and absence of grass competition. Nutrient supplementation increased sapling growth, especially in terms of basal diameter and total biomass. Grass competition is detrimental to tree sapling growth. The negative effects of grass competition are considerable relative to the benefits of nutrient addition on sapling growth (see also Kambatuku et al. 2013). Sapling growth did not depend on the frequency of rainfall as the mean annual precipitation is high in humid savannas. Environmental conditions in humid savannas are conducive for the persistence of tree saplings (*survival*) without the necessity of any additional resource benefits such as frequent water availability, canopy shade and nutrients. Sapling *survival* was also not affected by the presence of grass competition.

Elevated CO₂ levels associated with climate change, regardless of water availability or nutrients may impose woody canopy closure in humid savannas (Murphy and Bowman 2012). However, we found that shade inhibits savanna tree establishment in humid savanna ecosystems and therefore canopy closure might be hard for savanna tree species to attain. However, forest tree species are adapted to shade and can flourish under dense vegetation cover, particularly in areas receiving high rainfall (Hoffmann et al. 2004). In humid savannas, frequent fires can be a

major hindrance to forest tree establishment, but canopy shade can protect saplings and facilitate recruitment. In this context, we will further examine the factorial drivers of savanna-forest tree sapling establishment in a South African humid savanna. This will help us understand the existence of savanna-forest ecotone boundaries in African humid zones.

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TABLE 1. Linear mixed model (lme) analysis using maximum likelihood, showing the individual and interactive effects of water, shade, nutrient and grass treatments (two levels each) on all species, excluding *Acacia nigrescens*. Interaction effects of nutrient and grass (sub-plot) within water and shade (whole plot) within replicates (random effects of block units) was considered as split-plot error. We analyzed final-initial relative growth rate of stem length (RGR_L in mm mm⁻¹ week⁻¹), final-initial relative growth rate in stem basal diameter (RGR_D in mm mm⁻¹ week⁻¹), final proportion of leaf to sapling dry biomass (LP), final log₁₀ transformed sapling dry biomass (SDB in mg). The numerator degrees of freedom (df), error df, F-value and significance are indicated per factor and factor interactions. The values in bold are significant using a Bonferroni correction (p < 0.01)

Source	df	RGR _L			RGR _D			LP			SDB		
		Err df	F	p	Err df	F	p	Err df	F	p	Err df	F	p
Spp	6	359	41.99	<.0001	360	44.07	<.0001	360	44.72	<.0001	361	122.07	<.0001
W	1	12	5.81	0.0329	12	3.07	0.1052	12	0.52	0.4863	12	4.18	0.0635
S	1	12	0.02	0.8960	12	6.18	0.0287	12	1.04	0.3288	12	10.94	0.0062
N	1	48	24.24	<.0001	48	35.48	<.0001	48	4.14	0.0473	48	55.28	<.0001
G	1	48	12.48	0.0009	48	76.29	<.0001	48	27.90	<.0001	48	116.64	<.0001
Spp:W	6	359	2.52	0.0211	360	1.43	0.2025	360	2.73	0.0133	361	1.98	0.0678
Spp:S	6	359	4.08	0.0006	360	5.97	<.0001	360	2.61	0.0173	361	4.45	0.0002
W:S	1	12	0.01	0.9066	12	1.11	0.3130	12	2.05	0.1777	12	0.76	0.4007
Spp:N	6	359	0.31	0.9338	360	2.50	0.0220	360	1.14	0.3401	361	2.60	0.0177
W:N	1	48	0.67	0.4156	48	0.04	0.8382	48	0.40	0.5284	48	0.45	0.5076
S:N	1	48	32.71	<.0001	48	15.07	0.0003	48	3.83	0.0563	48	12.18	0.0010
Spp:G	6	359	0.64	0.6986	360	1.42	0.2067	360	1.91	0.0789	361	3.73	0.0013
W:G	1	48	0.05	0.8213	48	0.69	0.4086	48	4.41	0.0409	48	0.60	0.4431
S:G	1	48	11.93	0.0012	48	46.92	<.0001	48	18.56	0.0001	48	58.04	<.0001
N:G	1	48	0.54	0.4659	48	0.04	0.8358	48	6.75	0.0124	48	0.42	0.5214
Spp:W:S	6	359	1.31	0.2510	360	2.47	0.0238	360	2.38	0.0289	361	1.44	0.1977
Spp:W:N	6	359	2.54	0.0202	360	0.86	0.5255	360	1.51	0.1730	361	0.85	0.5332
Spp:S:N	6	359	0.77	0.5938	360	2.08	0.0554	360	1.60	0.1467	361	1.73	0.1119
W:S:N	1	48	0.07	0.7914	48	1.57	0.2164	48	0.00	0.9577	48	0.01	0.9210
Spp:W:G	6	359	0.89	0.5021	360	0.53	0.7875	360	0.42	0.8665	361	1.24	0.2842
Spp:S:G	6	359	1.60	0.1464	360	2.21	0.0412	360	0.42	0.8646	361	2.36	0.0298
W:S:G	1	48	0.40	0.5286	48	0.03	0.8602	48	2.40	0.1283	48	0.03	0.8713

Spp:N:G	6	359	0.68	0.6630	360	0.97	0.4449	360	1.14	0.3383	361	0.21	0.9729
W:N:G	1	48	0.01	0.9175	48	1.82	0.1836	48	2.34	0.1325	48	0.15	0.6963
S:N:G	1	48	1.20	0.2786	48	0.00	0.9791	48	0.17	0.6805	48	1.21	0.2764
Spp:W:S:N	6	359	1.07	0.3796	360	0.70	0.6521	360	1.09	0.3702	361	0.68	0.6658
Spp:W:S:G	6	359	1.36	0.2279	360	0.50	0.8064	360	0.79	0.5821	361	0.57	0.7581
Spp:W:N:G	6	359	1.37	0.2247	360	1.27	0.2724	360	1.67	0.1282	361	0.58	0.7482
Spp:S:N:G	6	359	0.52	0.7943	360	0.55	0.7672	360	0.33	0.9229	361	1.22	0.2971
W:S:N:G	1	48	0.43	0.5172	48	2.20	0.1444	48	0.01	0.9183	48	1.78	0.1881
Spp:W:S:N:G	6	359	2.06	0.0571	360	1.16	0.3259	360	0.93	0.4730	361	1.14	0.3404

TABLE 2. Linear mixed model (lme) analysis using maximum likelihood, showing the individual and interactive effects of water, shade, nutrient and grass treatments (two levels each) on *Acacia nigrescens* saplings. Interaction effects of nutrient and grass (sub-plot) within water and shade (whole plot) within replicates (random effects of block units) was considered as split-plot error. We analyzed final-initial relative growth rate of stem length (RGR_L in mm mm⁻¹ week⁻¹), final-initial relative growth rate in stem basal diameter (RGR_D in mm mm⁻¹ week⁻¹), final proportion of leaf to sapling dry biomass (LP), final log₁₀ transformed sapling dry biomass (SDB in mg). The numerator degrees of freedom (df), error df, F-value and significance are indicated per factor and factor interactions. The values in bold are significant using a Bonferroni correction ($p < 0.01$)

Source	<i>Acacia nigrescens</i>												
	df	RGR _L			RGR _D			LP			SDB		
		Err df	F	p	Err df	F	p	Err df	F	p	Err df	F	p
W	1	8	1.290	0.3150	8	0.248	0.6323	8	3.101	0.1163	8	2.319	0.1663
S	1	8	0.858	0.4286	8	0.241	0.6366	8	14.975	0.0047	8	0.744	0.4134
N	1	26	6.878	0.0123	27	7.029	0.0133	27	0.368	0.5491	27	11.017	0.0026
G	1	26	0.014	0.9067	27	11.746	0.0020	27	1.962	0.1727	27	21.150	0.0001
W:S	1	8	0.070	0.7894	8	0.471	0.5119	8	0.400	0.5449	8	2.505	0.1521
W:N	1	26	0.072	0.7703	27	0.162	0.6907	27	2.524	0.1238	27	1.635	0.2119
S:N	1	26	0.002	0.9656	27	1.967	0.1722	27	0.339	0.5654	27	2.087	0.1601
W:G	1	26	3.168	0.0761	27	4.217	0.0498	27	9.896	0.0040	27	3.590	0.0689
S:G	1	26	0.905	0.3502	27	5.236	0.0302	27	0.315	0.5792	27	10.756	0.0029
N:G	1	26	0.483	0.5004	27	0.301	0.5876	27	0.000	0.9848	27	0.270	0.6077
W:S:N	1	26	0.206	0.6541	27	0.591	0.4487	27	1.615	0.2146	27	0.302	0.5872
W:S:G	1	26	1.745	0.1957	27	0.023	0.8805	27	0.729	0.4007	27	1.803	0.1905
W:N:G	1	26	1.276	0.2653	27	0.073	0.7896	27	0.313	0.5806	27	0.002	0.9610
S:N:G	1	26	0.782	0.3700	27	0.275	0.6041	27	1.102	0.3031	27	0.649	0.4277
W:S:N:G	–	–	–	–	–	–	–	–	–	–	–	–	–

List of figures

FIG. 1. Mean (\pm 1SE) relative growth rate of stem length (RGR_L) values of (a) humid and (b) mesic species showing the effects of shade. Negative effects of shade on sapling RGR_L were observed only in *Acacia tortilis* and *Colophospermum mopane* (mesic spp.).

FIG. 2. Mean (\pm 1SE) relative growth rate of stem length (RGR_L) values of (a) humid and (b) mesic species showing the effects of shade and nutrients. The treatment levels of nutrients (N1- nutrient addition and N0 – no nutrients) are represented on the x-axis, for each species. To be read as a two-way interactive effect of shade and nutrients on RGR_L of each species separately. Positive effects of nutrient addition on sapling RGR_L were observed only in the presence of natural light (no shade).

FIG. 3. Mean (\pm 1SE) relative growth rate of stem length (RGR_L) values of (a) humid and (b) mesic species showing the effects of shade and grass. The treatment levels of grass (G1- grass competition and G0 – no grass) are represented on the x-axis, for each species. To be read as a two-way interactive effect of shade and grass on RGR_L of each species separately. Negative effects of grass competition on sapling RGR_L were observed only in the presence of natural light (no shade), except for *Combretum apiculatum* (mesic sp.) and *Strychnos spinosa* (humid sp.).

FIG. 4. Mean (\pm 1SE) relative growth rate of stem basal diameter (RGR_D) values of (a) humid and (b) mesic species showing the effects of shade. Shade had a negative effect on sapling RGR_D of all species, except for *Strychnos spinosa* (humid sp.).

FIG. 5. Mean (\pm 1SE) relative growth rate stem basal diameter (RGR_D) values of (a) humid and (b) mesic species showing the effects of shade and nutrients. The treatment levels of nutrients (N1- nutrient addition and N0 – no nutrients) are represented on the x-axis for each species. To be read as a two-way interactive effect of shade and nutrients on RGR_D of each species separately. Positive effects of nutrient addition on sapling RGR_D were observed only in the presence of natural light (no shade), except for *Strychnos spinosa* (humid sp.).

FIG. 6. Mean (\pm 1SE) relative growth rate of stem basal diameter (RGR_D) values of (a) humid and (b) mesic species showing the effects of shade and grass. The treatment levels of grass (G1- grass competition and G0 – no grass) are represented on the x-axis, for each species. To be read as a two-way interactive effect of shade and grass on RGR_D of each species separately. Negative effects of grass competition on sapling RGR_D were observed only in the presence of natural light (no shade).

FIG. 7. The \log_{10} mean (\pm 1SE) sapling dry biomass (SDB) values (mg) of (a) humid and (b) mesic species showing the effects of shade. Shade had a negative effect on SDB of all species, except *Acacia karroo* and *Strychnos spinosa* (humid spp.).

FIG. 8. The \log_{10} mean (\pm 1SE) sapling dry biomass (SDB) values (mg) of (a) humid and (b) mesic species showing the effects of shade and nutrients. The treatment levels of nutrients (N1- nutrient addition and N0 – no nutrients) are represented on the x-axis, for each species. To be read as a two-way interactive effect of shade and nutrients on SDB of each species separately. Positive effects of nutrient addition on SDB were observed only in the presence of natural light (no shade), except for *Strychnos spinosa* (humid sp.).

FIG. 9. The \log_{10} mean (± 1 SE) sapling dry biomass (SDB) values (mg) of (a) humid and (b) mesic species showing the effects of shade and grass. The treatment levels of grass (G1- grass competition and G0 – no grass) are represented on the x-axis, for each species. To be read as a two-way interactive effect of shade and grass on SDB of each species separately. Negative effects of grass competition on SDB were observed only in the presence of natural light (no shade).

FIG. 10. Mean (± 1 SE) leaf proportion (ratio of leaf dry biomass to total sapling dry biomass) values of (a) humid and (b) mesic species showing the effects of shade and grass. The treatment levels of grass (G1- grass competition and G0 – no grass) are represented on the x-axis, for each species. To be read as a two-way interactive effect of shade and grass on leaf proportion of each species separately. Negative effects of grass competition on sapling leaf proportion were observed only in the presence of natural light (no shade), except for *Strychnos spinosa* (humid sp.).

FIG. 11. Mean (± 1 SE) sapling dry biomass (SDB) values of *Acacia nigrescens*, showing the effects of shade and grass. Negative effects of grass competition on SDB were observed only in the presence of natural light (no shade).

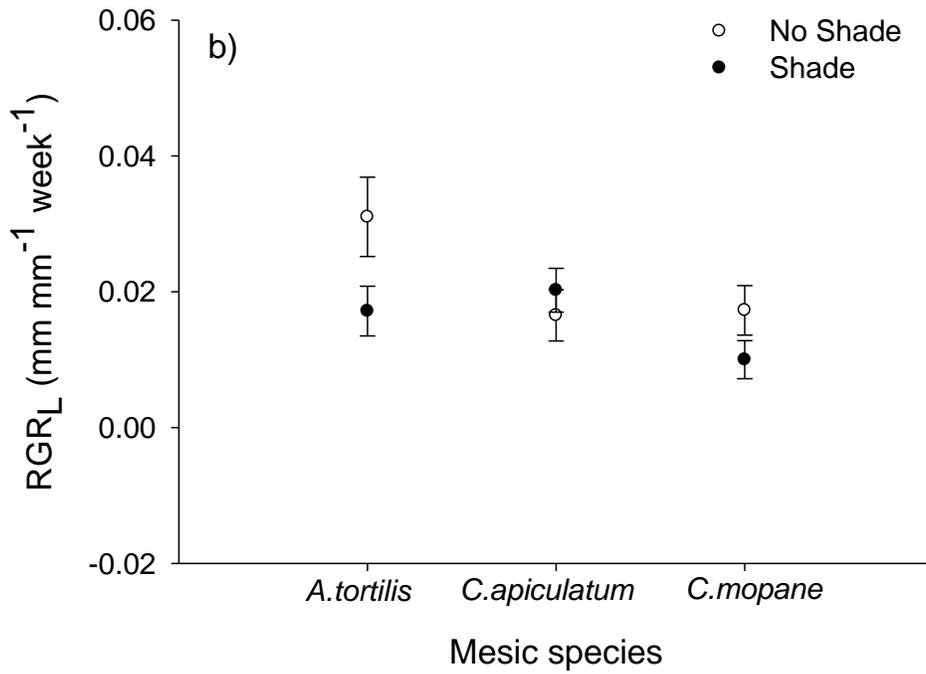
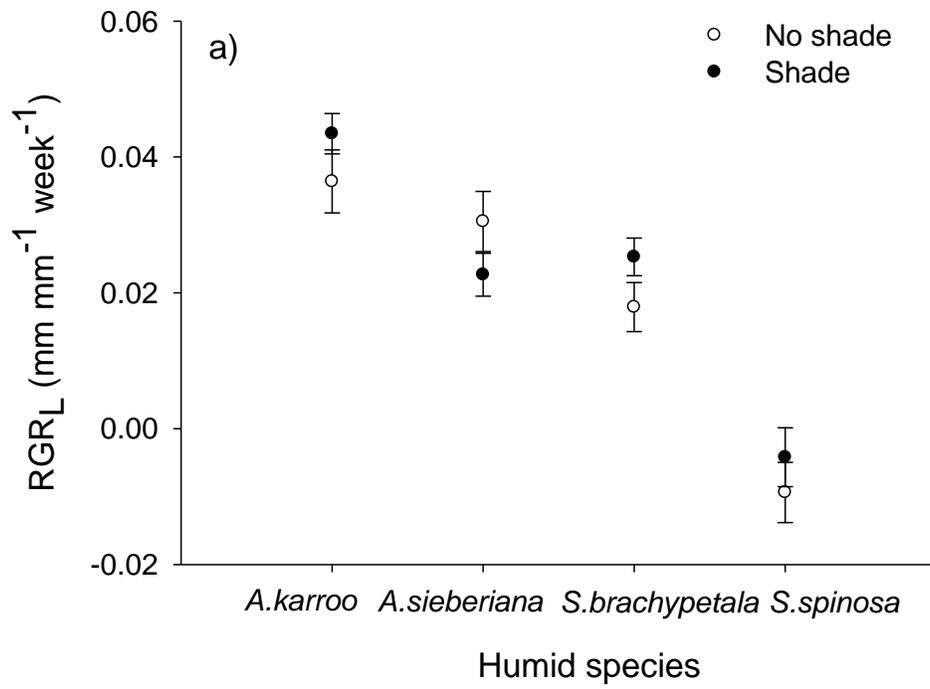


FIG. 1.

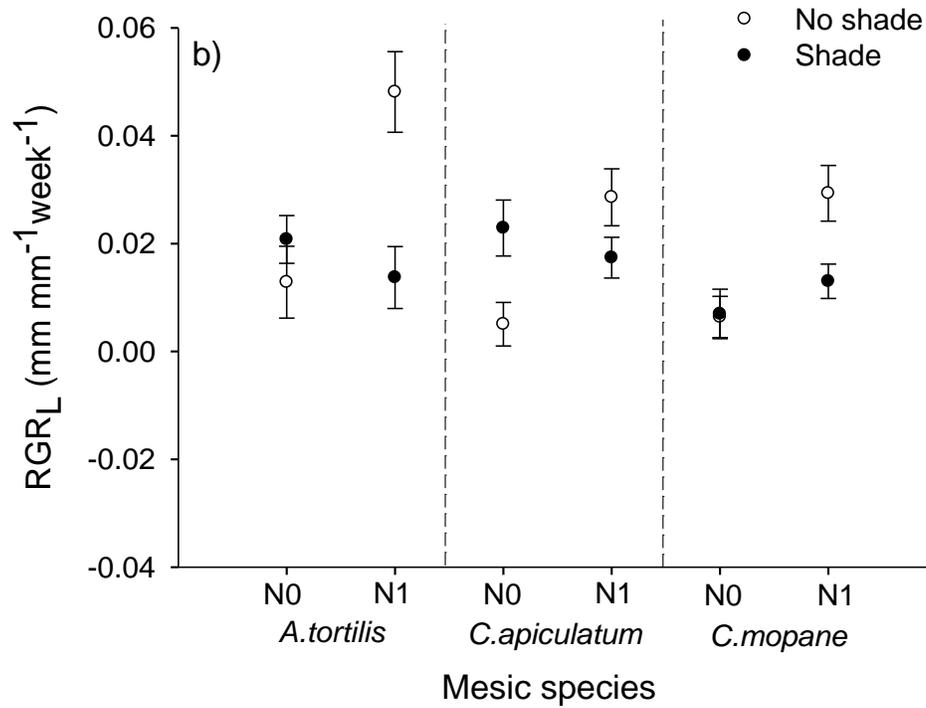
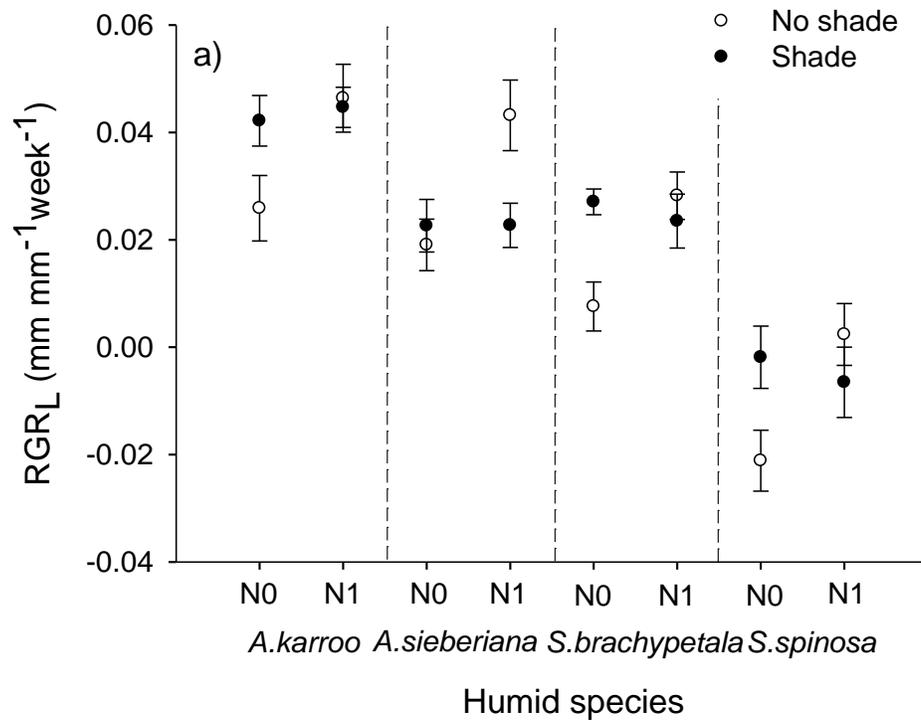


FIG. 2.

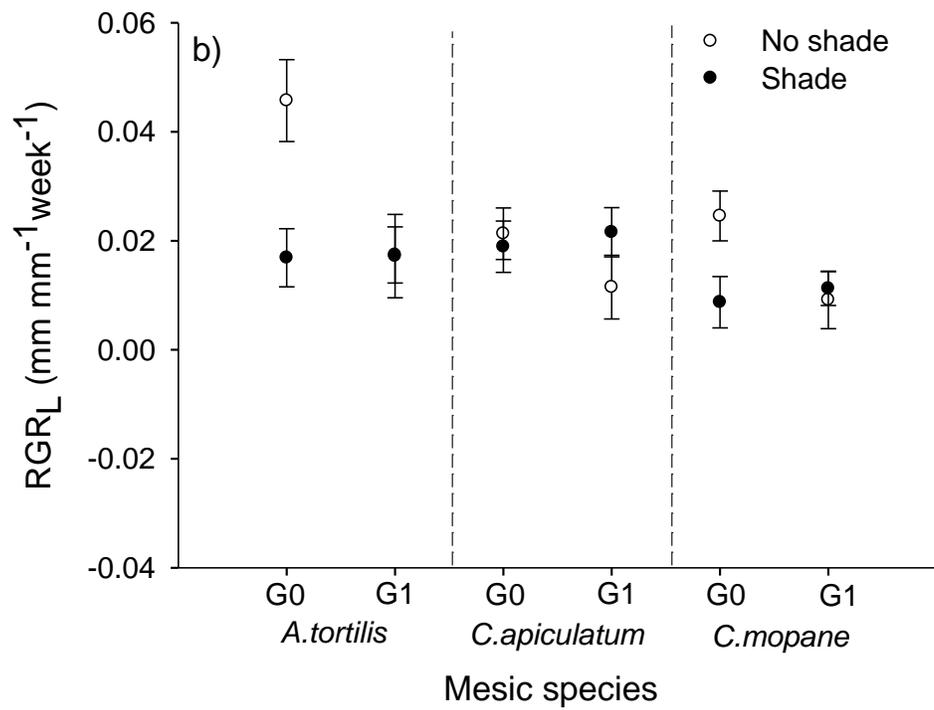
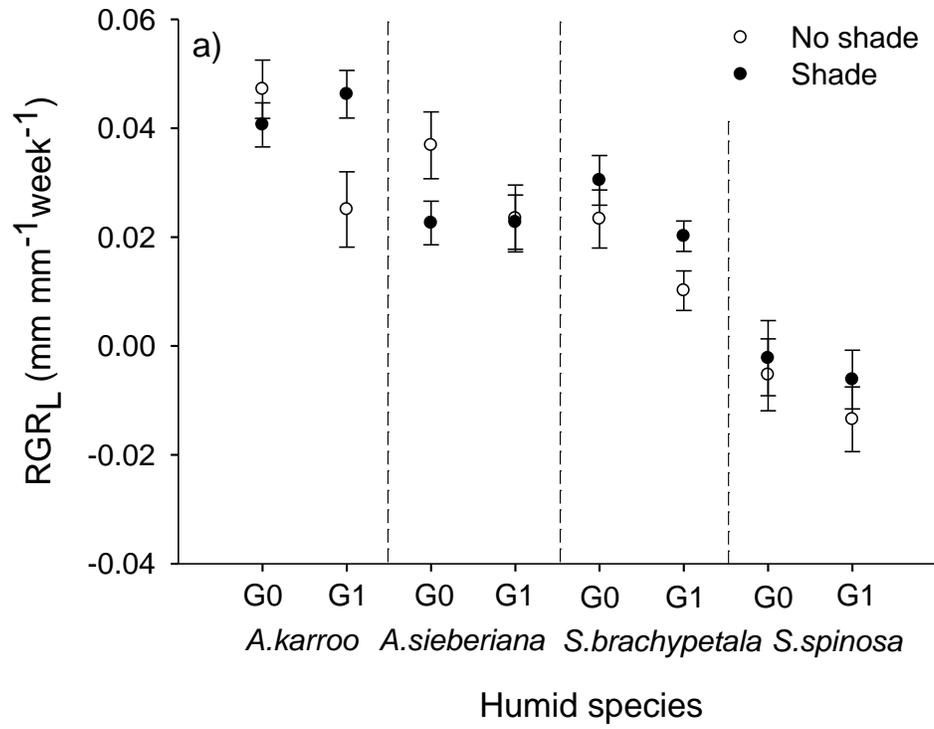


FIG. 3.

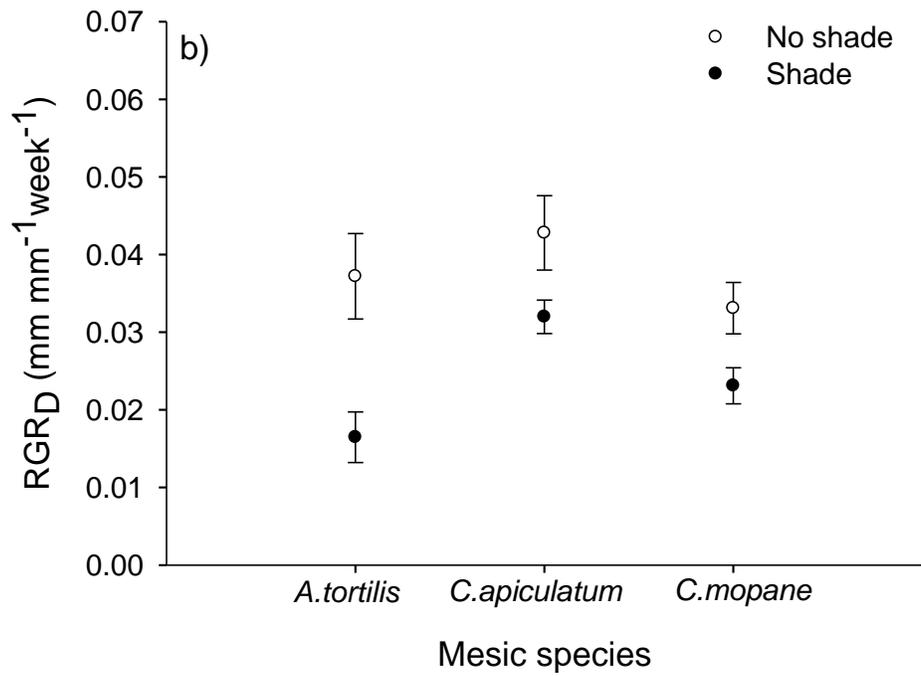
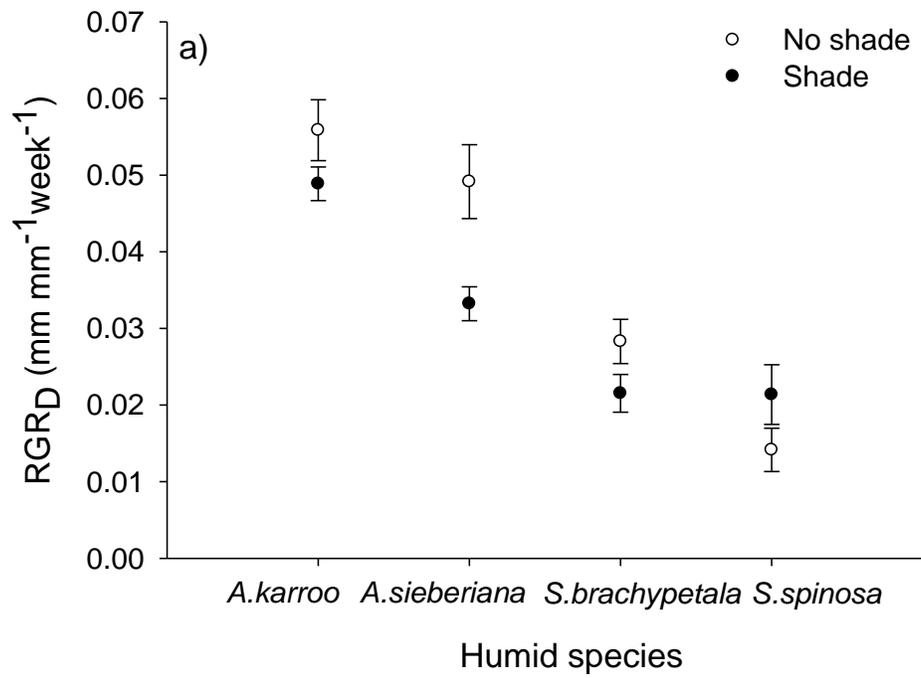


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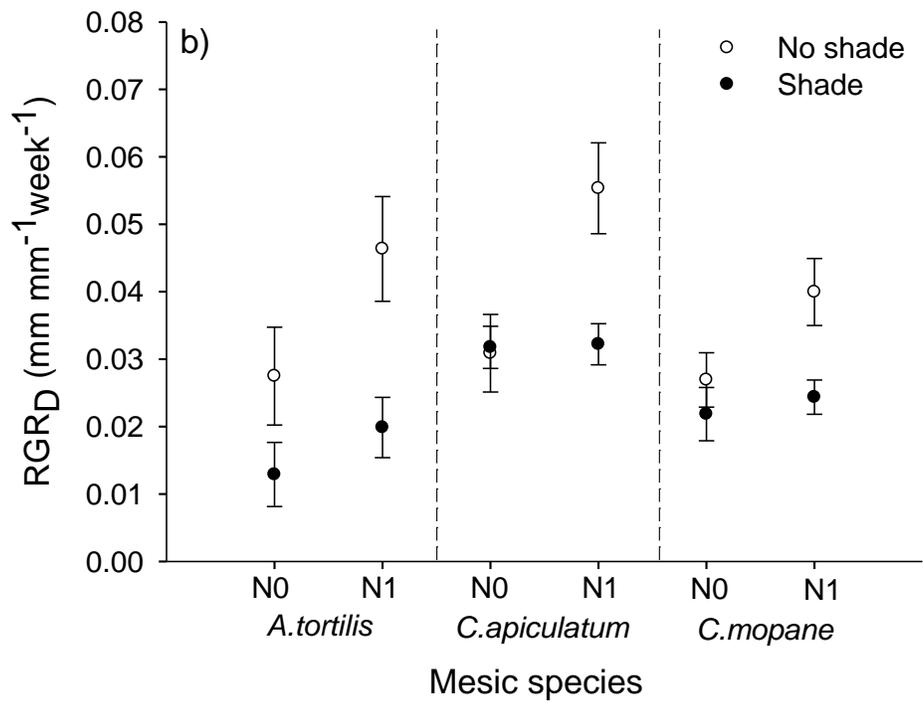
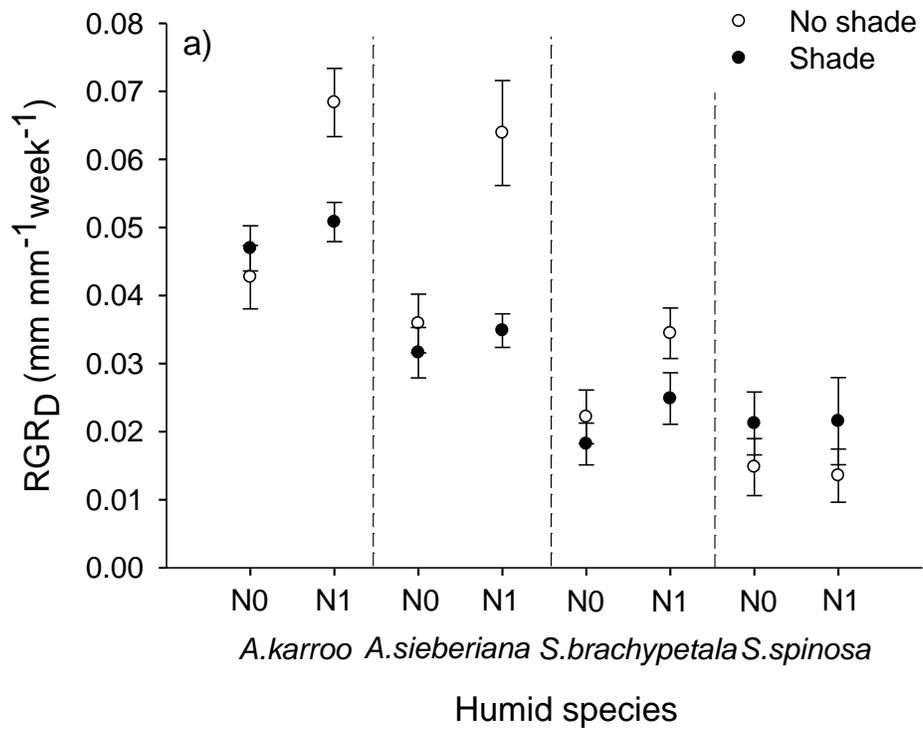


FIG. 5.

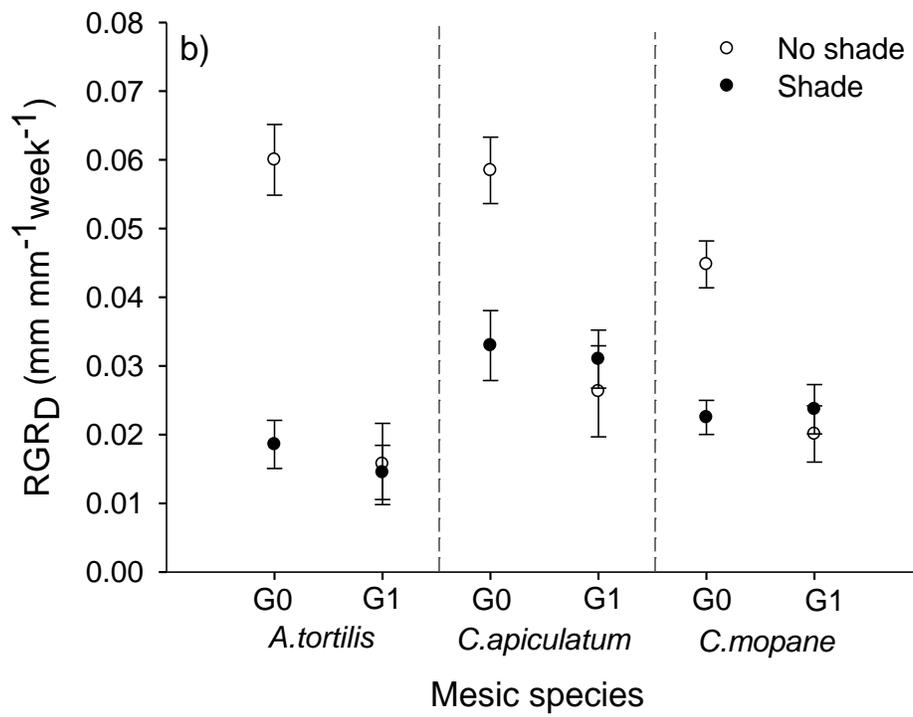
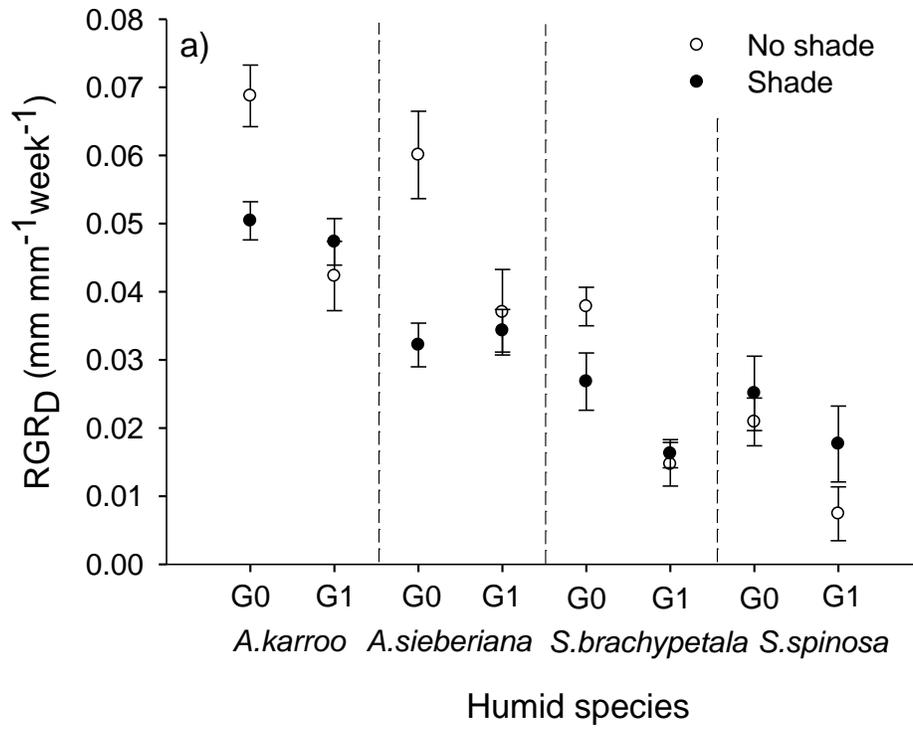


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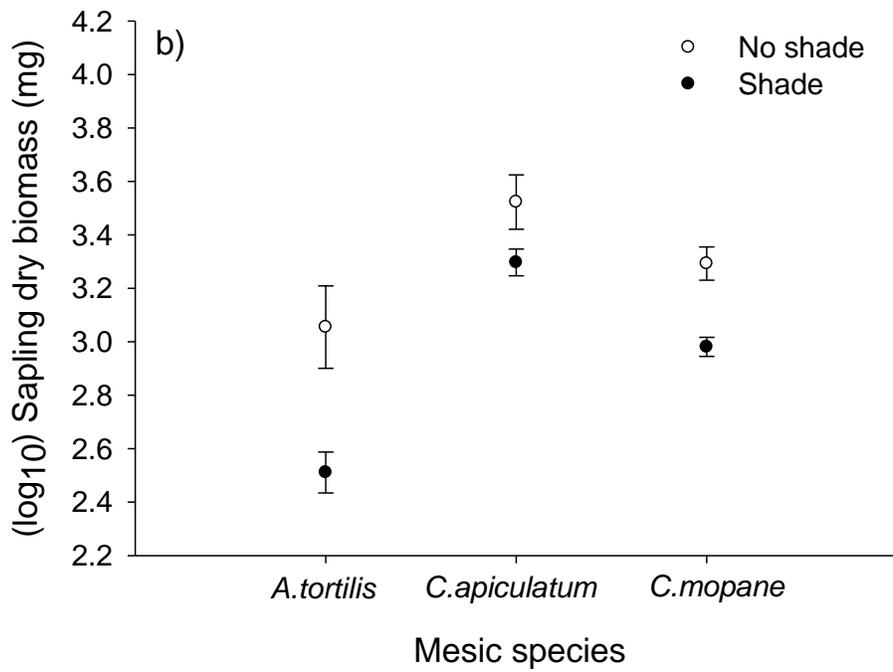
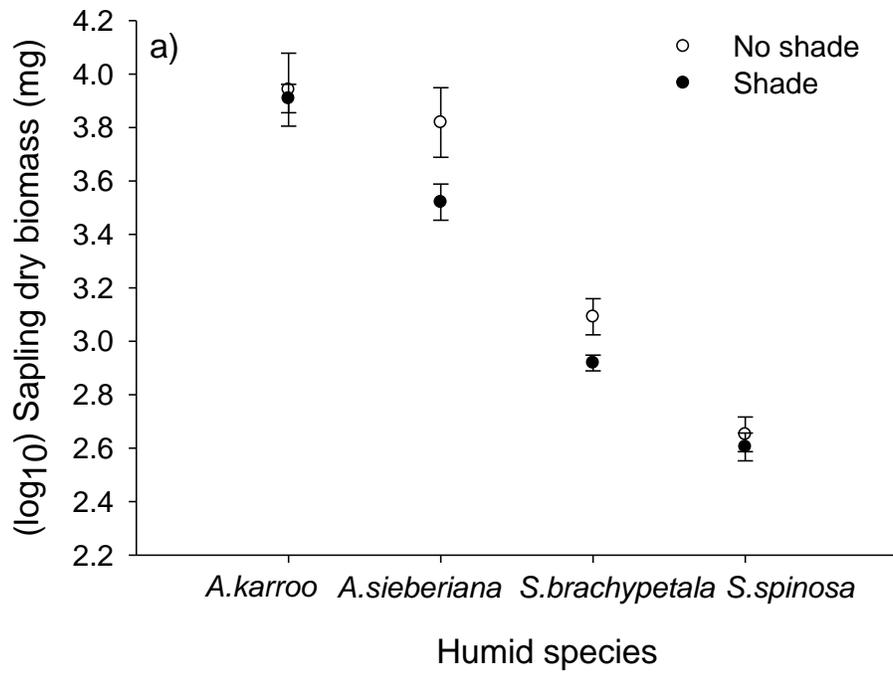


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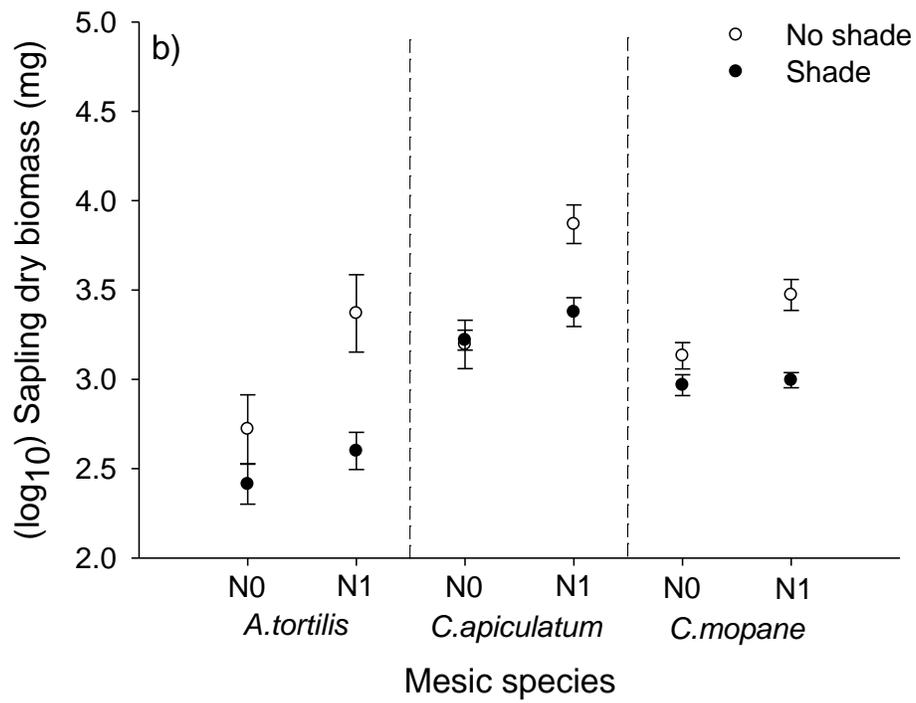
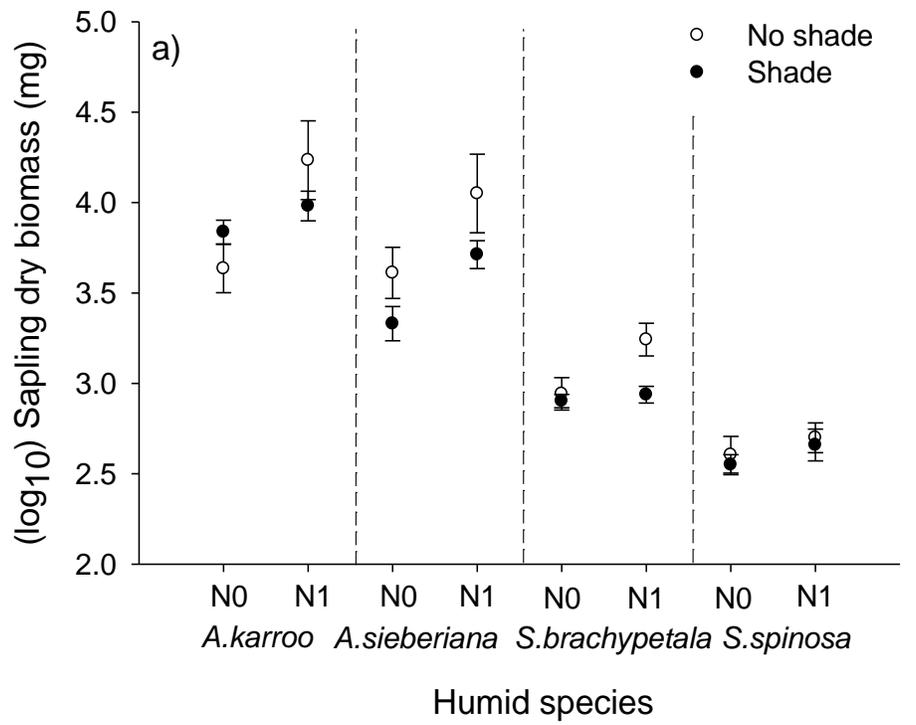


FIG. 8.

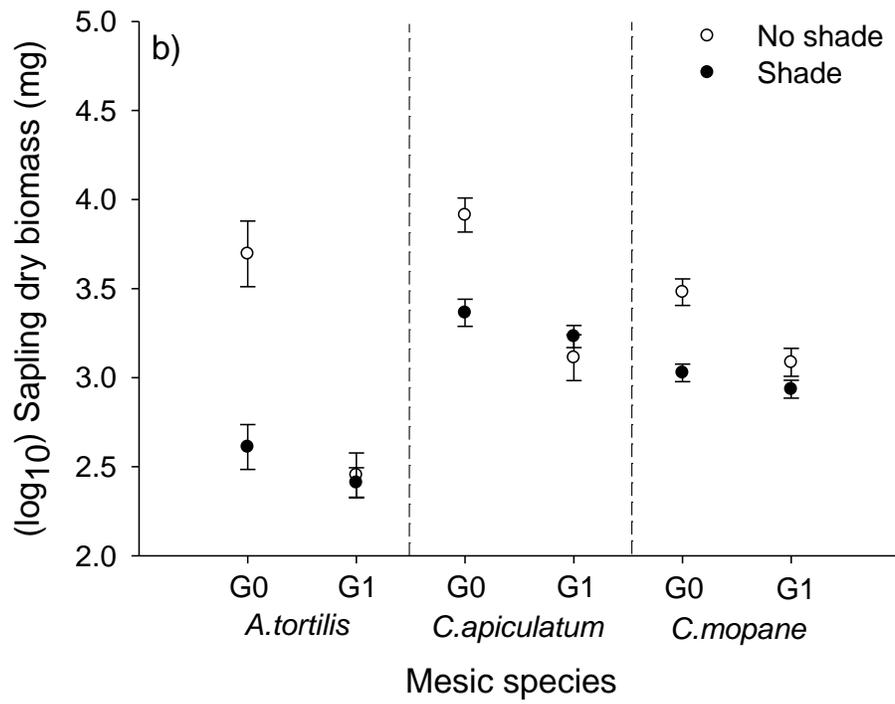
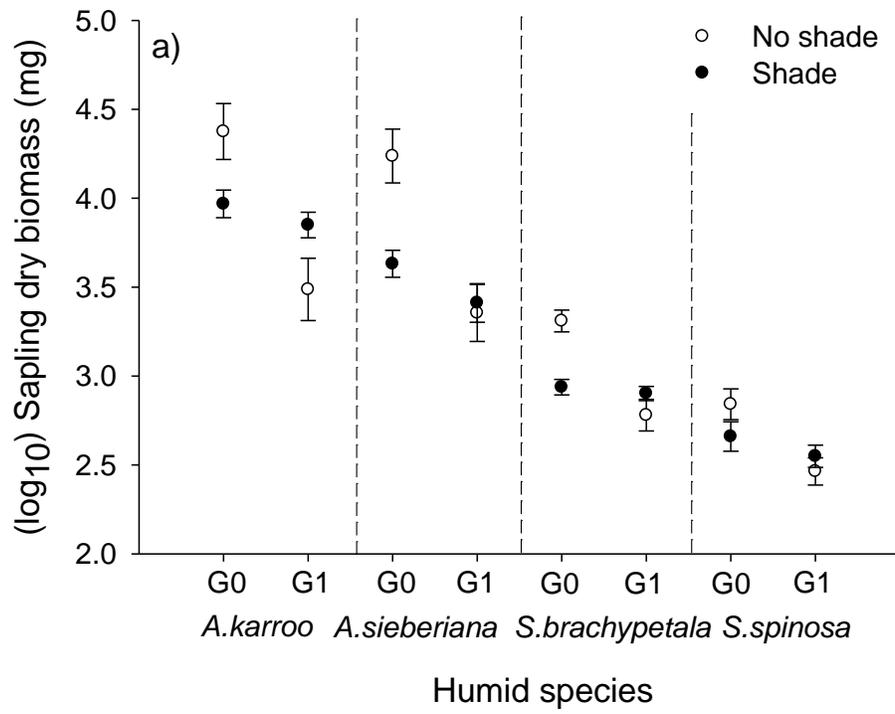


FIG. 9.

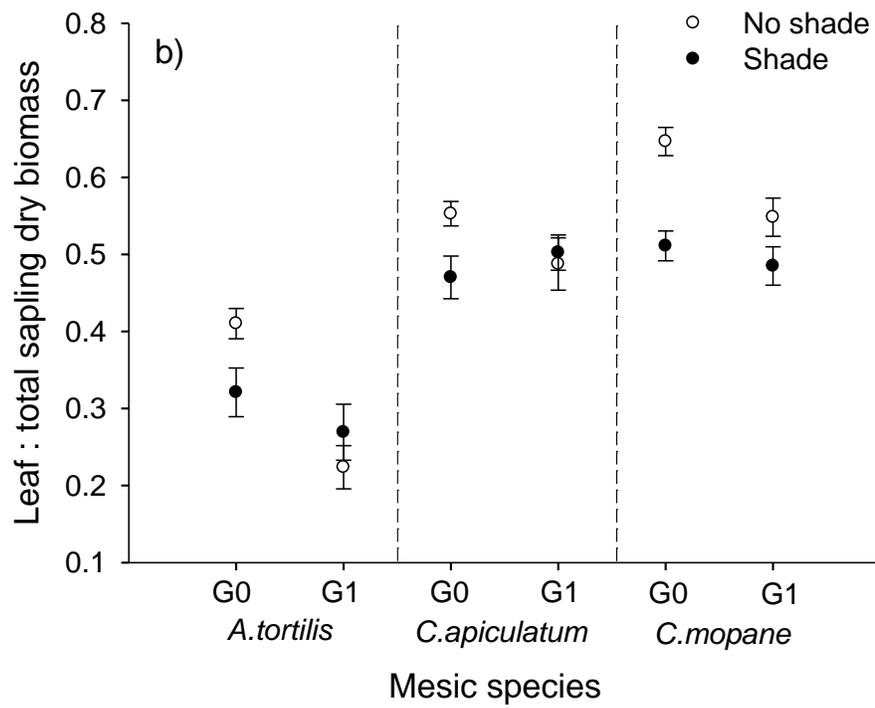
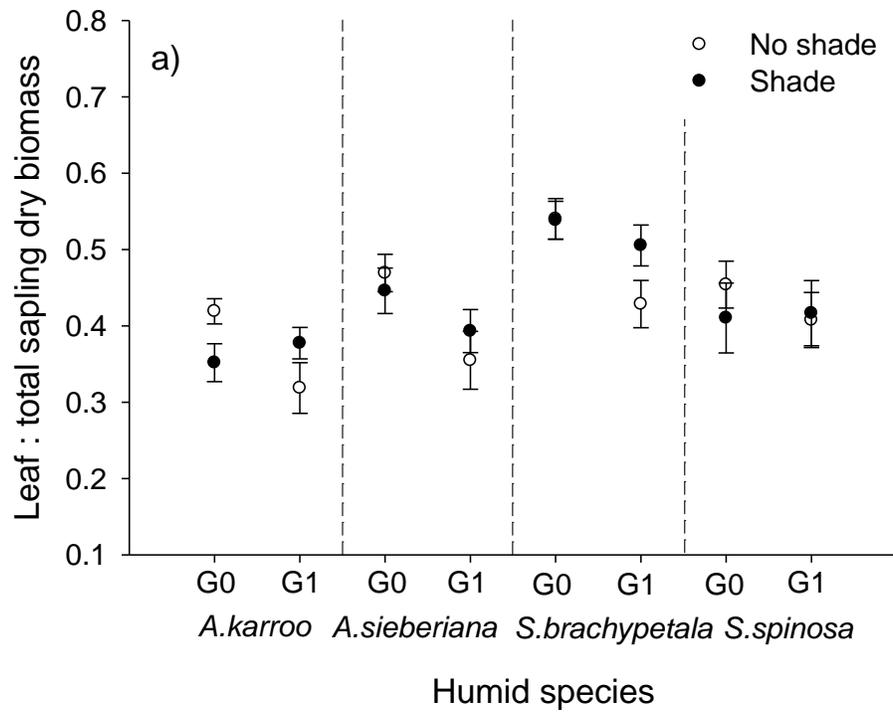


FIG. 10.

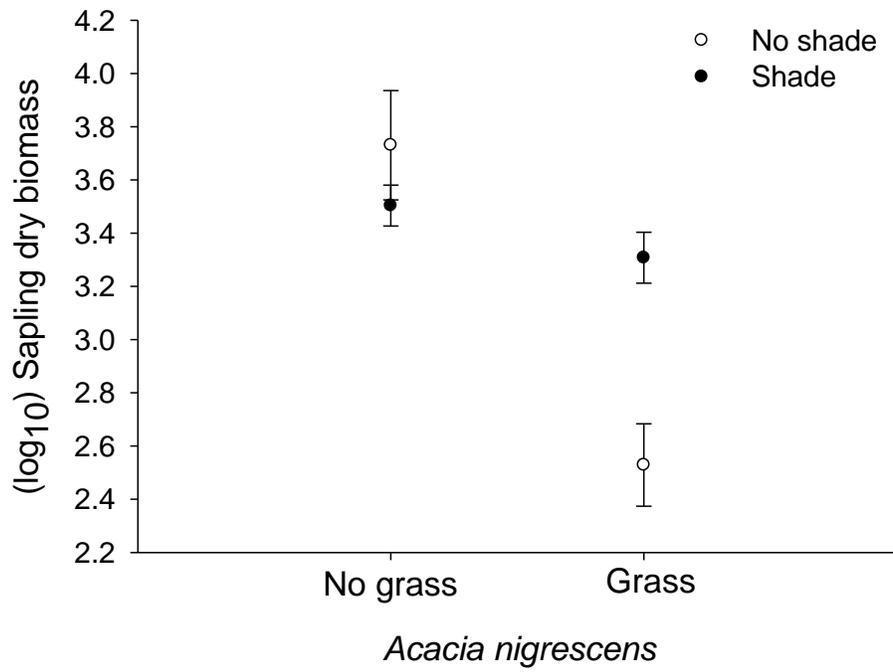


FIG. 11.

SUPPLEMENTAL MATERIAL

Appendix A

Table A1-A7 Results of linear mixed model analysis for each species

TABLE A1. Linear mixed model (lme) analysis using maximum likelihood, showing the individual and interactive effects of water, shade, nutrient and grass treatments (two levels each) on *Acacia karroo* saplings. Interaction effects of nutrient and grass (sub-plot) within water and shade (whole plot) within replicates (random effects of block units) was considered as split-plot error. We analyzed final-initial relative growth rate of stem length (RGR_L in mm mm⁻¹ week⁻¹), final-initial relative growth rate in stem basal diameter (RGR_D in mm mm⁻¹ week⁻¹), final proportion of leaf to sapling dry biomass (LP), final log₁₀ transformed sapling dry biomass (SDB in mg). The numerator degrees of freedom (df), error df, F-value and significance are indicated per factor and factor interactions. The values in bold are significant, using a Bonferroni correction ($p < 0.01$)

Source	<i>Acacia karroo</i>												
	df	RGR _L			RGR _D			LP			SDB		
		Err df	F	p	Err df	F	p	Err df	F	p	Err df	F	p
W	1	12	1.441	0.2531	12	0.924	0.3554	12	0.011	0.9182	12	0.014	0.9072
S	1	12	1.533	0.2393	12	2.961	0.1109	12	0.053	0.8216	12	0.116	0.7395
N	1	47	6.341	0.0153	47	21.587	<.0001	47	0.572	0.4533	47	9.969	0.0028
G	1	47	3.302	0.0756	47	22.215	<.0001	47	2.687	0.1079	47	18.811	0.0001
W:S	1	12	0.293	0.5982	12	0.559	0.4689	12	0.001	0.9802	12	0.133	0.7213
W:N	1	47	0.178	0.6746	47	0.542	0.4652	47	0.339	0.5632	47	0.219	0.6423
S:N	1	47	4.076	0.0492	47	12.436	0.001	47	0.107	0.7445	47	3.955	0.0526
W:G	1	47	0.564	0.4562	47	1.085	0.3028	47	4.160	0.0470	47	0.535	0.4683
S:G	1	47	9.922	0.0028	47	14.824	0.0004	47	8.269	0.0060	47	11.504	0.0014
N:G	1	47	0.126	0.7244	47	0.060	0.8082	47	8.531	0.0054	47	0.150	0.7006
W:S:N	1	47	1.544	0.2202	47	0.080	0.7792	47	0.784	0.3803	47	0.254	0.6166
W:S:G	1	47	4.543	0.0383	47	0.013	0.9086	47	0.220	0.6414	47	0.334	0.5658
W:N:G	1	47	0.106	0.7461	47	0.003	0.9552	47	7.524	0.0086	47	0.032	0.8592
S:N:G	1	47	1.751	0.1922	47	0.082	0.7765	47	0.901	0.3473	47	1.305	0.2591
W:S:N:G	1	47	5.465	0.0237	47	0.603	0.4412	47	0.080	0.7791	47	1.477	0.2304

TABLE A2. Linear mixed model (lme) analysis using maximum likelihood, showing the individual and interactive effects of water, shade, nutrient and grass treatments (two levels each) on *Acacia sieberiana* saplings. Interaction effects of nutrient and grass (sub-plot) within water and shade (whole plot) within replicates (random effects of block units) was considered as split-plot error. We analyzed final-initial relative growth rate of stem length (RGR_L in $mm\ mm^{-1}\ week^{-1}$), final-initial relative growth rate in stem basal diameter (RGR_D in $mm\ mm^{-1}\ week^{-1}$), final proportion of leaf to sapling dry biomass (LP), final \log_{10} transformed sapling dry biomass (SDB in mg). The numerator degrees of freedom (df), error df, F-value and significance are indicated per factor and factor interactions. The values in bold are significant, using a Bonferroni correction ($p < 0.01$)

Source	<i>Acacia sieberiana</i>												
	df	RGR_L			RGR_D			LP			SDB		
		Err df	F	p	Err df	F	p	Err df	F	p	Err df	F	p
W	1	12	4.559	0.0541	12	8.050	0.0150	12	2.377	0.1491	12	4.772	0.0495
S	1	12	1.664	0.2214	12	14.528	0.0025	12	0.005	0.9479	12	5.201	0.0416
N	1	46	5.728	0.0208	46	13.378	0.0007	45	3.564	0.0655	46	15.600	0.0003
G	1	46	1.872	0.1779	46	6.047	0.0178	45	9.281	0.0039	46	27.152	<.0001
W:S	1	12	0.791	0.3914	12	6.075	0.0298	12	11.095	0.0060	12	3.570	0.0832
W:N	1	46	0.019	0.8910	46	1.389	0.2446	45	0.398	0.5316	46	1.223	0.2745
S:N	1	46	5.709	0.0210	46	7.660	0.0081	45	0.703	0.4062	46	0.000	0.9980
W:G	1	46	0.212	0.6477	46	1.026	0.3165	45	0.0003	0.9857	46	3.809	0.0571
S:G	1	46	1.875	0.1775	46	9.321	0.0038	45	1.400	0.2430	46	10.177	0.0026
N:G	1	46	0.119	0.7319	46	2.381	0.1297	45	0.007	0.9357	46	0.043	0.8369
W:S:N	1	46	2.596	0.114	46	3.472	0.0688	45	0.977	0.3282	46	1.429	0.2380
W:S:G	1	46	0.0001	0.9941	46	0.295	0.5894	45	0.003	0.9548	46	1.106	0.2985
W:N:G	1	46	0.141	0.7088	46	0.152	0.6983	45	0.157	0.6937	46	0.005	0.9450
S:N:G	1	46	0.068	0.7961	46	0.289	0.5932	45	0.014	0.9064	46	3.560	0.0655
W:S:N:G	1	46	2.030	0.1610	46	1.544	0.2203	45	1.422	0.2393	46	1.533	0.2220

TABLE A3. Linear mixed model (lme) analysis using maximum likelihood, showing the individual and interactive effects of water, shade, nutrient and grass treatments (two levels each) on *Schotia brachypetala* saplings. Interaction effects of nutrient and grass (sub-plot) within water and shade (whole plot) within replicates (random effects of block units) was considered as split-plot error. We analyzed final-initial relative growth rate of stem length (RGR_L in mm mm⁻¹ week⁻¹), final-initial relative growth rate in stem basal diameter (RGR_D in mm mm⁻¹ week⁻¹), final proportion of leaf to sapling dry biomass (LP), final log₁₀ transformed sapling dry biomass (SDB in mg). The numerator degrees of freedom (df), error df, F-value and significance are indicated per factor and factor interactions. The values in bold are significant, using a Bonferroni correction (p < 0.01)

Source	<i>Schotia brachypetala</i>												
	df	RGR _L			RGR _D			LP			SDB		
		Err df	F	p	Err df	F	p	Err df	F	p	Err df	F	p
W	1	12	0.721	0.4125	12	0.575	0.4629	12	0.934	0.3530	12	0.001	0.9803
S	1	12	3.664	0.0797	12	5.935	0.0314	12	1.049	0.3259	12	9.678	0.0090
N	1	42	3.537	0.0670	42	11.798	0.0013	42	2.805	0.1014	42	9.429	0.0037
G	1	42	8.831	0.0049	42	35.885	<.0001	42	8.896	0.0047	42	23.513	<.0001
W:S	1	12	1.777	0.2073	12	1.307	0.2752	12	4.844	0.0481	12	3.078	0.1049
W:N	1	42	3.062	0.0874	42	0.073	0.7891	42	4.238	0.0458	42	0.006	0.9408
S:N	1	42	8.199	0.0065	42	1.214	0.2769	42	8.688	0.0052	42	6.771	0.0127
W:G	1	42	0.758	0.3890	42	1.960	0.1689	42	0.819	0.3707	42	3.243	0.0789
S:G	1	42	0.049	0.8256	42	4.480	0.0403	42	2.823	0.1003	42	18.450	0.0001
N:G	1	42	0.596	0.4445	42	0.194	0.6617	42	1.014	0.3197	42	0.002	0.9676
W:S:N	1	42	0.355	0.5548	42	0.003	0.9590	42	2.710	0.1072	42	1.070	0.3069
W:S:G	1	42	0.298	0.5883	42	0.705	0.4058	42	2.146	0.1504	42	0.032	0.8588
W:N:G	1	42	0.325	0.5716	42	0.007	0.9319	42	0.739	0.3947	42	0.001	0.9748
S:N:G	1	42	1.038	0.3140	42	2.157	0.1494	42	1.333	0.2548	42	0.001	0.9816
W:S:N:G	1	42	0.655	0.4229	42	0.082	0.7761	42	0.447	0.5072	42	0.627	0.4330

TABLE A4. Linear mixed model (lme) analysis using maximum likelihood, showing the individual and interactive effects of water, shade, nutrient and grass treatments (two levels each) on *Strychnos spinosa* saplings. Interaction effects of nutrient and grass (sub-plot) within water and shade (whole plot) within replicates (random effects of block units) was considered as split-plot error. We analyzed final-initial relative growth rate of stem length (RGR_L in mm mm⁻¹ week⁻¹), final-initial relative growth rate in stem basal diameter (RGR_D in mm mm⁻¹ week⁻¹), final proportion of leaf to sapling dry biomass (LP), final log₁₀ transformed sapling dry biomass (SDB in mg). The numerator degrees of freedom (df), error df, F-value and significance are indicated per factor and factor interactions. The values in bold are significant, using a Bonferroni correction (p < 0.01)

Source	<i>Strychnos spinosa</i>												
	df	RGR _L			RGR _D			LP			SDB		
		Err df	F	p	Err df	F	p	Err df	F	p	Err df	F	p
W	1	12	11.955	0.0047	12	0.205	0.6591	12	2.295	0.1557	12	4.572	0.0538
S	1	12	1.082	0.3188	12	1.023	0.3317	12	0.081	0.7808	12	0.256	0.6217
N	1	46	2.713	0.1064	46	0.001	0.9793	46	0.709	0.4041	46	2.102	0.1539
G	1	46	1.158	0.2875	46	9.655	0.0032	46	0.408	0.5263	46	10.765	0.0020
W:S	1	12	1.063	0.3230	12	2.212	0.1627	12	0.023	0.8828	12	0.151	0.7040
W:N	1	46	5.493	0.0235	46	0.062	0.8041	46	1.466	0.2322	46	3.069	0.0865
S:N	1	46	6.150	0.0169	46	0.038	0.8461	46	2.849	0.0982	46	0.045	0.8327
W:G	1	46	0.183	0.6710	46	0.246	0.6223	46	0.005	0.9440	46	0.450	0.5059
S:G	1	46	0.138	0.7121	46	0.656	0.4223	46	0.727	0.3982	46	3.357	0.0734
N:G	1	46	0.705	0.4055	46	0.501	0.4825	46	0.615	0.4369	46	0.144	0.7061
W:S:N	1	46	0.439	0.5108	46	0.005	0.9466	46	0.003	0.9549	46	0.627	0.4326
W:S:G	1	46	0.019	0.8924	46	1.386	0.2451	46	1.935	0.1709	46	1.165	0.2861
W:N:G	1	46	3.999	0.0515	46	0.358	0.5524	46	2.863	0.0974	46	1.491	0.2283
S:N:G	1	46	0.145	0.7049	46	0.477	0.4934	46	0.056	0.8141	46	0.085	0.7724
W:S:N:G	1	46	0.109	0.7429	46	0.226	0.6368	46	1.483	0.2296	46	0.697	0.4082

TABLE A5. Linear mixed model (lme) analysis using maximum likelihood, showing the individual and interactive effects of water, shade, nutrient and grass treatments (two levels each) on *Acacia tortilis* saplings. Interaction effects of nutrient and grass (sub-plot) within water and shade (whole plot) within replicates (random effects of block units) was considered as split-plot error. We analyzed final-initial relative growth rate of stem length (RGR_L in mm mm⁻¹ week⁻¹), final-initial relative growth rate in stem basal diameter (RGR_D in mm mm⁻¹ week⁻¹), final proportion of leaf to sapling dry biomass (LP), final log₁₀ transformed sapling dry biomass (SDB in mg). The numerator degrees of freedom (df), error df, F-value and significance are indicated per factor and factor interactions. The values in bold are significant, using a Bonferroni correction (p < 0.01)

Source	<i>Acacia tortilis</i>												
	df	RGR _L			RGR _D			LP			SDB		
		Err df	F	p	Err df	F	p	Err df	F	p	Err df	F	p
W	1	12	7.484	0.0181	12	10.809	0.0065	12	1.435	0.2540	12	9.660	0.0091
S	1	12	5.770	0.0334	12	20.362	0.0007	12	0.357	0.5615	12	19.151	0.0009
N	1	38	5.739	0.0216	38	8.031	0.0073	39	0.016	0.8997	39	10.301	0.0027
G	1	38	5.125	0.0294	38	26.704	<.0001	39	14.766	0.0004	39	30.081	<.0001
W:S	1	12	0.707	0.4168	12	0.318	0.5835	12	2.895	0.1146	12	1.107	0.3135
W:N	1	38	1.428	0.2396	38	1.433	0.2388	39	2.912	0.0959	39	0.066	0.7983
S:N	1	38	14.551	0.0005	38	1.454	0.2354	39	0.004	0.9516	39	3.478	0.0697
W:G	1	38	2.292	0.1384	38	0.031	0.8604	39	0.437	0.5127	39	0.432	0.5151
S:G	1	38	8.568	0.0057	38	20.610	0.0001	39	6.175	0.0174	39	18.030	0.0001
N:G	1	38	0.192	0.6634	38	0.250	0.6203	39	2.186	0.1473	39	0.605	0.4414
W:S:N	1	38	0.362	0.5511	38	1.822	0.1851	39	2.088	0.1564	39	0.008	0.9278
W:S:G	1	38	0.524	0.4736	38	0.123	0.7278	39	0.000	0.9981	39	0.234	0.6316
W:N:G	1	38	0.638	0.4294	38	0.006	0.9407	39	1.778	0.1901	39	0.168	0.6846
S:N:G	1	38	0.102	0.7514	38	0.551	0.4623	39	0.070	0.7924	39	0.224	0.6387
W:S:N:G	1	38	0.021	0.8863	38	5.451	0.0249	39	0.411	0.5252	39	1.665	0.2046

TABLE A6. Linear mixed model (lme) analysis using maximum likelihood, showing the individual and interactive effects of water, shade, nutrient and grass treatments (two levels each) on *Colophospermum mopane* saplings. Interaction effects of nutrient and grass (sub-plot) within water and shade (whole plot) within replicates (random effects of block units) was considered as split-plot error. We analyzed final-initial relative growth rate of stem length (RGR_L in $mm\ mm^{-1}\ week^{-1}$), final-initial relative growth rate in stem basal diameter (RGR_D in $mm\ mm^{-1}\ week^{-1}$), final proportion of leaf to sapling dry biomass (LP), final \log_{10} transformed sapling dry biomass (SDB in mg). The numerator degrees of freedom (df), error df, F-value and significance are indicated per factor and factor interactions. The values in bold are significant, using a Bonferroni correction ($p < 0.01$)

Source	<i>Colophospermum mopane</i>												
	df	RGR_L			RGR_D			LP			SDB		
		Err df	F	p	Err df	F	p	Err df	F	p	Err df	F	p
W	1	12	0.020	0.8894	12	0.325	0.5794	12	1.781	0.2068	12	0.394	0.5422
S	1	12	3.137	0.1019	12	4.923	0.0465	12	18.894	0.0010	12	21.241	0.0006
N	1	46	13.942	0.0005	46	6.333	0.0154	46	2.040	0.1599	46	8.953	0.0044
G	1	46	2.562	0.1163	46	13.530	0.0006	46	9.108	0.0041	46	15.776	0.0002
W:S	1	12	0.137	0.7181	12	0.162	0.694	12	1.914	0.1917	12	0.161	0.6953
W:N	1	46	2.457	0.1238	46	1.335	0.2539	46	0.179	0.6746	46	0.140	0.7097
S:N	1	46	4.860	0.0325	46	2.740	0.1047	46	0.257	0.6149	46	6.100	0.0173
W:G	1	46	0.436	0.5124	46	2.081	0.1559	46	2.593	0.1142	46	0.380	0.5406
S:G	1	46	4.765	0.0342	46	16.597	0.0002	46	3.301	0.0757	46	5.779	0.0203
N:G	1	46	3.543	0.0661	46	0.550	0.4619	46	0.246	0.6224	46	0.172	0.6807
W:S:N	1	46	0.025	0.8744	46	0.001	0.9787	46	0.174	0.6786	46	0.158	0.6929
W:S:G	1	46	3.471	0.0689	46	1.285	0.2628	46	4.466	0.0400	46	0.346	0.5595
W:N:G	1	46	0.007	0.9360	46	4.570	0.0379	46	0.939	0.3375	46	0.405	0.5276
S:N:G	1	46	0.226	0.6367	46	0.309	0.5810	46	0.280	0.5994	46	0.003	0.9590
W:S:N:G	1	46	3.883	0.0548	46	0.198	0.6586	46	0.279	0.5997	46	1.890	0.1759

TABLE A7. Linear mixed model (lme) analysis using maximum likelihood, showing the individual and interactive effects of water, shade, nutrient and grass treatments (two levels each) on *Combretum apiculatum* saplings. Interaction effects of nutrient and grass (sub-plot) within water and shade (whole plot) within replicates (random effects of block units) was considered as split-plot error. We analyzed final-initial relative growth rate of stem length (RGR_L in mm mm⁻¹ week⁻¹), final-initial relative growth rate in stem basal diameter (RGR_D in mm mm⁻¹ week⁻¹), final proportion of leaf to sapling dry biomass (LP), final log₁₀ transformed sapling dry biomass (SDB in mg). The numerator degrees of freedom (df), error df, F-value and significance are indicated per factor and factor interactions. The values in bold are significant, using a Bonferroni correction (p < 0.01)

Source	<i>Combretum apiculatum</i>												
	df	RGR _L			RGR _D			LP			SDB		
		Err df	F	p	Err df	F	p	Err df	F	p	Err df	F	p
W	1	12	0.005	0.9447	12	1.308	0.2751	12	0.370	0.5542	12	0.402	0.5380
S	1	12	0.510	0.4888	12	4.163	0.064	12	1.115	0.3118	12	4.039	0.0675
N	1	46	5.044	0.0296	47	13.970	0.0005	47	2.870	0.0968	47	45.377	<.0001
G	1	46	0.535	0.4682	47	23.459	<.0001	47	0.414	0.5230	47	53.823	<.0001
W:S	1	12	1.338	0.2699	12	0.851	0.3746	12	0.201	0.6620	12	0.256	0.6222
W:N	1	46	3.177	0.0813	47	0.192	0.6632	47	0.047	0.8296	47	0.813	0.3718
S:N	1	46	12.407	0.0010	47	12.810	0.0008	47	2.731	0.1051	47	17.090	0.0001
W:G	1	46	0.590	0.4462	47	0.165	0.6860	47	1.662	0.2037	47	0.214	0.6459
S:G	1	46	1.905	0.1742	47	18.185	0.0001	47	4.477	0.0397	47	27.256	<.0001
N:G	1	46	0.007	0.9332	47	1.143	0.2906	47	4.577	0.0376	47	0.449	0.5061
W:S:N	1	46	0.246	0.6225	47	0.037	0.8477	47	0.060	0.8076	47	0.001	0.9732
W:S:G	1	46	0.008	0.9309	47	0.685	0.412	47	0.008	0.9281	47	0.004	0.9493
W:N:G	1	46	0.527	0.4714	47	6.253	0.0159	47	0.005	0.9462	47	3.386	0.0721
S:N:G	1	46	2.773	0.1027	47	0.395	0.5325	47	0.157	0.6936	47	2.090	0.1550
W:S:N:G	1	46	0.897	0.3487	47	0.560	0.4581	47	1.160	0.2869	47	0.834	0.3659

Appendix B

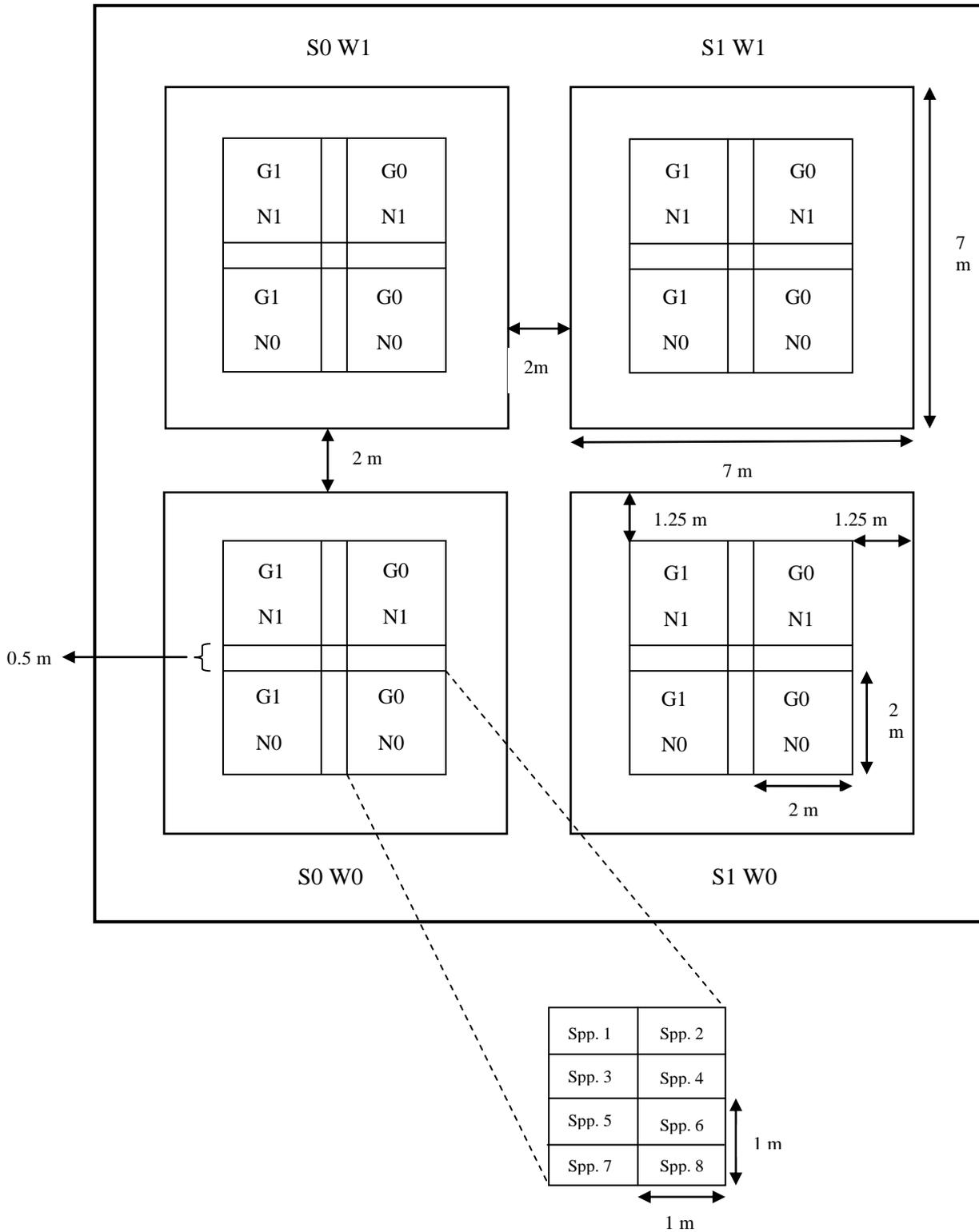


FIG. B1. Schematic treatment plot layout

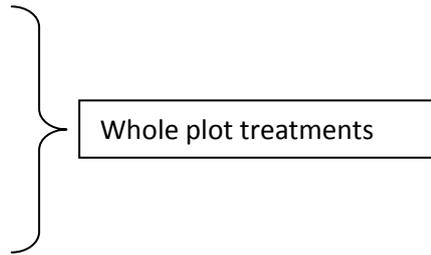
Treatments provided

W1: Regular watering.

W0: Natural rainfall.

S1: 80 % Shade level.

S0: Natural light.

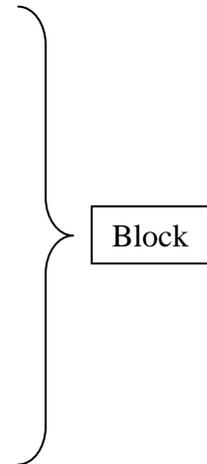
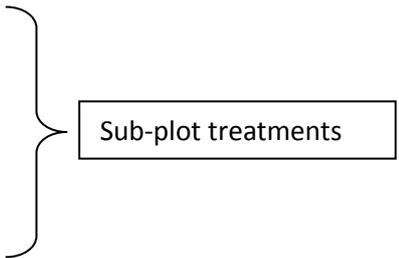


G1: Grass present.

G0: Grass absent.

N1: With fertilizer.

N0: Without fertilizer.



Chapter 4: Herbivory effects on saplings are influenced by nutrients and grass competition in a humid South African savanna

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Summary

1. Herbivory is a common disturbance influencing savanna vegetation composition and structure. Grazers and browsers interfere with sapling establishment dynamics by direct consumption of plant tissue and changing soil nutrient status and grass competition. Studies evaluating the effects of herbivory on sapling establishment have mostly been extrapolated from single species.
2. In a controlled field experiment, we studied the effects of clipping (presence vs absence of simulated grazing and browsing), nutrients (presence vs absence), grass competition (presence vs absence) and their interactive effects on sapling survival and growth of four humid and four mesic savanna species. We conducted this experiment in a humid South African savanna.
3. Sapling survival was not affected by the treatments provided. Clipped saplings of all species increased their investment in relative growth rate of stem length (RGR_L). On the other hand, clipping had a greater negative impact on relative growth rate of more humid than mesic species in terms of stem diameter (RGR_D) (of 2 humid species), total dry biomass (of 2 humid and 1 mesic species) and proportion of leaf biomass (of 4 humid and 2 mesic species).
4. Nutrients had a positive effect on the RGR_L of *Acacia tortilis*, *Combretum apiculatum* and *Colophospermum mopane* and sapling biomass of *Schotia brachypetala*, *Acacia nigrescens* and *Combretum apiculatum*. Positive effects of nutrients on RGR_L of *Acacia karroo*, *Acacia tortilis* and *Colophospermum mopane* were observed in their clipped saplings only.

5. Grass competition had a strong negative impact on all growth parameters measured. The defoliated saplings of three species, *Acacia sieberiana*, *Combretum apiculatum* and *Colophospermum mopane* had lower RGR_L with grass competition whereas intact saplings showed no significant response. The presence of both grass and nutrients had a significant negative effect on the RGR_L of *Strychnos spinosa* saplings.
6. *Synthesis*. Saplings grow faster in length but the overall investment in shoots is reduced, after clipping. Nutrients and grass competition have positive and negative effects on sapling growth, respectively. After clipping, humid species were more vulnerable to grass competition with reduced ability to use nutrients than mesic species. In conclusion, herbivory increases sapling vulnerability to further disturbances, with humid species being more susceptible than mesic species.

Key-words: *Acacia*, browsing, grazing, mesic savanna, relative growth rate, tree sapling biomass.

Introduction

Variability in tree demography largely determines the structure of savannas (Staver, Bond & February 2011). Recruitment of tree saplings into adults is a critical phase of woody plant existence in savannas (Scholes & Archer 1997; Chidumayo 2008, Wakeling, Cramer & Bond 2012). Tree sapling establishment is a rare event in the presence of fire, herbivory and grass competition (Prins & van der Jeugd 1993; Ward 2005; Lehmann, Prior & Bowman 2009). However, an undue increase in tree abundance or woody plant encroachment causes a decline in species richness (Smit 2004; Ratajczak, Nippert & Collins 2012). Woody plant encroachment is caused mainly by the removal of grasses by intense grazing and subsequent decrease in the fuel loads for fires (Scholes & Archer 1997; van Auken 2000; Roques, O'Connor & Watkinson 2001; van Langevelde *et al.* 2003; Briggs *et al.* 2005). An increase in plant-available resources and a reduction in ungulate browsers can also significantly increase tree recruitment (Prins & van der Jeugd 1993; Ward 2005).

African savannas host a wide range of mammalian herbivores, constituting grazers and browsers (Holdo, Holt & Fryxell 2009). Large generalist herbivores such as elephants can have devastating effects on closed canopy woodlands and convert them to open savannas (Cumming *et al.* 1997; van de Koppel & Prins 1998; Shrader *et al.* 2012), although it may largely depend on woody vegetation response to rainfall, elephant densities and distribution (Guldmond & Van Aarde 2008). Tree recruitment rates can also be greater than browser-induced adult tree mortality (Dublin, Sinclair & McGlade 1990). Mortality of large trees creates open spaces with fertile sub-canopy soils, which promotes growth of tree saplings or nutritive grasses (Riginos & Young 2007; Ludwig, de Kroon & Prins 2008). Therefore, large mammalian herbivores may alter savanna vegetation structure without necessarily reducing woody plant densities (van de Vijver, Foley & Olf 1999). However, woody vegetation

encroachment can be suppressed in the presence of both small specialist and large generalist mammal browsers (Augustine & McNaughton 2004). Major effects driven by invertebrate herbivory on tree seedlings in Africa can increase mortality and restrict recruitment (Shaw *et al.* 2002). However, Western & Maitumo (2004) have found that large mammalian herbivores had more negative effects on woody plants than invertebrates in Africa.

Herbivores can have a direct positive effect on savanna tree growth. For example, heavy browsing stimulated shoot re-growth with high nutritive quality in *Acacia nigrescens* growing in fertile soils (du Toit, Bryant & Frisby 1990). Indirectly, large herbivores enrich local soil nutrient levels, through dung and urine deposits, affecting savanna vegetation structure (van der Waal *et al.* 2011). Nutrient-rich areas support herbivores by offering high-quality forage and maintain the soil fertility in a positive feedback system (du Toit *et al.* 1990; Augustine, McNaughton & Frank 2003; Asner *et al.* 2009; Mbatha & Ward 2010; van der Waal *et al.* 2011). In contrast, intense herbivore preference of plant species with high leaf nitrogen content, particularly in nutrient poor soils, may reduce plant available resources (Ritchie, Tilman & Knops 1998). Although nutrients directly promote tree growth, high utilization of nutrients for grass growth might increase the negative competitive effects on woody saplings (van der Waal *et al.* 2009). Grass competition is among the potential factors restricting tree recruitment especially when the rainfall is high (Goheen *et al.* 2010; Grellier *et al.* 2012). Large herbivore grazers enhance tree sapling establishment by removing grasses (Roques *et al.* 2001). On the other hand, grasses may exert potential positive effects on tree establishment by protecting the saplings from browsers (Seymour 2008). Therefore, both grazers and browsers interact to alter the competitive interactions between trees and grasses (Scholes & Archer 1997).

Many studies evaluating herbivory effects on the role of sapling establishment in woody plant encroachment have been based on single species (Kraaij & Ward 2006; Riginos & Young 2007; Scogings & Mopipi 2008; Goheen *et al.* 2010). Owing to the probability that any indigenous tree species may proliferate under favourable conditions, understanding sapling establishment dynamics of multiple species with respect to herbivory can help predict changes in vegetation at an ecosystem level. Persistence of savanna saplings amidst disturbances is mainly determined by their ability to resprout utilising stored root reserves (Bond & van Wilgen 1996). Success of the saplings is mainly affected by their ability to resist, viz., allocation to stems (Clarke *et al.* 2013), particularly fast growth rates in height (Higgins, Bond & Trollope 2000) and diameter (Gignoux, Clobert & Menaut 1997), recurring disturbances such as fire and herbivory. Replenishment of sapling reserves for re-establishment is mainly derived from undamaged leaves (Sakai & Sakai 1998) and new leaves produced after disturbance. Therefore, investment in plant photosynthetic material also has a deterministic role in promoting tree success.

We conducted a field experiment to evaluate the effects of clipping grass and tree saplings (i.e. both grazing and browsing), nutrient availability and grass competition on sapling survival and growth of eight indigenous savanna tree species in a humid South African savanna. In order to test whether savanna tree species from different climate types have a similar or different growth patterns, we selected four humid savanna species (> 1000 mm mean annual precipitation) i.e. *Acacia karroo* (Hayne) (a subtropical coastal variety from Richards Bay (Ward 2011)), *Acacia sieberiana* (Burt Davy), *Schotia brachypetala* (Sond.) and *Strychnos spinosa* (Lam.) and four species from mesic savannas (approx. 750 mm mean annual precipitation), i.e. *Acacia nigrescens* (Oliv.), *Acacia tortilis* (Hayne), *Combretum apiculatum* (Sond.) and *Colophospermum mopane* (J.Kirk ex J.Léonard), as our study

species. Tree species were selected based on their dominance in their respective climate types. The most widespread grass species in South Africa, *Eragrostis curvula*, was used to study the effects of grass competition in relation to herbivory on the tree sapling growth. We performed our study under irrigated conditions to control for variation in rainfall which may directly interfere with our study by affecting sapling survival and growth. Water, amounting to the long-term mean annual rainfall (1250 mm) of the study area, was supplied to plants at regular time intervals over the 6 month study period. Based on the individual factor effects followed by the interactive effects on sapling survival and growth, we predicted that:

1. Tree saplings will resprout and grow fast after clipping. Very young savanna tree seedlings have the ability to resprout and invest root reserves rapidly in growth after herbivory (Bergström 1992; Dube, Mlambo & Sebata 2009). Herbivore impact on plants is greatest on nutrient-rich soils as they prefer feeding on plants with high nutrient content (Levick & Rogers 2008; Asner *et al.* 2009). Mesic tree species evolved in eutrophic soils with high browsing pressure (du Toit 1995). Therefore, mesic species should be well adapted to herbivory and grow better with clipping treatment than humid species.
2. Nutrient addition will increase the survival and growth of tree saplings (Wakeling, Cramer & Bond 2010). Plants invest in root biomass more than stems with soil nutrient limitation (Poorter *et al.* 2012). Humid species grow under high rainfall conditions and on nutrient-poor soils, whereas mesic species occur in areas with low rainfall and nutrient-rich soils (du Toit 1995). Therefore mesic species, adapted to their native soil conditions, will perform better than humid species in the presence of nutrients.

3. Grass competition should have a strong negative effect on tree sapling survival and growth (Kambatuku, Cramer & Ward 2011; Grellier *et al.* 2012). Savanna seedlings increase their investment in root mass at the expense of shoots in the presence of grass competition (Kambatuku *et al.* 2011). Grasses may provide moist microclimatic conditions (Grellier *et al.* 2012) conducive for sapling establishment in mesic savannas (less evapotranspiration), but may be inhibitory to sapling establishment (due to low light availability) in humid savannas. Thus, species that evolved under humid conditions will be more negatively affected by grass competition than mesic species.
4. Survival and growth will be highest in clipped saplings treated with nutrients and no grass competition whereas intact saplings will exhibit lowest survival and growth with no nutrients and in the presence of grass competition.

Materials and Methods

STUDY SITE

The experimental study was conducted on Mondi nursery grounds, KwaMbonambi which is situated in the north-eastern coastal region of KwaZulu-Natal, South Africa (28°35'59.20" S, 32°10'47.22" E). The long-term mean annual rainfall (1984 – 2010) of the study area is 1250 mm. Rainfall occurs predominantly in the summer months, with peaks in November and February. The terrain at KwaMbonambi is generally flat and is comprised of Recent sediments of Quaternary, clayey, alluvial sands of aeolian deposition overlying the Cretaceous conglomerates of the Berea formation or Maputaland group (King 1982, Mucina and Rutherford 2006). The high permeability of the soils allows rapid leaching of the

nutrients due to the heavy rainfall in these areas. Vegetation is classified as Maputaland wooded grassland (Mucina and Rutherford 2006).

EXPERIMENTAL DESIGN AND TREATMENTS

The experimental site was fenced to exclude large mammalian herbivores (> 5 kg) from the area. The site was cleared of all vegetation prior to the application of the treatments and was not previously fertilized. Topsoil samples (up to 15 cm depth) were randomly collected from the experimental site and were sent to the University of KwaZulu-Natal laboratory, Pietermaritzburg, South Africa for soil N and P analysis. Nitrogen was analysed in a LECO Truspec Nitrogen Analyser (LECO corporation, Michigan) using the Dumas combustion method and phosphorous was analysed using a Technicon Autoanalyser II (Technicon Industrial Systems, Tarrytown, N.Y.). The topsoil in the study area contained a mean (\pm SE) of 0.1051 (\pm 0.013) % total N and 0.0236 (\pm 0.0021) % total P on a dry matter basis (n = 12 samples).

We adopted a split-plot design with irrigation provided at a whole-plot level. Ten 7 m \times 7 m whole-plots were randomly scattered in an open, non-wooded and topographically flat 70 m \times 50 m area. Clipping treatment with two levels, clipped and non-clipped, was performed at a whole-plot level. The two sub-plot treatment factors of nutrient fertilization (N) and grass (G), each had two levels, *viz.* presence and absence, within a given whole-plot treatment. Four sub-plots of 2 m \times 2 m area with 0.5 m distance between them were situated at the centre, leaving a peripheral width of 1.25 m, of the whole-plot.

“Osmocote exact” was the fertiliser applied to all sub-plots provided with nutrient treatment. It was a water soluble, granular, controlled-release fertilizer. The nutrient composition was 15 % of N, 9 % of P₂O₃, 11 % of K₂O, 2.5 % of MgO and other trace elements. This fertilizer was chosen for the steady and continuous release pattern over a 12

month period. Nitrogen is an important limiting factor for plant growth in tropical savannas (Bremen & de Wit 1983). The fertilizer was applied once-off in October 2009 at the rate of $4 \text{ g N m}^{-2} \text{ month}^{-1}$. This amount was approx. double the highest level of nitrogen ($2.3 \text{ g m}^{-2} \text{ month}^{-1}$) provided by Tilman (1987) and $2.8 \text{ g N m}^{-2} \text{ month}^{-1}$ applied by Kraaij & Ward (2006) in their study. The fertilizer applied amounted to $640 \text{ g sub-plot}^{-1}$.

Ten days after the fertilizer was added, 300 g of grass seeds were sown in each grass treatment sub-plot. Within each grass sub-plot, the soil was compacted after the grass seeds were added. Grasses were allowed to establish for two months before tree saplings were transplanted into the plots. However, grass was cut to 2 cm above ground prior to tree sapling transplantation to reduce grass competition and give some time for the tree saplings to establish. All plots were weeded every month to keep other herbaceous plants from interfering with the experiment. Four saplings of each species were planted at least 15 cm apart in sub-squares within each sub-plot. The two whole plot clipping treatments along with four sub-plots (nutrient and grass treatment combinations) were taken as a unit and replicated five times. Hence, the four saplings of each species were planted in $[2C \times (2N \times 2G)] \times 5 = 40$ sub-plots.

Plastic-roofed structures, made of 200 μm -thick clear greenhouse plastic film approx. 2 m aboveground were used to prevent the entry of rainwater in the irrigated whole-plots. Each whole-plot structure was surrounded by wire mesh at 0.50 m high aboveground, to exclude small mammalian herbivores. Percentage transmission of photosynthetically active radiation (PAR) through the plastic was calculated (based on Bauhus, van Winden & Nicotra 2004) at 1 m above the ground using a Sunfleck PAR Ceptometer model SF-80 (Decagon Devices Inc., Washington, DC, USA). We recorded an average percentage transmission approx. 55 % ($504.1 \mu\text{mol m}^{-2} \text{ s}^{-1}$) of PAR in the open ($905.9 \mu\text{mol m}^{-2} \text{ s}^{-1}$) under the plastic

roofed structures. Salazar *et al.* (2012) have found that 32 % of natural sun light was sufficient for seedling emergence in various Neotropical woody savanna species. The plastic used in our study did not inhibit the saplings from receiving an adequate PAR. Irrigation was provided such that each sub-plot within the experimental period of six months (24 weeks) received an amount of water (5000 L) equal to the long-term average annual rainfall (1250 mm) at regular time intervals.

Clipping was done once in half the number of whole plots, three months after transplanting the 2-3 month-old tree saplings in April 2010. In the whole-plots provided with clipping treatment each sapling was clipped at the second internode. In addition, grass was cut to 2 cm above ground in the grassy sub-plots within the whole-plots of the clipping treatment.

SAPLING DEVELOPMENT AND TRANSPLANTING

The hard-coated seeds of *Acacia karroo*, *Acacia nigrescens*, *Acacia sieberiana*, *Acacia tortilis* and *Schotia brachypetala* were soaked in concentrated sulphuric acid and scarified for 1 h. They were then washed thoroughly with water to remove the traces of acid and then soaked in water for 12 h. The soft-coated seeds of *Colophospermum mopane*, *Combretum apiculatum*, and *Strychnos spinosa* were not treated with acid but were soaked in 0.2 % HgCl₂ solution for 5 min to decontaminate the seeds and were then washed thoroughly and soaked in water for 12 h. Each seed was planted in 750 mL potting bags, were then allowed to grow in a mixture of soil, vermiculite and coconut coir. In order for the seeds to acclimate to natural soil conditions, soil (up to 15 cm depth) from the experimental site was used. Vermiculite and coconut coir mixture was used to increase the porosity and water

retention of the soil. After planting the seeds, potting bags were placed in mist beds under shade, ensuring moist conditions throughout the day. However, *Colophospermum mopane* seeds do not require a high water supply, as they are highly permeable to water and germinate easily (Mlambo et al. 2007) and therefore were watered only once every week. Saplings were grown for 2 – 3 months and were transplanted into the treatment plots. All the saplings were transplanted after the first heavy rainfall for the season occurred; transplantation was completed by mid-January 2010. The potting bags were sprayed with a general preventative fungicide once every week until the saplings were transplanted. For them to establish, saplings were watered immediately after transplantation. Some replacement of dead saplings (n = 146) within a month after transplanting was done.

DATA COLLECTION AND COMPILATION

Sapling survival status (live or dead), was noted at the end of the experiment in June 2010 on all the saplings that were planted at the beginning of the experiment in February 2010. Sapling growth was measured following clipping treatment; initial measurements were taken immediately after clipping in April 2010 and at the end of the experiment in June 2010. Stem length (from the base to apex), basal stem diameter (two perpendicular readings) were the pre-harvest measurements taken on each sapling. In June 2010, aboveground plant material of half the number of live saplings was harvested. The other half of the saplings was allowed to grow to the next season. Plant material collected was allowed to dry in a drying oven for 48 h at 60 °C. Harvested grass material was sun-dried and weighed in g. Post-harvest dried leaf and stem material of each tree sapling was weighed separately in mg.

Rapid attainment of overall sapling growth is essential to ensure its establishment in a savanna ecosystem (Vadigi & Ward 2012). Hence, we analysed sapling growth in terms of relative growth rate of the stem length (RGR_L), relative growth rate of the stem basal diameter (RGR_D), total aboveground dry weight or sapling dry biomass (SDB), as well as proportion of leaf dry biomass to the total aboveground plant dry weight (LP). The total aboveground dry biomass for each tree sapling was calculated as the sum of leaf and stem dry weight. Calculations for relative growth rate of sapling stem length (RGR_L) and relative growth rate of sapling basal stem diameter (RGR_D) were based on the increment in \ln (stem length) and \ln (basal diameter), respectively, per unit time (Kohi *et al.* 2009). The initial stem length used to calculate RGR_L was taken after clipping.

STATISTICAL ANALYSIS

We used linear mixed modelling with multilevel regression and analysed our data using the R statistical program (R Development Core Team 2011). A multilevel model is not only capable of integrating predictors that vary across different spatio-temporal scales but can also incorporate non-predictor variables explaining the between-group variation (Qian *et al.* 2010). Furthermore, linear mixed models are capable of dealing better with missing values in the data than conventional ANOVA (Piepho, Büchse & Emrich 2003; Schielzeth & Nakagawa 2012). The predictor factor effects on binomial survival data were estimated by using “glmer” (generalized linear mixed model) function and effects on sapling growth were analyzed by using ANOVA output of “lme” (linear mixed model) function.

In our full model, we tested the fixed effects of species (Spp), clipping (C), nutrients (N) and grass (G) treatments on sapling survival and growth in order to understand species

differences in response to treatment factors given. Species were considered as fixed effects because they were selected based on their dominance in either humid or mesic habitats. The outcomes measured at the sub-plot scale in the hierarchical structure are affected by factors measured at that scale and factors operating at the whole-plot level within the randomized blocks (Qian *et al.* 2010; Schielzeth & Nakagawa 2012). A random factor was modelled to adjust variations in the data due to a complicated spatial pseudo-replication arising from the split-plot design (Piepho *et al.* 2003, Crawley 2007). We considered a split-plot error term associated with the treatment factors, representing a sub-plot level interaction of nutrient and grass within a whole-plot level of clipping within block / replicate (Blk) in our maximum likelihood models. In other words, block and the block \times treatment interactions were considered as random effects in the models. We analysed the sub-plot means for each measured growth response variable of each species to avoid pseudo-replication at the sapling level. Equation (1) shows an example of the ‘lme’ model used in R for the overall analysis:

$$\text{Overall RGR}_L \sim \text{Spp} * \text{C} * \text{N} * \text{G}, \text{ random} = \sim 1 | \text{Blk} / \text{C} / \text{N} : \text{G} \quad \text{Eqn (1)}$$

The above model represents predictor parameter effects on relative growth rate in sapling stem length (RGR_L) as a linear function of all species interacting with full factorial treatment factors, viz. clipping (C), nutrients (N), and grass (G) with a split-plot error. To test for the effects of treatments on individual species, we modelled individual species growth as a linear function of the full factorial treatment effects with a split-plot error term in Eqn (2).

$$\text{Individual spp RGR}_L \sim \text{C} * \text{N} * \text{G}, \text{ random} = \sim 1 | \text{Blk} / \text{C} / \text{N} : \text{G} \quad \text{Eqn (2)}$$

To avoid Type I errors we only present individual species results where they are consistent with the overall analysis. We also performed similar generalized linear mixed

model (*glmer*) analyses on sapling *survival*, but observed no overall effects of the various treatments provided. Hence, the results of survival analyses are not presented.

All growth variables were checked for general linear model assumptions and the variable, sapling dry biomass, was \log_{10} transformed to ensure normal distribution and homogeneity of variances. All main effects, interactions and treatment differences were considered to be significant at $\alpha = 0.01$, after Bonferroni corrections. Additionally, we tested for the investment trade-off between RGR_L and RGR_D using a simple linear regression analysis.

Results

RELATIVE GROWTH RATE IN STEM LENGTH

Species differed significantly in all of the growth parameters measured (Table 1). The clipping treatment had an overall positive effect on relative growth rate in stem length (RGR_L), which differed significantly among species (Table 1). All species showed a significantly higher RGR_L with clipping (see Tables S1 to S8 in Supporting Information, Fig. 1a,b). Nutrients had an overall positive effect on sapling RGR_L although species differed significantly in their RGR_L with nutrient treatment (Table 1). When each species was tested separately, a significant positive effect of nutrients was observed only in *Acacia tortilis* (without nutrients = 0.068 ± 0.017 (mean \pm S.E.), with nutrients = 0.086 ± 0.026 mm mm⁻¹ week⁻¹; Table S6), *Combretum apiculatum* (without nutrients = 0.076 ± 0.020 , with nutrients = 0.106 ± 0.025 mm mm⁻¹ week⁻¹; Table S7) and *Colophospermum mopane* (without nutrients = 0.068 ± 0.015 , with nutrients = 0.088 ± 0.020 mm mm⁻¹ week⁻¹; Table S8).

The interaction of clipping treatment with nutrients had an overall significant effect on sapling RGR_L, which differed significantly among the species (Table 1). When individual species results were considered, the interactive effects of clipping and nutrients were significant only for the sapling RGR_L of *Acacia karroo*, *Strychnos spinosa* (humid species), *Acacia tortilis* and *Colophospermum mopane* (mesic species) (see Tables S1 to S8). Among these species, positive effects of nutrients were observed only in clipped saplings except *S. spinosa* which showed a decrease in RGR_L of clipped saplings with nutrients (Fig. 2a,b).

Grass competition had an overall negative effect on sapling RGR_L. Species differed significantly in their RGR_L with grass treatment (Table 1). When each species was tested separately, significant negative effect of grass competition was observed in five species out of eight, viz. *A. karroo* (without grass = 0.093 ± 0.020 , with grass = 0.076 ± 0.017 mm mm⁻¹ week⁻¹; Table S1), *Acacia sieberiana* (without grass = 0.107 ± 0.024 , with grass = 0.083 ± 0.019 mm mm⁻¹ week⁻¹; Table S2), *A. tortilis* (without grass = 0.088 ± 0.023 , with grass = 0.064 ± 0.020 mm mm⁻¹ week⁻¹; Table S6), *Combretum apiculatum* (without grass = 0.114 ± 0.027 , with grass = 0.068 ± 0.016 mm mm⁻¹ week⁻¹; Table S7) and *C. mopane* (without grass = 0.091 ± 0.019 , with grass = 0.066 ± 0.016 mm mm⁻¹ week⁻¹; Table S8).

The interactive effects of clipping and grass had an overall significant effect on sapling RGR_L and differed among the species (Table 1). Clipping and grass interactions were significant only in *A. sieberiana*, *C. apiculatum* and *C. mopane*, as observed after each species was tested separately (see Tables S1 to S8). In these species, the negative effect of grass competition was observed only in clipped saplings (Fig. 3a,b).

The interaction of nutrients with grass had a significant overall effect on sapling RGR_L (Table 1). When each species were tested separately, the results were significant for *S. spinosa* only (see Tables S1 to S8). RGR_L of *S. spinosa* was the lowest in the presence of

both nutrients and grass ($0.055 \pm 0.019 \text{ mm mm}^{-1} \text{ week}^{-1}$) when compared to the other three combinations (i.e. without nutrients and grass = 0.062 ± 0.029 , with nutrients and without grass = 0.075 ± 0.026 , without nutrients and with grass = $0.075 \pm 0.028 \text{ mm mm}^{-1} \text{ week}^{-1}$).

RELATIVE GROWTH RATE IN STEM BASAL DIAMETER

Clipping had an overall significant negative effect on sapling relative growth rate in stem basal diameter (RGR_D), which differed significantly among the species (Table 1). When individual species analyses were considered, relative growth rate in stem basal diameter was found to be significant only for *Acacia karroo* and *Acacia sieberiana* (see Tables S1 to S8, Fig. 4a,b). Grass competition had a significant overall negative effect on RGR_D (Table 1). Based on individual species results, the significant negative effect of grass competition was observed on sapling RGR_D of *A. karroo* (without grass = 0.023 ± 0.002 , with grass = $0.015 \pm 0.002 \text{ mm mm}^{-1} \text{ week}^{-1}$; Table S1), *Schotia brachypetala* (without grass = 0.013 ± 0.002 (mean \pm S.E.), with grass = $0.003 \pm 0.002 \text{ mm mm}^{-1} \text{ week}^{-1}$; Table S3), *Acacia tortilis* (without grass = 0.016 ± 0.002 , with grass = $0.003 \pm 0.002 \text{ mm mm}^{-1} \text{ week}^{-1}$; Table S6), *Combretum apiculatum* (without grass = 0.020 ± 0.001 , with grass = $0.010 \pm 0.003 \text{ mm mm}^{-1} \text{ week}^{-1}$; Table S7) and *Colophospermum mopane* (without grass = 0.016 ± 0.002 , with grass = $0.007 \pm 0.002 \text{ mm mm}^{-1} \text{ week}^{-1}$; Table S8).

The clipping treatment interacting with grass competition had a significant effect on overall sapling RGR_D (Table 1). Based on individual species results, intact saplings of only *S. brachypetala* exhibited significantly lower RGR_D whereas clipped saplings showed no significant effect of grass competition (see Table S3; Fig. 5a,b).

ALLOCATION TRADE-OFF BETWEEN STEM LENGTH AND DIAMETER

Relative growth rate in stem length was negatively correlated with RGR_D on the non-clipped saplings of *Schotia brachypetala* ($r = -0.464$, $n = 20$, $P < 0.05$) and *Strychnos spinosa* ($r = -0.559$, $n = 20$, $P < 0.01$). However, in *Colophospermum mopane* we found a strong positive effect ($r = 0.782$, $n = 20$, $P < 0.0001$). Relative growth of stem length of clipped *S. brachypetala* saplings had a positive effect on its RGR_D ($r = 0.525$, $n = 20$, $P < 0.05$).

SAPLING DRY BIOMASS

Clipping significantly reduced the overall (\log_{10}) sapling dry biomass (SDB), which differed significantly among the species (Table 1). When each species was tested separately, the significant negative effect of clipping on SDB was observed only in *Acacia sieberiana*, *S. brachypetala* and *C. mopane* (see Tables S1 to S8, Fig. 6a,b). Nutrients had an overall significant positive effect on SDB (Table 1). Based on the individual species results, the positive effect of nutrients on SDB was observed only in *S. brachypetala* (without nutrients = 2.720 ± 0.076 (mean \pm S.E.), with nutrients = 2.923 ± 0.101 mm mm⁻¹ week⁻¹; Table S3), *Acacia nigrescens* (without nutrients = 2.800 ± 0.139 , with nutrients = 3.260 ± 0.167 mm mm⁻¹ week⁻¹; Table S5) and *Combretum apiculatum* (without nutrients = 3.073 ± 0.143 , with nutrients = 3.637 ± 0.151 mm mm⁻¹ week⁻¹; Table S7). Grass competition had an overall significant negative effect on sapling SDB, which differed significantly among species (Table 1). All species had a significantly lower SDB in the presence of grass competition (see Tables S1 to S8, Fig. 7a, b).

PROPORTION OF LEAF TO SAPLING DRY BIOMASS

The proportion of leaf to sapling dry biomass (LP) differed significantly among the species in response to clipping (Table 1). Clipped saplings had lower LP when compared to the intact plants in all humid and two mesic (*Combretum apiculatum* and *Colophospermum mopane*) species (Fig. 8a,b). Grass competition had a significant negative effect on overall sapling LP (Table 1). The negative effect of grass competition, based on each species' results, was observed only in *Acacia karroo* (without grass = 0.365 ± 0.023 (mean \pm S.E.), with grass = 0.286 ± 0.034 mm mm⁻¹ week⁻¹; Table S1), *Acacia sieberiana* (without grass = 0.336 ± 0.026 , with grass = 0.276 ± 0.039 mm mm⁻¹ week⁻¹; Table S2) and *Acacia tortilis* (without grass = 0.364 ± 0.023 , with grass = 0.210 ± 0.024 mm mm⁻¹ week⁻¹; Table S6).

Discussion

SAPLING SURVIVAL

We observed no significant differences in sapling survival with respect to any treatment provided. In other words, saplings survived clipping and are capable of tolerating intense herbivory. Similarly, Hean & Ward (2012) found that clipping produced no mortality on the seedlings of *Acacia* species. Nutrients also had no effect on sapling survival, similar to the results obtained by Vadigi & Ward (2012). The increase in negative effects of grass competition in the presence of nutrients, as shown by van der Waal *et al.* (2009), also produced no significant mortality among tree saplings in our study. Grass competition was found to be a major factor limiting tree recruitment (Ward & Esler 2011; Grellier *et al.* 2012). Frequent and intense grazing may indirectly facilitate tree seedling survival by suppressing

grass competition (Hagenah *et al.* 2009). In our study, clipping was performed only once and despite being watered daily, grasses did not significantly eliminate tree saplings. Fires can critically impair tree sapling survival in savannas, and certain species may respond poorly to burning (Vadigi & Ward 2012). Zida *et al.* (2007) have also provided evidence that tree species richness is significantly reduced under annual fire regimes as sapling response to fires is species-specific. However, lack of a sapling survival response to clipping and other environmental factors (Vadigi & Ward, unpublished data) may emphasize that fires are more important than browsing in determining the composition of humid savanna vegetation.

EFFECTS OF CLIPPING ON SAPLING GROWTH

Saplings of all species grew faster in terms of stem length after clipping. However, clipping had a negative effect in certain species with respect to all other growth parameters measured. Similarly, Fornara & du Toit (2007) observed that browsed *Acacia nigrescens* plants in the wet season exhibited pulsed shoot growth which depended on stored nutrient reserves within the tree. However, as also indicated by Fornara & du Toit (2007), we did not find an increased investment in biomass compared to non-clipped saplings, at least not within the period of our study. Previous studies have noted a positive effect of browsing or clipping on compensatory shoot or leaf regrowth in trees (Bergström 1992; Scogings & Mopipi 2008; Scogings, Mamashela & Zobolo 2012). Contrastingly, Augustine & McNaughton (2004) found a decrease in leaf biomass and growth rate of twigs, and consequently reduced recruitment among saplings that were browsed by the small mammalian herbivore, the dik-dik (*Madoqua kirkii*). In our study, the negative effects of clipping on sapling growth (as measured by the functional traits of RGR_D, SDB and LP) were observed more often in humid than mesic species. Hence, saplings of humid species could be more vulnerable to the effects

of browsing than mesic species. This supports our prediction that mesic species are well adapted to herbivory, as they evolved in eutrophic soils and under high browsing pressure (du Toit 1995). In this context, we also note that clipping did not induce sapling investment in basal diameter as we found after fire treatment (Vadigi & Ward 2012). With one exception (*Schotia brachypetala*), the clipped saplings invested simultaneously in stem length and diameter but their intact saplings exhibited a trade-off between these two parameters. This may indicate that *S. brachypetala* compensates for the loss of tissue by investing heavily in shoot growth (volume), thereby overcoming the trade-off between the investment in stem length and diameter.

EFFECTS OF NUTRIENTS ON SAPLING GROWTH

Sankaran, Ratnam & Hanan (2008) have indicated that, apart from water, soil nutrient availability influences woody cover in African savannas. Soil nutrients and water increase tree regenerative capacity and forage quality for herbivores (Grant & Scholes 2006; Scogings & Mopipi 2008; Asner *et al.* 2009). In our study, the majority of species did not show any effect of nutrients on the various growth parameters measured. However, positive effects of nutrients were observed on RGR_L of *Acacia tortilis*, *Combretum apiculatum* and *Colophospermum mopane* and sapling biomass of *S. brachypetala*, *Acacia nigrescens* and *C. apiculatum*. This could mean that only a few species among many may benefit from the presence of nutrients, which in turn determines specific landscape usage by herbivores based on the quality of the forage species. Beneficial effects of nutrient supplementation were observed in *Acacia mellifera* seedlings despite grass competition (Kambatuku, Cramer & Ward 2013). In accordance with our expectation, more mesic species benefited from nutrients than humid species in terms of sapling growth. Humid savanna species typically adapted to

nutrient-poor conditions, may be investing more in roots than shoots in the presence of favourable conditions.

Positive effects of nutrients on RGR_L of *Acacia karroo*, *Acacia tortilis* and *Colophospermum mopane* were observed only on their clipped saplings. These species may respond to herbivory by rapid investments in shoot regrowth by an effective utilization of soil resources. Moreover, the negative effect of nutrient addition on the clipped saplings of *Strychnos spinosa* could mean that these saplings may be investing more in defences than in regrowth. Within a plant, a trade-off may exist between regrowth and investment in defence (Rohner & Ward 1997), although this may depend on whether the plants occur in high or low-nutrient environments (Ward & Young 2002). Savanna trees may frequently invest in defences in the presence of herbivory (e.g. Rohner & Ward 1997; Ward & Young 2002; Zinn, Ward & Kirkman 2007).

EFFECTS OF GRASS COMPETITION ON SAPLING GROWTH

Negative effects of grass competition are as important as fire and herbivory in determining woody plant demography (Riginos 2009; Grellier *et al.* 2012). Although grasses are known to strongly suppress tree sapling establishment (Jurena & Archer 2003; Riginos & Young 2007; Kambatuku *et al.* 2011; Grellier *et al.* 2012), they also exert negative competitive effects on the growth of large trees (Riginos 2009). Sapling growth in all parameters we measured was reduced in the presence of grass competition. In particular, the RGR_L of two humid and three mesic species, RGR_D of two humid and three mesic species, SDB of all species, and LP of two humid and one mesic species were lower with grass competition. The competitive effects of grass were found in both mesic and humid species.

Therefore, a strong negative impact on sapling growth in our study indicates that grass competition is a major hurdle for overall tree recruitment in savannas. In addition, we note that *Acacia karroo* saplings are highly vulnerable to grass competition because this species' growth was significantly reduced in all parameters measured. Consistent with this result, Chirara, Frost & Gwarazimba (1998) found that, under irrigated conditions, grass clipping promoted seedling growth in *A. karroo*, an encroaching species in many savannas. However, grasses may protect tree saplings by concealing them from herbivores (Riginos & Young 2007) and by reducing their transpiration (Grellier *et al.* 2012).

The clipped saplings of three species, *Acacia sieberiana*, *Combretum apiculatum* and *Colophospermum mopane* invested less in RGR_L in the presence of grass competition whereas intact saplings showed no significant response (Fig. 4a,b). Contrastingly, we found a reduction in the RGR_D of intact saplings of *Schotia brachypetala* with grass competition but not in the clipped plants (Fig. 5a,b). This result could mean that in the presence of grass competition and herbivory, saplings increase their investment rates in stem diameter at the cost of length.

Synthesis

By comparing the main results found here with our previous study (Vadigi & Ward 2012) we found that saplings have invested in stem length in response to burning and clipping whereas positive investment in basal diameter was observed only after burning but not after clipping (negative). In addition, clipped saplings had invested less in total shoot biomass. These characteristic responses to fire and herbivory were distinctly found in *Acacia sieberiana*. This leads to increased sapling susceptibility to further disturbances, particularly

fire, as stem diameter or thickness may be more important than height in determining tree sensitivity to disturbances (Midgley, Lawes & Chamaillé-Jammes 2010). Additionally, we found that humid species were more vulnerable than mesic species to clipping and grass competition with a reduced ability to utilize nutrients. Therefore, sapling resilience to disturbances is reduced more after herbivory than by fire, with humid species more susceptible than mesic species.

Herbivory (by browsers) and fire are the major factors curbing tree sapling establishment and subsequent encroachment (Trollope 1980; Prins & van der Jeugd 1993; Roques *et al.* 2001). Woody plant invasion reduces biodiversity and palatable grass species (Trollope 1980; Ward 2005) although trees in an open savanna are important in maintaining species diversity (Cumming *et al.* 1997; Fenton *et al.* 1998; Smit 2004). Management of savannas is challenged by the need to sustain large mammalian herbivore populations and maintain vegetation biodiversity (Asner *et al.* 2009). Based on our results, we assume that herbivory can effectively curb tree recruitment only when it is followed by fire, i.e. after saplings coppice. Trollope (1980) suggested that woody plant encroachment can best be managed by allowing browsing after fires, particularly when burning is made practically easy in humid savannas with the accumulation of excess grass fuel load. Therefore, tree-encroached areas may require a management combination of fire and herbivory, particularly when nutrient resources are not limited (Staver *et al.* 2009). Patch-wise alternation of browsing and fire treatments will create an opportunity for the establishment of trees of different species with the maintenance of diversity. However to achieve this, studies considering different landscapes need to be conducted in order to assess the individual species' responses to fire and forage-species preferences of herbivores (Gordijn *et al.* 2012).

These studies will enable us to identify the species vulnerable to both disturbances (viz. fire and herbivory) and accommodate them into management plans.

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Supporting Information (see below Figures)

Additional Supporting Information may be found in the online version of this article:

Table S1. Linear mixed model analysis results of *Acacia karroo* growth parameters

Table S2. Linear mixed model analysis results of *Acacia sieberiana* growth parameters

Table S3. Linear mixed model analysis results of *Schotia brachypetala* growth parameters

Table S4. Linear mixed model analysis results of *Strychnos spinosa* growth parameters

Table S5. Linear mixed model analysis results of *Acacia nigrescens* growth parameters

Table S6. Linear mixed model analysis results of *Acacia tortilis* growth parameters

Table S7. Linear mixed model analysis results of *Combretum apiculatum* growth parameters

Table S8. Linear mixed model analysis results of *Colophospermum mopane* growth parameters

Table 1. Linear mixed model (lme) analysis using maximum likelihood, showing the individual and interactive effects of clipping (D), nutrient (N) and grass (G) treatments (two levels each) on all species. Treatments were provided in the presence of irrigation (high water availability) and natural light. Interactive effects of nutrient and grass (sub-plot) within the treatment clipping (performed at a whole plot level) within replicates (block units) were considered as split-plot error. We analyzed final - initial relative growth rate of stem length (RGR_L in $mm\ mm^{-1}\ week^{-1}$) and final - initial relative growth rate in stem basal diameter (RGR_D in $mm\ mm^{-1}\ week^{-1}$), after clipping in April 2010. The final proportion of leaf to sapling dry biomass (LP) and final \log_{10} transformed sapling dry biomass (SDB in mg), taken in June 2010, were analyzed. The numerator degrees of freedom (df), error df, F-value and significance are indicated per factor and factor interactions. The values in bold are significant, using a Bonferroni correction ($p < 0.01$)

Source	df	RGR_L			RGR_D			LP			SDB		
		Err df	F	P	Err df	F	P	Err df	F	P	Err df	F	P
Spp	7	205	10.42	<.0001	205	14.83	<.0001	204	30.74	<.0001	205	36.63	<.0001
D	1	4	775.75	<.0001	4	36.49	0.0038	4	3.14	0.1509	4	23.15	0.0086
N	1	24	21.14	0.0001	24	1.31	0.2632	24	1.35	0.2559	24	33.52	<.0001
G	1	24	54.53	<.0001	24	40.98	<.0001	24	20.03	0.0002	24	138.08	<.0001
Spp:D	7	205	12.86	<.0001	205	3.56	0.0012	204	2.88	0.0069	205	5.09	<.0001
Spp:N	7	205	3.22	0.0029	205	1.51	0.1649	204	0.55	0.7991	205	2.18	0.0372
D:N	1	24	10.38	0.0036	24	1.97	0.1734	24	0.03	0.8547	24	7.64	0.0108
Spp:G	7	205	3.18	0.0032	205	0.92	0.4904	204	1.22	0.2943	205	5.56	<.0001
D:G	1	24	38.86	<.0001	24	12.46	0.0017	24	0.53	0.4720	24	3.16	0.0883
N:G	1	24	18.09	0.0003	24	0.00	0.9745	24	0.12	0.7353	24	2.75	0.1103
Spp:D:N	7	205	4.61	0.0001	205	0.77	0.6139	204	1.74	0.1009	205	0.36	0.9244
Spp:D:G	7	205	3.36	0.0021	205	0.57	0.7834	204	1.31	0.2462	205	1.35	0.2279
Spp:N:G	7	205	1.61	0.1343	205	0.40	0.9017	204	0.50	0.8321	205	0.64	0.7183
D:N:G	1	24	4.27	0.0498	24	5.41	0.0289	24	0.66	0.4237	24	1.47	0.2379
Spp:D:N:G	7	205	1.92	0.0677	205	0.46	0.8632	204	1.02	0.4184	205	0.95	0.4664

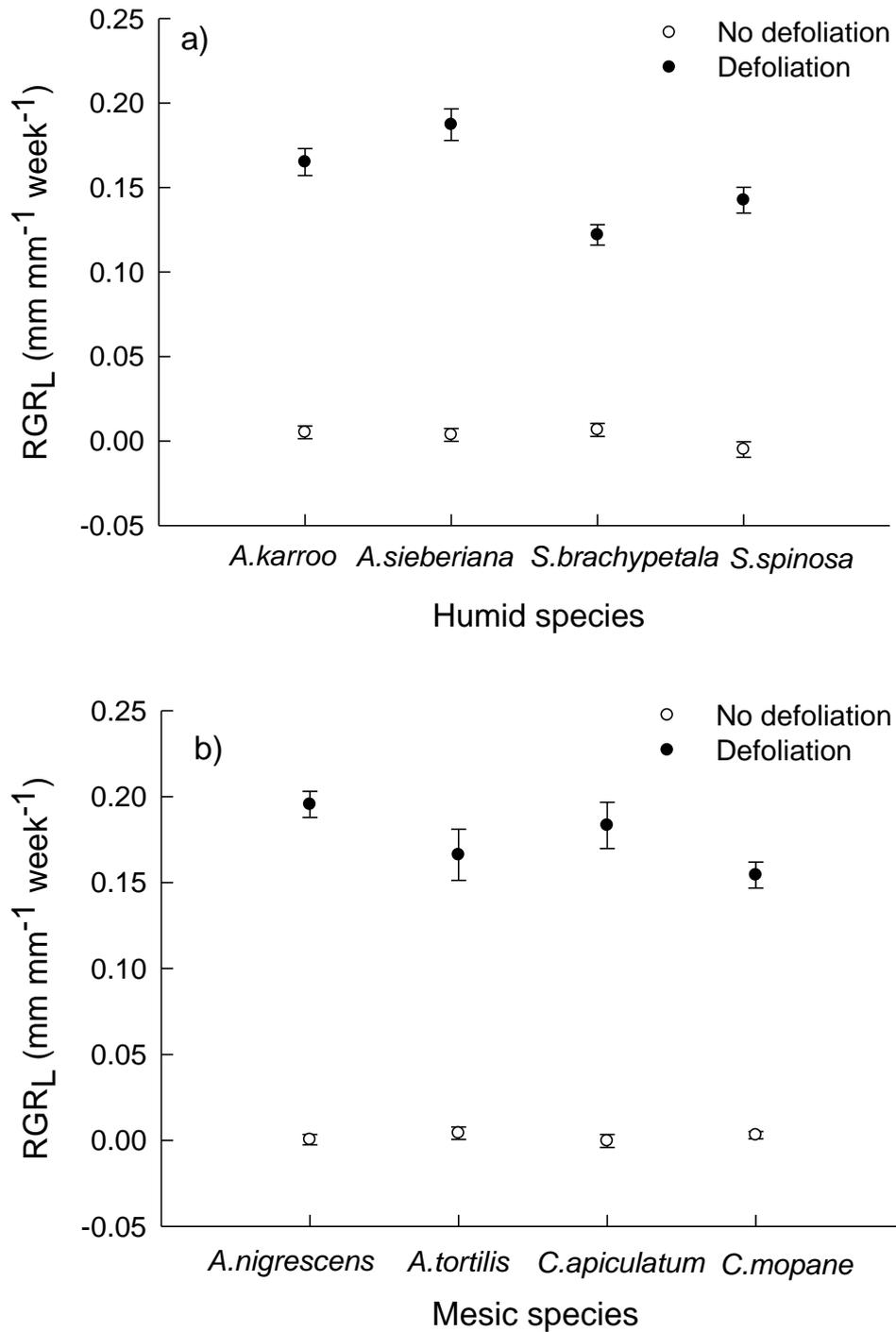


Fig. 1. Mean (\pm 1SE) relative growth rate of sapling stem length (RGR_L) of (a) humid and (b) mesic species in response to the clipping treatment. Stem lengths of saplings of all species grew significantly faster after clipping.

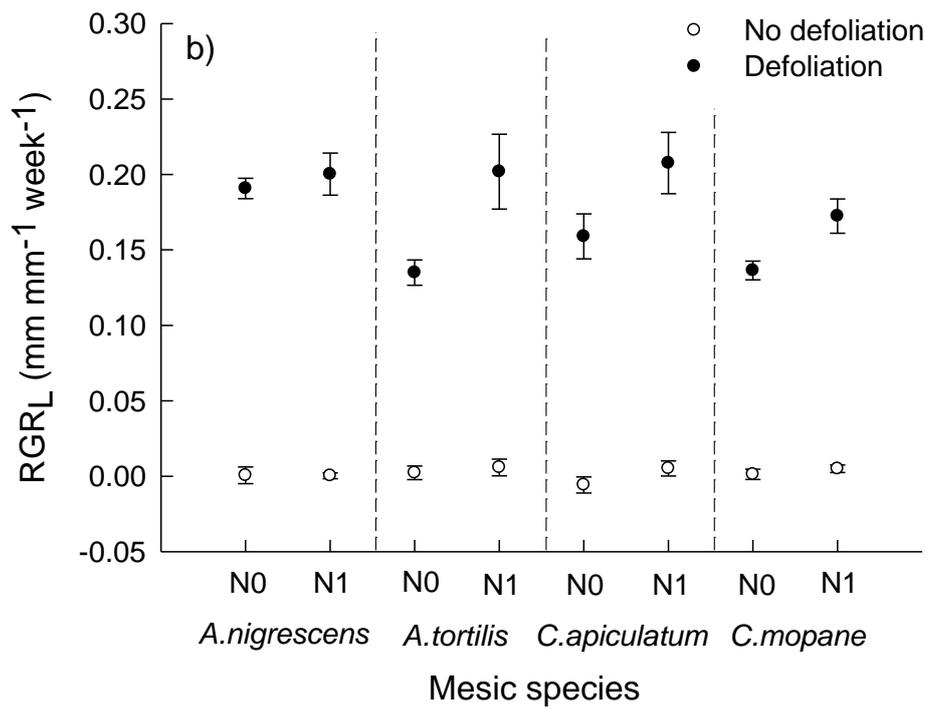
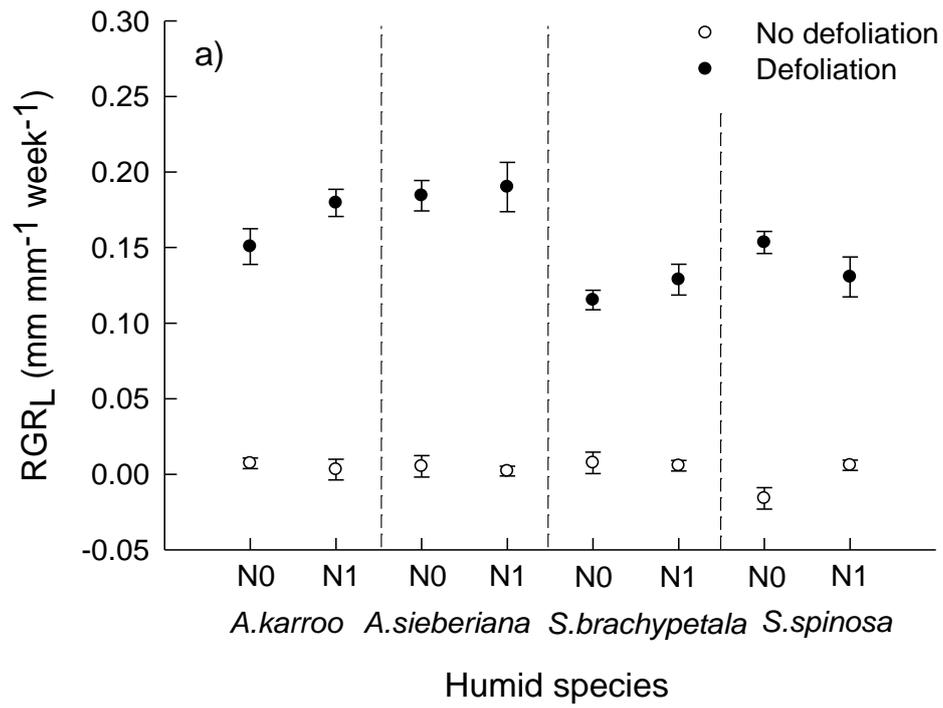


Fig. 2. Mean (\pm 1SE) relative growth rate sapling stem length (RGR_L) of (a) humid and (b) mesic species in response to clipping and nutrient availability. The treatment levels of nutrients (N1- nutrient addition and N0 – no nutrients) are represented on the x-axis, for each species. To be read as a two-way interactive effect of clipping and nutrients on RGR_L of each species separately. Significant increases in RGR_L with nutrients were observed only in defoliated saplings of *Acacia karroo*, *Acacia tortilis* and *Colophospermum mopane* whereas *Strychnos spinosa* showed a decrease in defoliated sapling RGR_L with nutrients, relative to unfertilized plants.

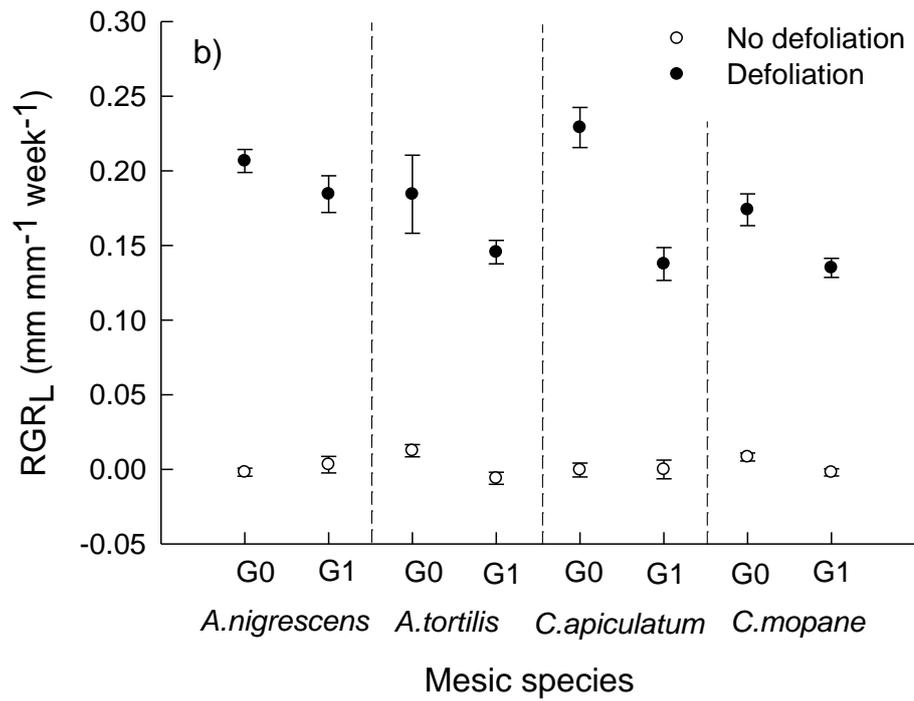
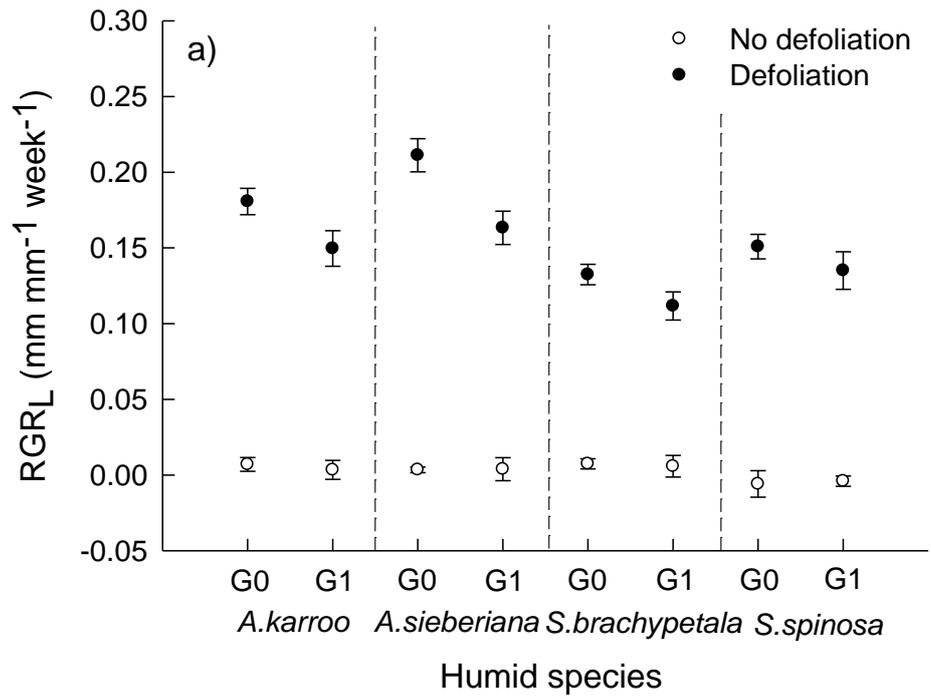


Fig. 3. Mean (\pm 1SE) relative growth rate of sapling stem length (RGR_L) of (a) humid and (b) mesic species in response to clipping and grass competition. The treatment levels of grass (G1- grass competition and G0 – no grass) are represented on the x-axis, for each species. To be read as a two-way interactive effect of clipping and grass on RGR_L of each species separately. A negative effect of grass competition on RGR_L was observed only in defoliated saplings of *Acacia sieberiana*, *Combretum apiculatum* and *Colophospermum mopane* relative to the sapling RGR_L with no grass competition.

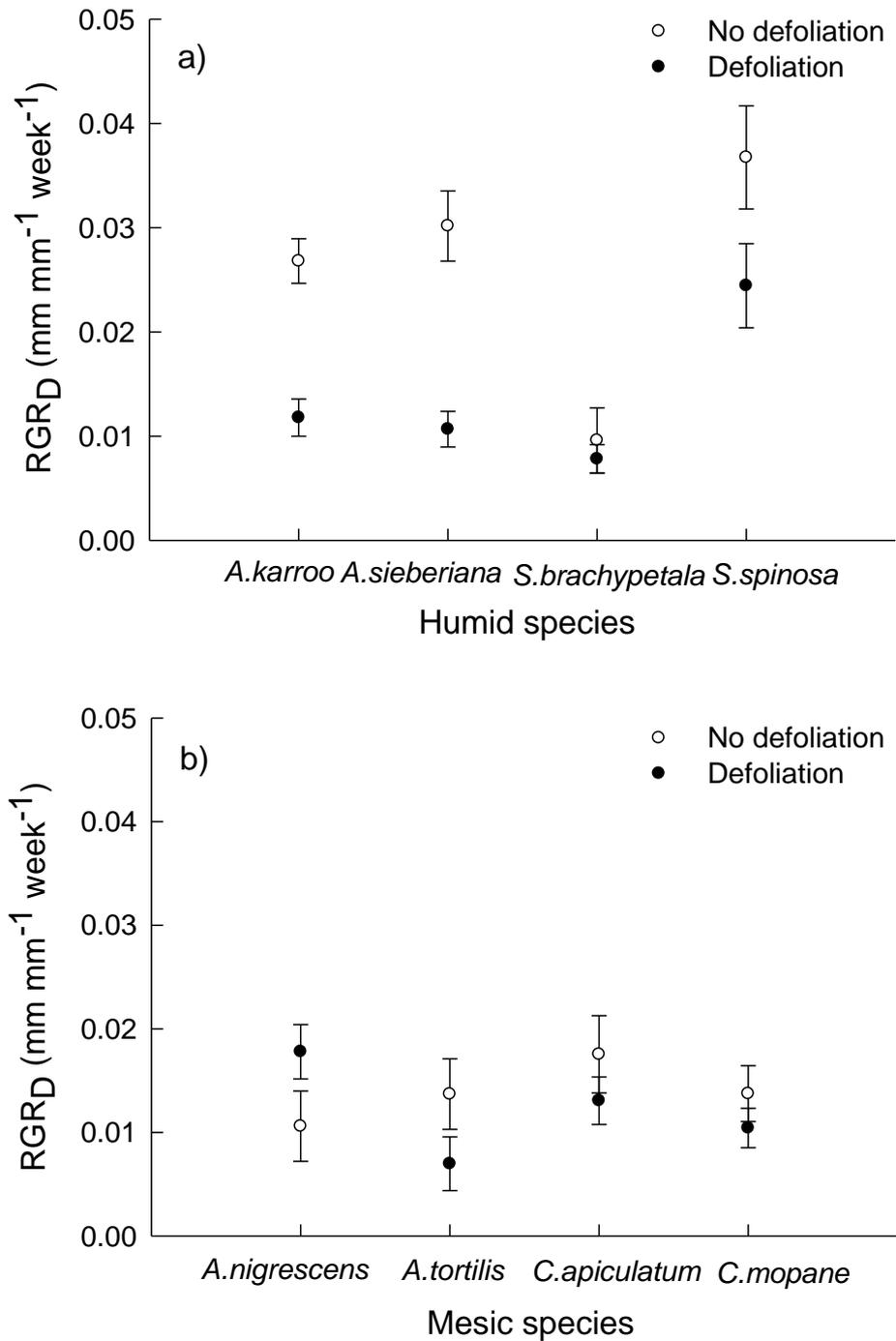


Fig. 4. Mean (\pm 1SE) relative growth rate of sapling stem basal diameter (RGR_D) of (a) humid and (b) mesic species in response to the clipping treatment. Relative growth rate in stem basal diameter was significantly lower in defoliated *Acacia karroo* and *Acacia sieberiana* saplings.

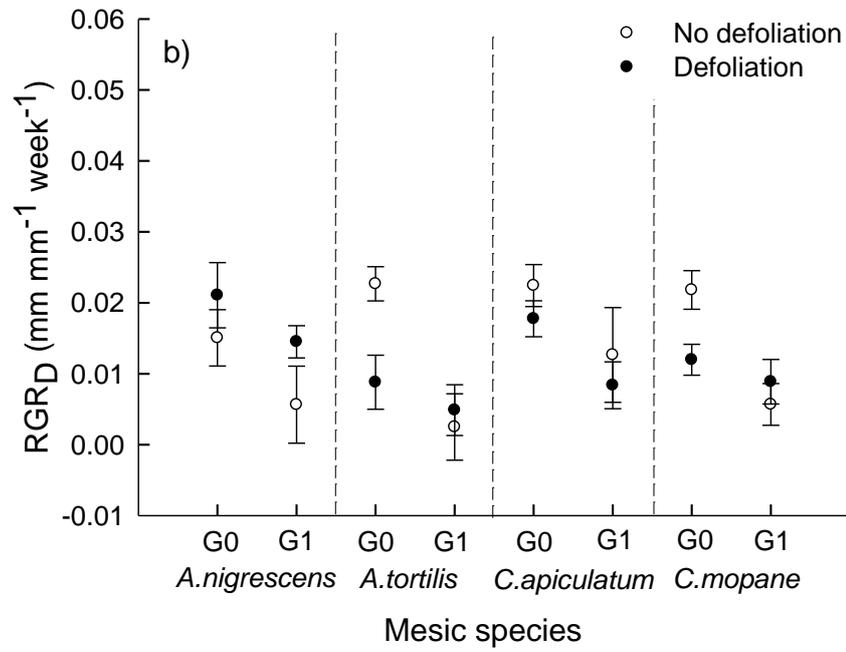
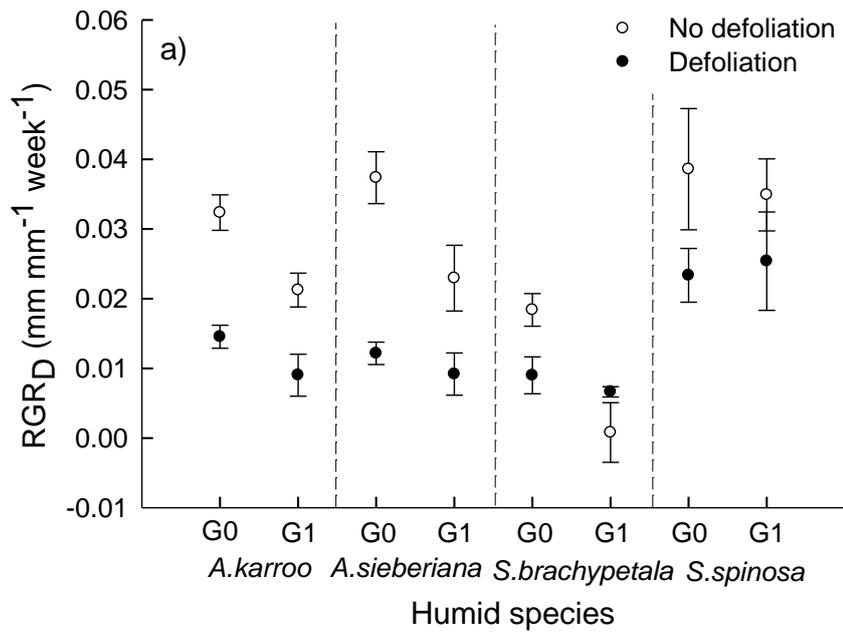


Fig. 5. Mean (\pm 1SE) relative growth rate of sapling stem basal diameter (RGR_D) of (a) humid and (b) mesic species in response to clipping and grass competition. The treatment levels of grass (G1- grass competition and G0 – no grass) are represented on the x-axis, for each species. To be read as a two-way interactive effect of clipping and grass on RGR_D of each species separately. A significant negative effect of grass competition on sapling RGR_D was observed in the absence of clipping in *Schotia brachypetala*.

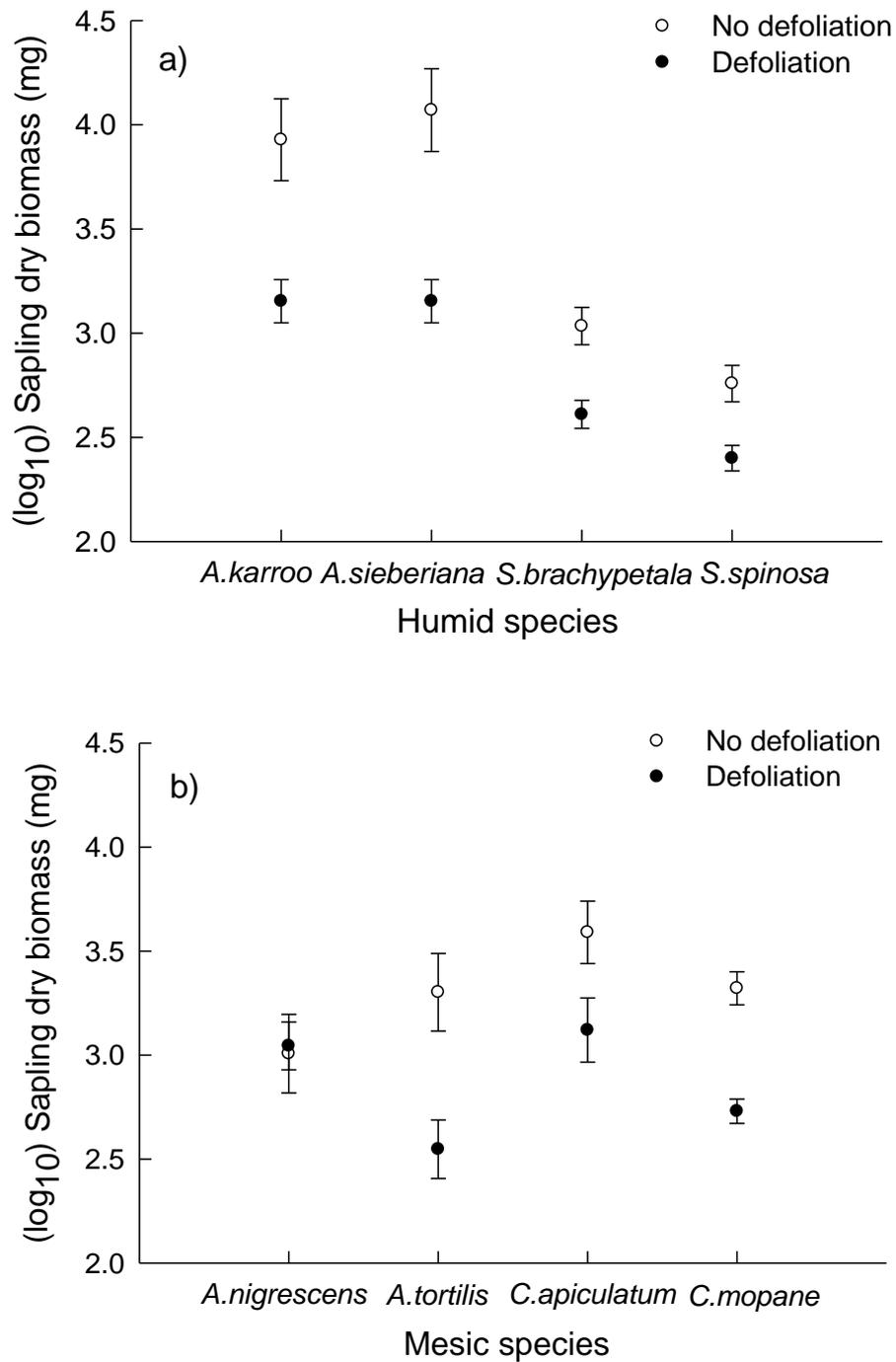


Fig. 6. The log₁₀ mean (\pm 1SE) sapling dry biomass (SDB) values (mg) of (a) humid and (b) mesic species in response to the clipping treatment. Sapling dry biomass was significantly lower in defoliated *Acacia sieberiana*, *Schotia brachypetala* and *Colophospermum mopane* saplings.

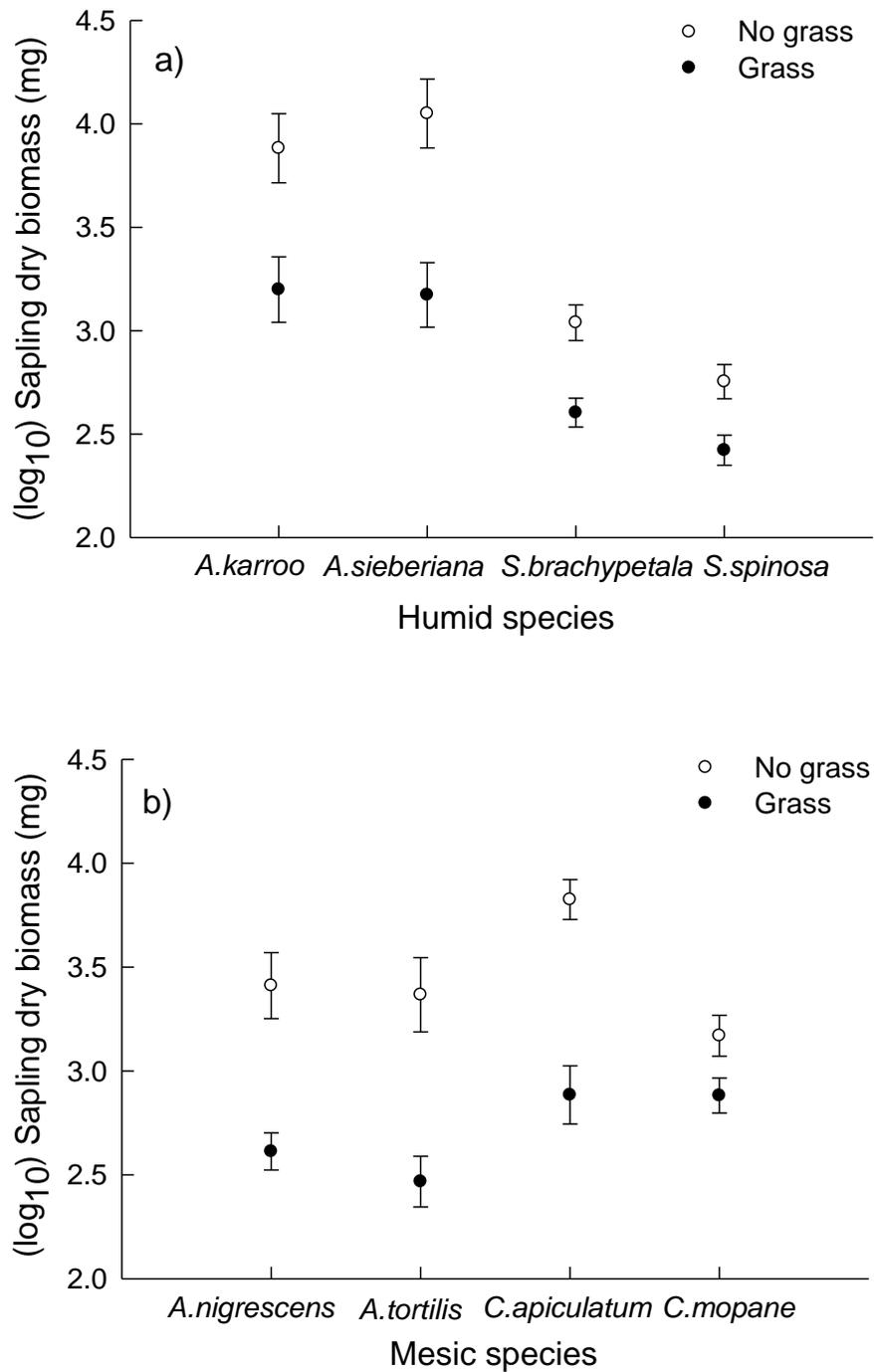


Fig. 7. The log₁₀ mean (\pm 1SE) sapling dry biomass (SDB) values (mg) of (a) humid and (b) mesic species in response to grass competition. All species showed significantly lower SDB in the presence of grass competition.

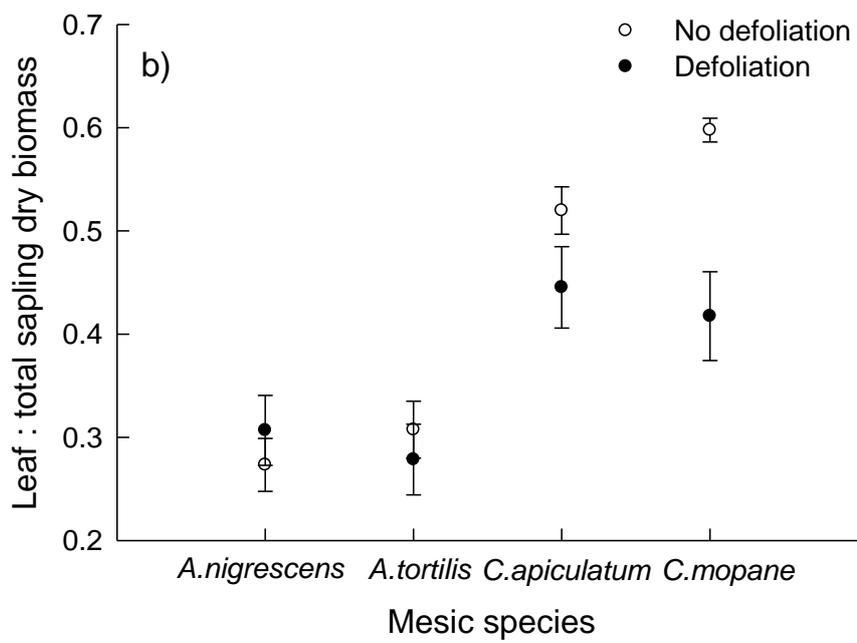
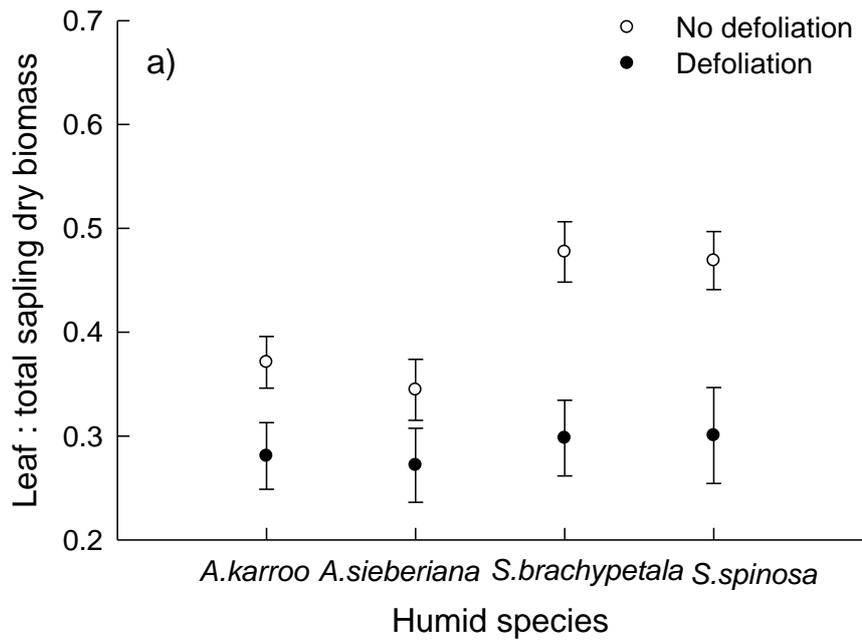


Fig. 8. Mean (\pm 1SE) leaf proportion (LP - taken as a ratio of leaf dry biomass to total sapling dry biomass) values of (a) humid and (b) mesic species in response to the clipping treatment. Defoliated saplings had lower LP when compared to the intact plants in *Acacia karroo*, *Acacia sieberiana*, *Schotia brachypetala*, *Strychnos spinosa*, *Combretum apiculatum* and *Colophospermum mopane*.

Supporting information

Table S1. Linear mixed model (lme) analysis using maximum likelihood, showing the individual and interactive effects of clipping (D), nutrient (N) and grass (G) treatments (two levels each) on *Acacia karroo* saplings. Treatments were provided in the presence of irrigation (high water availability) and natural light. Interactive effects of nutrient and grass (sub-plot) within the treatment clipping (performed at a whole plot level) within replicates (block units) was considered as split plot error. We analyzed final-initial relative growth rate of stem length (RGR_L in $\text{mm mm}^{-1} \text{ week}^{-1}$) and final-initial relative growth rate in stem basal diameter (RGR_D in $\text{mm mm}^{-1} \text{ week}^{-1}$) after clipping in April 2010. The final proportion of leaf to sapling dry biomass (LP) and final \log_{10} transformed sapling dry biomass (SDB in mg), taken in June 2010, were analyzed. The numerator degrees of freedom (df), error df, F-value and significance are indicated per factor and factor interactions. The values in bold are significant, using a Bonferroni correction ($p < 0.01$)

<i>Acacia karroo</i>													
Source	df	RGR_L			RGR_D			LP			SDB		
		Err df	F	p	Err df	F	p	Err df	F	p	Err df	F	p
D	1	4	164.7995	0.0002	4	38.9369	0.0034	4	1.7631	0.2549	4	10.8405	0.0301
N	1	24	5.7784	0.0243	24	0.3512	0.5590	24	0.219	0.6440	24	5.4105	0.0288
G	1	24	11.3578	0.0025	24	11.9446	0.0021	24	10.8977	0.0030	24	14.9996	0.0007
D:N	1	24	10.2968	0.0038	24	0.2008	0.6581	24	4.3184	0.0486	24	0.22	0.6433
D:G	1	24	7.1135	0.0135	24	1.3485	0.2570	24	0.2381	0.6300	24	0.1124	0.7403
N:G	1	24	0.2645	0.6118	24	0.0774	0.7832	24	0.6702	0.4210	24	0.8192	0.3744
D:N:G	1	24	5.5234	0.0273	24	4.176	0.0521	24	0.0002	0.9892	24	0.3419	0.5642

Table S2. Linear mixed model (lme) analysis using maximum likelihood, showing the individual and interactive effects of clipping (D), nutrient (N) and grass (G) treatments (two levels each) on *Acacia sieberiana* saplings. Treatments were provided in the presence of irrigation (high water availability) and natural light. Interactive effects of nutrient and grass (sub-plot) within the treatment clipping (performed at a whole plot level) within replicates (block units) was considered as split plot error. We analyzed final-initial relative growth rate of stem length (RGR_L in mm mm⁻¹ week⁻¹) and final-initial relative growth rate in stem basal diameter (RGR_D in mm mm⁻¹ week⁻¹) after clipping in April 2010. The final proportion of leaf to sapling dry biomass (LP) and final log₁₀ transformed sapling dry biomass (SDB in mg), taken in June 2010, were analyzed. The numerator degrees of freedom (df), error df, F-value and significance are indicated per factor and factor interactions. The values in bold are significant, using a Bonferroni correction (p < 0.01)

Source	<i>Acacia sieberiana</i>												
	df	RGR _L			RGR _D			LP			SDB		
		Err df	F	p	Err df	F	p	Err df	F	p	Err df	F	p
D	1	4	509.8795	<.0001	4	33.9561	0.0043	4	0.9417	0.3868	4	24.9335	0.0075
N	1	24	0.0347	0.8539	24	1.4615	0.2385	23	0.0003	0.9867	24	5.3191	0.0300
G	1	24	11.196	0.0027	24	6.772	0.0156	23	2.808	0.1073	24	33.6515	<.0001
D:N	1	24	0.3936	0.5363	24	1.2017	0.2839	23	0.8986	0.3530	24	2.9697	0.0977
D:G	1	24	11.6317	0.0023	24	2.9284	0.0999	23	2.5244	0.1258	24	3.9787	0.0576
N:G	1	24	7.7577	0.0103	24	0.0086	0.9268	23	0.0791	0.7810	24	1.2874	0.2677
D:N:G	1	24	4.1142	0.0538	24	0.0785	0.7818	23	0.0027	0.9589	24	0.308	0.5840

Table S3. Linear mixed model (lme) analysis using maximum likelihood, showing the individual and interactive effects of clipping (D), nutrient (N) and grass (G) treatments (two levels each) on *Schotia brachypetala* saplings. Treatments were provided in the presence of irrigation (high water availability) and natural light. Interactive effects of nutrient and grass (sub-plot) within the treatment clipping (performed at a whole plot level) within replicates (block units) was considered as split plot error. We analyzed final-initial relative growth rate of stem length (RGR_L in mm mm⁻¹ week⁻¹) and final-initial relative growth rate in stem basal diameter (RGR_D in mm mm⁻¹ week⁻¹) after clipping in April 2010. The final proportion of leaf to sapling dry biomass (LP) and final log₁₀ transformed sapling dry biomass (SDB in mg), taken in June 2010, were analyzed. The numerator degrees of freedom (df), error df, F-value and significance are indicated per factor and factor interactions. The values in bold are significant, using a Bonferroni correction ($p < 0.01$)

<i>Schotia brachypetala</i>													
Source	df	RGR _L			RGR _D			LP			SDB		
		Err df	F	p	Err df	F	p	Err df	F	p	Err df	F	p
D	1	4	143.5584	0.0003	4	0.5684	0.4928	4	10.8334	0.0302	4	22.7708	0.0088
N	1	24	1.0931	0.3062	24	2.9621	0.0981	24	0.2683	0.6092	24	8.0157	0.0092
G	1	24	4.0652	0.0551	24	17.9153	0.0003	24	6.3282	0.0190	24	36.4627	<.0001
D:N	1	24	1.9293	0.1776	24	0.2961	0.5914	24	0.4308	0.5179	24	3.8648	0.0610
D:G	1	24	2.9936	0.0964	24	10.4143	0.0036	24	0.8286	0.3717	24	3.309	0.0814
N:G	1	24	2.1488	0.1557	24	1.1103	0.3025	24	0.6495	0.4282	24	0.001	0.9754
D:N:G	1	24	0.0005	0.9821	24	6.3467	0.0188	24	2.7229	0.1119	24	0.2275	0.6377

Table S4. Linear mixed model (lme) analysis using maximum likelihood, showing the individual and interactive effects of clipping (D), nutrient (N) and grass (G) treatments (two levels each) on *Strychnos spinosa* saplings. Treatments were provided in the presence of irrigation (high water availability) and natural light. Interactive effects of nutrient and grass (sub-plot) within the treatment clipping (performed at a whole plot level) within replicates (block units) was considered as split plot error. We analyzed final-initial relative growth rate of stem length (RGR_L in mm mm⁻¹ week⁻¹) and final-initial relative growth rate in stem basal diameter (RGR_D in mm mm⁻¹ week⁻¹) after clipping in April 2010. The final proportion of leaf to sapling dry biomass (LP) and final log₁₀ transformed sapling dry biomass (SDB in mg), taken in June 2010, were analyzed. The numerator degrees of freedom (df), error df, F-value and significance are indicated per factor and factor interactions. The values in bold are significant, using a Bonferroni correction ($p < 0.01$)

Source	<i>Strychnos spinosa</i>												
	df	RGR _L			RGR _D			LP			SDB		
		Err df	F	p	Err df	F	p	Err df	F	p	Err df	F	p
D	1	4	167.9813	0.0002	4	3.1486	0.1507	4	3.7745	0.1240	4	7.2289	0.0547
N	1	23	0.0211	0.8859	23	2.4787	0.1291	23	1.3758	0.2528	23	0.4367	0.5153
G	1	23	1.0248	0.3219	23	0	0.9993	23	0.9803	0.3324	23	16.1504	0.0005
D:N	1	23	16.774	0.0004	23	0.9634	0.3365	23	3.728	0.0659	23	1.1888	0.2869
D:G	1	23	1.5443	0.2265	23	0.528	0.4748	23	0.8121	0.3768	23	0.1001	0.7545
N:G	1	23	11.1992	0.0028	23	0.3238	0.5748	23	0.1837	0.6722	23	2.3594	0.1382
D:N:G	1	23	2.5728	0.1224	23	0.0048	0.9455	23	1.3078	0.2646	23	0.1249	0.7270

Table S5. Linear mixed model (lme) analysis using maximum likelihood, showing the individual and interactive effects of clipping (D), nutrient (N) and grass (G) treatments (two levels each) on *Acacia nigrescens* saplings. Treatments were provided in the presence of irrigation (high water availability) and natural light. Interactive effects of nutrient and grass (sub-plot) within the treatment clipping (performed at a whole plot level) within replicates (block units) was considered as split plot error. We analyzed final-initial relative growth rate of stem length (RGR_L in mm mm⁻¹ week⁻¹) and final-initial relative growth rate in stem basal diameter (RGR_D in mm mm⁻¹ week⁻¹) after clipping in April 2010. The final proportion of leaf to sapling dry biomass (LP) and final log₁₀ transformed sapling dry biomass (SDB in mg), taken in June 2010, were analyzed. The numerator degrees of freedom (df), error df, F-value and significance are indicated per factor and factor interactions. The values in bold are significant, using a Bonferroni correction (p < 0.01)

Source	<i>Acacia nigrescens</i>												
	df	RGR _L			RGR _D			LP			SDB		
		Err df	F	p	Err df	F	p	Err df	F	p	Err df	F	p
D	1	2	450.8569	0.0022	2	0.123	0.7594	2	0.0472	0.8481	2	0.2617	0.6599
N	1	15	0.1591	0.6955	15	2.7104	0.1205	15	1.1965	0.2913	15	10.1154	0.0062
G	1	15	1.292	0.2735	15	6.9382	0.0188	15	0.5461	0.4713	15	32.1384	<.0001
D:N	1	15	0.1637	0.6915	15	0.743	0.4022	15	2.1365	0.1645	15	2.8126	0.1142
D:G	1	15	4.9481	0.0419	15	1.024	0.3276	15	0.0563	0.8157	15	0.2062	0.6562
N:G	1	15	1.143	0.3019	15	2.2868	0.1513	15	0.3413	0.5678	15	0.7897	0.3882
D:N:G	1	15	0.0291	0.8669	15	0.2764	0.6067	15	0.0239	0.8792	15	0.3613	0.5568

Table S6. Linear mixed model (lme) analysis using maximum likelihood, showing the individual and interactive effects of clipping (D), nutrient (N) and grass (G) treatments (two levels each) on *Acacia tortilis* saplings. Treatments were provided in the presence of irrigation (high water availability) and natural light. Interactive effects of nutrient and grass (sub-plot) within the treatment clipping (performed at a whole plot level) within replicates (block units) was considered as split plot error. We analyzed final-initial relative growth rate of stem length (RGR_L in mm mm⁻¹ week⁻¹) and final-initial relative growth rate in stem basal diameter (RGR_D in mm mm⁻¹ week⁻¹) after clipping in April 2010. The final proportion of leaf to sapling dry biomass (LP) and final log₁₀ transformed sapling dry biomass (SDB in mg), taken in June 2010, were analyzed. The numerator degrees of freedom (df), error df, F-value and significance are indicated per factor and factor interactions. The values in bold are significant, using a Bonferroni correction ($p < 0.01$)

Source	<i>Acacia tortilis</i>												
	df	RGR _L			RGR _D			LP			SDB		
		Err df	F	p	Err df	F	p	Err df	F	p	Err df	F	p
D	1	3	199.6878	0.0008	3	2.0539	0.2473	3	0.5175	0.5239	3	23.2825	0.0170
N	1	18	12.8224	0.0021	18	0.0405	0.8427	18	0.0771	0.7845	18	5.4247	0.0317
G	1	18	9.7044	0.0060	18	15.5461	0.0010	18	28.499	<.0001	18	32.6542	<.0001
D:N	1	18	10.2902	0.0049	18	1.7373	0.2040	18	0.1723	0.6830	18	1.6078	0.2210
D:G	1	18	0.571	0.4596	18	6.4513	0.0205	18	0.4672	0.5030	18	3.4705	0.0789
N:G	1	18	7.7684	0.0122	18	0.2729	0.6078	18	2.9712	0.1019	18	1.5663	0.2268
D:N:G	1	18	3.3023	0.0859	18	0.0903	0.7673	18	5.9991	0.0248	18	2.4784	0.1328

Table S7. Linear mixed model (lme) analysis using maximum likelihood, showing the individual and interactive effects of clipping (D), nutrient (N) and grass (G) treatments (two levels each) on *Combretum apiculatum* saplings. Treatments were provided in the presence of irrigation (high water availability) and natural light. Interactive effects of nutrient and grass (sub-plot) within the treatment clipping (performed at a whole plot level) within replicates (block units) was considered as split plot error. We analyzed final-initial relative growth rate of stem length (RGR_L in $mm\ mm^{-1}\ week^{-1}$) and final-initial relative growth rate in stem basal diameter (RGR_D in $mm\ mm^{-1}\ week^{-1}$) after clipping in April 2010. The final proportion of leaf to sapling dry biomass (LP) and final \log_{10} transformed sapling dry biomass (SDB in mg), taken in June 2010, were analyzed. The numerator degrees of freedom (df), error df, F-value and significance are indicated per factor and factor interactions. The values in bold are significant, using a Bonferroni correction ($p < 0.01$)

Source	<i>Combretum apiculatum</i>												
	df	RGR_L			RGR_D			LP			SDB		
		Err df	F	p	Err df	F	p	Err df	F	p	Err df	F	p
D	1	4	593.0952	<.0001	4	0.5753	0.4904	4	1.8045	0.2503	4	8.3381	0.0447
N	1	24	15.6141	0.0006	24	3.4105	0.0772	24	1.2992	0.2656	24	31.598	<.0001
G	1	24	36.4946	<.0001	24	8.3144	0.0082	24	7.4197	0.0118	24	87.7157	<.0001
D:N	1	24	6.2374	0.0198	24	1.4357	0.2425	24	1.3654	0.2541	24	2.2258	0.1487
D:G	1	24	37.0992	<.0001	24	0.0036	0.9528	24	3.2121	0.0857	24	2.009	0.1692
N:G	1	24	1.8671	0.1845	24	0.159	0.6936	24	0.0793	0.7806	24	0.3718	0.5477
D:N:G	1	24	1.6573	0.2102	24	1.2973	0.2659	24	0.3358	0.5676	24	5.5901	0.0265

Table S8. Linear mixed model (lme) analysis using maximum likelihood, showing the individual and interactive effects of clipping (D), nutrient (N) and grass (G) treatments (two levels each) on *Colophospermum mopane* saplings. Treatments were provided in the presence of irrigation (high water availability) and natural light. Interactive effects of nutrient and grass (sub-plot) within the treatment clipping (performed at a whole plot level) within replicates (block units) was considered as split plot error. We analyzed final-initial relative growth rate of stem length (RGR_L in mm mm⁻¹ week⁻¹) and final-initial relative growth rate in stem basal diameter (RGR_D in mm mm⁻¹ week⁻¹) after clipping in April 2010. The final proportion of leaf to sapling dry biomass (LP) and final log₁₀ transformed sapling dry biomass (SDB in mg), taken in June 2010, were analyzed. The numerator degrees of freedom (df), error df, F-value and significance are indicated per factor and factor interactions. The values in bold are significant, using a Bonferroni correction (p < 0.01)

<i>Colophospermum mopane</i>													
Source	df	RGR _L			RGR _D			LP			SDB		
		Err df	F	p	Err df	F	p	Err df	F	p	Err df	F	p
D	1	4	688.8154	<.0001	4	1.7106	0.2610	4	6.6396	0.0615	4	67.2889	0.0012
N	1	24	27.6064	<.0001	24	1.1712	0.2899	24	0.1846	0.6713	24	3.9554	0.0582
G	1	24	42.4533	<.0001	24	14.3835	0.0009	24	1.1794	0.2883	24	15.959	0.0005
D:N	1	24	18.3622	0.0003	24	2.5949	0.1203	24	0.0291	0.8661	24	3.0484	0.0936
D:G	1	24	14.396	0.0009	24	6.6203	0.0167	24	0.1854	0.6706	24	1.5471	0.2256
N:G	1	24	5.1601	0.0324	24	1.2235	0.2796	24	0.021	0.8861	24	0.0905	0.7661
D:N:G	1	24	7.7311	0.0104	24	5.7051	0.0251	24	0.0022	0.9627	24	0.4628	0.5028

Chapter 5: Conclusions

General discussion

The physiognomy of savannas ranges from open grasslands with few trees to tall closed woodlands with underlying grass layer (Cole 1986, Jeltsch et al. 2000). A unified theory explaining the mechanism behind long-term coexistence of trees and grass in savannas remains elusive (Sankaran et al. 2004, Wiegand et al. 2006, Bond 2008, Higgins et al. 2010). Early ecological models concentrated on competition-based niche separation between the two life forms to explain coexistence (Walter 1939, Walker et al. 1981). The niche separation mechanism invoked by Walter (1939) assumes that water is the primary factor that limits plant growth in semi-arid savannas, with trees and grasses avoiding competition by accessing water at different depths of soil. Grasses access upper soil layers whereas trees access deeper layers of soil. However, this model largely ignores the competition that might arise between tree seedlings and grasses when their roots are confined to the upper layers of soil profile (Jurena and Archer 2003, Ward 2005). Walter's (1939) two-layer hypothesis also ignores the role of other factors that might play an important role in maintaining tree-grass ratios in areas receiving heavy rainfall or where there is no water limitation. More recent models have recognized the fundamental roles of disturbance (fire and herbivory) and climatic variability (mainly unpredictable variations in rainfall) in explaining the coexistence of trees and grasses in savannas (Higgins et al. 2000; Jeltsch et al. 2000). These models advocate coexistence through various environmental effects on different life history stages of trees (Sankaran et al. 2004) and concur that tree sapling recruitment is critical to the long-term dynamics of savannas. It is commonly recognized that the

sapling stage is the most vulnerable phase in the life history cycle of trees (Harper 1977, Bond 2008, Chidumayo 2008), particularly due to their slow growth.

Primarily, tree sapling recruitment is affected by the limitation of resources such as water, nutrients and light (O'Connor 1995, Davis et al. 1999, Kraaij and Ward 2006) and disturbances such as fire and herbivory (Jeltsch et al. 2000, Sankaran et al. 2008, Midgley et al. 2010). All these factors influence tree establishment either directly or indirectly by affecting grass competitive effects on sapling growth (Davis et al. 1999, van Langevelde et al. 2011). The importance of fire and herbivory (disturbances) in restricting savanna sapling growth increases along the mean annual rainfall gradient (Sankaran et al. 2005). While sapling recruitment is mainly limited by water supply in arid savannas (Jeltsch et al. 1996, Higgins et al. 2000, Kraaij and Ward 2006), fire and herbivory have greater effects in humid savannas (van Langevelde et al. 2003, Bond 2008).

Physiologically, from a sapling perspective, the ability of savanna trees to utilize available resources and invest rapidly in aboveground growth will determine its establishment in the ecosystem despite the disturbances that may be encountered. Although savanna saplings have the ability to resprout and persist after disturbances (Bond and van Wilgen 1996, Gignoux et al. 1997), it is sapling stem growth rates (particularly in height) that determine escape frequency from fires (Higgins et al. 2000). The allocation to stem height occurs at the expense of diameter (Sumida et al. 1997), which is positively correlated with bark thickness (Gignoux et al. 1997). However, Lawes et al. (2011) have recently suggested that stem diameter and height are different pathways to achieve thick bark and suggest the importance of bark thickness in stem protection against fires. Savanna tree saplings utilize stored nonstructural carbohydrate root reserves to

resprout (Schutz et al. 2009, Wigley et al. 2009) and resources for storage are mainly derived from photosynthesis when the leaves are not damaged (Sakai and Sakai 1998). Thus, the key species-specific traits or proxy stem-growth measurements that determine the success of savanna tree saplings will be their total aboveground growth, stem growth rates (diameter and height), and investment in leaves. Wherever pertinent in this chapter, I refer to these plant functional growth traits (see e.g. Poorter et al. 2012, Siefert 2012) as ‘growth’ for convenience.

Research summary and specific future directions

Induction of defences in savanna tree species due to herbivory, even at the sapling stage (Hean and Ward 2012), is well known (Gowda 1997, Rohner and Ward 1997, Zinn et al. 2007). It is well understood that defence investment by saplings resprouting after fire is expensive but important to prevent further damage due to herbivory. However, there is a notably scarcity of studies investigating resprouter defence dynamics (Clarke et al. 2013). Hence, in **Chapter 2**, I investigated the effects of fire and nutrient gradient on the survival, growth and in particular defence investment of four *Acacia* species’ saplings under greenhouse conditions. In **Chapter 2**, I found that burning had no influence on resprout defence investment but the resprouts significantly increased stem height growth rate. This indicates that resprouts may remain very vulnerable to herbivores. My study also confirms that species differ considerably in their sapling tolerance to burning (Bond 2008). Within the humid species tested, *Acacia sieberiana* was tolerant with high resprout survival, whereas *Acacia karroo* showed complete intolerance to fire. It implies there could be a major shift in tree species composition of humid savannas even after a single fire event. Clarke et al. (2013) recognized an ongoing debate about bud-bank limitation

being more important than resource limitation in determining resprouting success of plants. My results show that resource limitation has no effect on a sapling's ability to resprout. This adds weight to the notion that species-specific ability to produce and protect buds determines sapling regenerative success. Based on these results, future research could focus on investigating the effects of different intensities of fire and frequency of fires on sapling regenerative capability of various species. Fire intensity determines tree dynamics in mesic and humid savanna (Govender et al. 2006). However, the frequency of fire at a particular intensity will help determine the time taken to exhaust the bud bank for a given species (Clarke et al. 2013). This will enable us to categorize species according to their resilience to fires and better predict landscape level changes in species structure and composition.

In **Chapter 3**, I explored the relative importance of water, nutrients, shade availability, grass competition and their interactions on tree sapling establishment in a humid savanna. I studied the survival and growth of four humid and four mesic savanna tree species. My study also contributes to the understanding of species-specific functional traits in a humid environment, which may provide a useful insight to savanna ecosystem functioning (Lehmann et al. 2009, Poorter et al. 2012, Siefert 2012). I found that, in humid savannas, sapling *survival* was not dependent on any of the factors provided. This could largely be due to the presence of favourable conditions such as low irradiance, temperature and high rainfall in humid savannas (Jagtap 1995). It is generally understood that humid savannas support high grass fuel loads, resulting in frequent fires inhibiting tree seedling establishment (Trollope 1980, Bond 2008, Sankaran et al. 2008). My results indicated that shade is the most important factor suppressing tree sapling growth in humid savannas, although grass competition also had a substantial

negative effect. Therefore, light could be a potential limiting factor affecting tree-grass coexistence in humid savannas.

In the wake of continuous and rapid increases in global atmospheric CO₂ (Norby and Zak 2011), further research could be streamlined to understand the effects of CO₂ concentration on sapling growth which may better predict ecosystem level changes in humid savannas. Increased concentrations of CO₂ activate plant photosynthesis and productivity and indirectly affect the ecosystem (Ainsworth and Rogers 2007, Kgope et al. 2010). An increase in plant growth with an increase in CO₂ produces a high demand for nutrients, leading to a rapid exhaustion of nutrients which in turn may limit further increases in plant growth even in the presence of elevated CO₂ levels (Körner 2006). Hence, benefits of CO₂ increase on plant productivity are contingent upon the availability of other resources such as water, light and nutrients (Körner 2006). In addition, numerous studies based on temperate ecosystems suggest that it is important to understand CO₂ interactions with other environmental factors such as temperature, precipitation and soil moisture (Leakey et al. 2012). However, Kgope et al. (2010) indicated that CO₂ may indirectly interact with fire and herbivory in tropical savannas by altering tree sapling growth, productivity and ability to recover from disturbances. Despite disturbances, higher growth rates of C₃ trees than C₄ grasses (dominant in savannas) under elevated CO₂ levels will enable woody canopy closure (Ward 2010) causing reduction in C₄ grasses which require open, high-light environments to flourish (Bond et al. 2003). However, based on the results obtained from **Chapter 3**, it will be worth determining whether the benefits accrued to tree saplings by increased CO₂ concentrations will overcome the negative effects of shading on tree sapling growth (to attain canopy closure) in humid savannas.

In **Chapter 4**, I tested the effects of clipping (i.e. simulated herbivory by clipping grass and tree saplings), nutrient availability, grass competition and their interactions on sapling survival and growth of four humid and four mesic savanna tree species. This study was performed under controlled field conditions in a humid savanna. My main observation is that clipping had no effect on sapling *survival*. However, defoliated saplings invested rapidly in stem length with a reduced investment in thickness and total biomass. Defoliated saplings were more sensitive to grass competition and benefitted more from nutrient addition than intact plants. Grass competition had a substantial negative effect on sapling growth. The results in **Chapter 4** support the perception that herbivory has a relatively low impact on tree population density (Roques et al. 2001, Sankaran et al. 2005) but can alter savanna vegetative distribution (van de Vijver et al. 1999, Bond 2008). Additionally, these results indicate that tree saplings can become more vulnerable to fire after herbivory (or browsing), particularly in the presence of high grass biomass in humid savannas (Bond 2008), despite the fact that investment in stem length is high. It is well understood that fire and herbivory act synergistically to restrict tree recruitment in savannas (Midgley et al. 2010; Hean and Ward 2011). Lack of investment in defenses after fire (**Chapter 2**) and aboveground biomass after clipping (**Chapter 4**) provides a clear physiological mechanism of sapling vulnerability, in terms of its aboveground functional traits. At an ecosystem level, large mammalian herbivore preferences play an important role in the distribution of woody plants (Levick and Rogers 2008; Gordijn et al. 2012). Therefore, further research on herbivory could include tree species interactions with herbivore preferences to understand woody plant establishment dynamics.

Interpretation and further general research directions

In our study, the mesic species had more or less similar aboveground growth whereas among humid species there was a high variation in growth; this particular pattern of variability was observed in all the functional traits measured (see **Chapter 3**). Clearly, more studies are needed of the intraspecific variation in plant functional traits (cf. Kattge et al. 2011, Poorter et al. 2012, Siefert 2012). Among the humid species I studied, the two *Acacia* species had higher growth than *Schotia brachypetala* and *Strychnos spinosa*. However, these differences were not very obvious when these plants were defoliated (see **Chapter 4**). Studying plant trait variation between humid and mesic savannas will also help us understand the importance of phylogenetic differences among trees in these savanna types (see e.g. Cavender-Bares et al. 2004, Cadotte et al. 2009). For example, in a common garden experiment, Tomlinson et al. (2012) have shown that humid species differed from semi-arid species by allocating higher biomass to roots than shoots, although they observed considerable variation in traits among humid species perhaps due to phylogenetic differences.

Based on our results in **Chapter 3**, tests including a light intensity gradient might provide more insight on plant functional traits. Specific traits of tree species can scale up to provide a better indication of characteristic features of savannas across a climate gradient. For example, forest species are well adapted to shade but lack sufficient root reserves to support sprouting after fires whereas savanna seedlings can resprout (Hoffmann et al. 2004). Hence, sprouting is the key trait difference between forest and savanna species' saplings that determines their success in their respective biomes (Bond 2008). However, factorial drivers for the existence of savanna-forest ecotone boundaries in African humid zones have not been studied. Similarly,

there are no studies comparing the traits of dry forest and arid savanna tree species (Bond 2008). Studies on constraints to tree distribution based on their functional traits (see e.g. Cornelissen et al. 2003, Kattge et al. 2011, Poorter et al. 2012, Siefert 2012) across the drier end of the moisture gradient coupled with studies of humid savannas and forest ecotones are required to understand tree demography among different savanna ecosystems.

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Appendix I: Tables of biological results

Chapter 2: Fire and nutrient gradient effects on the sapling ecology of four *Acacia* species in the presence of grass competition

Table 1. Mean growth, with standard error (SE), of *Acacia karroo* saplings under the influence of various treatment combinations after fire was applied. We measured ratio of final-initial relative growth rate of stem height (RGR_h in $\text{mm mm}^{-1} \text{ week}^{-1}$) to final-initial relative growth rate in stem basal diameter (RGR_d in $\text{mm mm}^{-1} \text{ week}^{-1}$), ratio of final log10 transformed root dry biomass (in mg) to shoot dry biomass (in mg), final log10 transformed leaf CT concentration (in mg mL^{-1} QE) and final power (-0.3) transformed mean thorn length (in mm). Two levels of fire provided are indicated as ‘0’ (control) and ‘1’ (burning). Five levels of nutrients indicate the concentration of fertiliser applied, 0 g (0); 13 g (0.5); 26 g (1); 52 g (2) and 104 g (4), per 0.16 m^2 . See **Methods** for further details

Treatment combination		$RGR_h : RGR_d$		Root : shoot biomass		CT Conc		Thorn length	
Nutrients	Fire	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0	-0.0130	0.1650	-0.2246	0.1102	1.7240	0.0054	0.5159	0.0173
0.5	0	0.0590	0.1030	-0.1399	0.0897	1.7470	0.0035	0.5214	0.0359
1	0	0.1350	0.0080	-0.2467	0.1109	1.7090	0.0101	0.5388	0.0396
2	0	0.0200	0.0520	0.1052	0.2367	1.7360	0.0026	0.5209	0.0165
4	0	0.2700	0.2370	-0.2575	0.1394	1.7090	0.0022	0.5098	0.0313
0	1	*	*	*	*	*	*	*	*
0.5	1	*	*	*	*	*	*	*	*
1	1	*	*	*	*	*	*	*	*
2	1	*	*	*	*	*	*	*	*
4	1	*	*	*	*	*	*	*	*

Table 2. Mean growth, with standard error (SE), of *Acacia nigrescens* saplings under the influence of various treatment combinations after fire was applied. We measured ratio of final-initial relative growth rate of stem height (RGR_h in $\text{mm mm}^{-1} \text{ week}^{-1}$) to final-initial relative growth rate in stem basal diameter (RGR_d in $\text{mm mm}^{-1} \text{ week}^{-1}$), ratio of final log10 transformed root dry biomass (in mg) to shoot dry biomass (in mg), final log10 transformed leaf CT concentration (in mg mL^{-1} QE) and final power (-0.3) transformed mean thorn length (in mm). Two levels of fire provided are indicated as ‘0’ (control) and ‘1’ (burning). Five levels of nutrients indicate the concentration of fertiliser applied, 0 g (0); 13 g (0.5); 26 g (1); 52 g (2) and 104 g (4), per 0.16 m^2 . See **Methods** for further details

Treatment combination		$RGR_h : RGR_d$		Root : shoot biomass		CT Conc		Thorn length	
Nutrients	Fire	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0	0.9100	0.7620	0.0923	0.1341	1.6820	0.0079	0.8295	0.0121
0.5	0	0.5620	0.2270	-0.0104	0.0399	1.6820	0.0101	0.7859	0.0254
1	0	1.6530	0.7950	-0.0402	0.0175	1.7010	0.0163	0.8323	0.0123
2	0	0.8080	0.6390	0.0550	0.0712	1.7150	0.0132	0.8158	0.0429
4	0	-7.5890	6.7210	-0.0651	0.0463	1.6970	0.0052	0.8365	0.0403
0	1	*	*	*	*	*	*	*	*
0.5	1	86.5130	*	0.0532	*	1.7360	*	0.8528	*
1	1	50.3850	*	0.1347	*	*	*	*	*
2	1	*	*	*	*	*	*	*	*
4	1	5.4750	*	0.0503	*	1.6860	*	0.9718	*

Table 3. Mean growth, with standard error (SE), of *Acacia sieberiana* saplings under the influence of various treatment combinations after fire was applied. We measured ratio of final-initial relative growth rate of stem height (RGR_h in $\text{mm mm}^{-1} \text{ week}^{-1}$) to final-initial relative growth rate in stem basal diameter (RGR_d in $\text{mm mm}^{-1} \text{ week}^{-1}$), ratio of final log10 transformed root dry biomass (in mg) to shoot dry biomass (in mg), final log10 transformed leaf CT concentration (in mg mL^{-1} QE) and final power (-0.3) transformed mean thorn length (in mm). Two levels of fire provided are indicated as ‘0’ (control) and ‘1’ (burning). Five levels of nutrients indicate the concentration of fertiliser applied, 0 g (0); 13 g (0.5); 26 g (1); 52 g (2) and 104 g (4), per 0.16 m^2 . See **Methods** for further details

Treatment combination		$RGR_h : RGR_d$		Root : shoot biomass		CT Conc		Thorn length	
Nutrients	Fire	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0	0.6340	0.3130	0.6683	0.1971	1.4030	0.0779	0.5376	0.0272
0.5	0	-0.0850	0.8270	0.4393	0.0253	0.8450	0.2708	0.5076	0.0451
1	0	2.4650	1.4780	0.5114	0.1086	1.3960	0.1552	0.6576	0.1027
2	0	-5.8500	6.5330	0.4864	0.0420	1.2010	0.1690	0.5324	0.0945
4	0	11.2650	10.6920	0.2264	0.0535	1.4080	0.0262	0.4911	0.2374
0	1	2.5070	0.3540	0.4435	0.1160	1.4820	0.1726	0.6919	0.0392
0.5	1	1.6310	0.2500	0.4974	0.1015	1.0870	0.2383	0.6272	0.0554
1	1	2.4250	0.1920	0.4029	0.0069	1.5830	*	0.6391	0.0688
2	1	2.2170	0.4860	0.4080	0.0017	1.2220	0.2143	0.6702	0.0555
4	1	1.2820	0.1700	0.5319	0.0848	1.3130	0.0759	0.6725	0.0922

Table 4. Mean growth, with standard error (SE), of *Acacia tortilis* saplings under the influence of various treatment combinations after fire was applied. We measured ratio of final-initial relative growth rate of stem height (RGR_h in $\text{mm mm}^{-1} \text{ week}^{-1}$) to final-initial relative growth rate in stem basal diameter (RGR_d in $\text{mm mm}^{-1} \text{ week}^{-1}$), ratio of final log10 transformed root dry biomass (in mg) to shoot dry biomass (in mg), final log10 transformed leaf CT concentration (in mg mL^{-1} QE) and final power (-0.3) transformed mean thorn length (in mm). Two levels of fire provided are indicated as ‘0’ (control) and ‘1’ (burning). Five levels of nutrients indicate the concentration of fertiliser applied, 0 g (0); 13 g (0.5); 26 g (1); 52 g (2) and 104 g (4), per 0.16 m^2 . See **Methods** for further details

Treatment combination		RGR _h : RGR _d		Root : shoot biomass		CT Conc		Thorn length	
Nutrients	Fire	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0	1.6430	0.5890	0.3645	0.0187	1.6900	0.0046	0.5698	0.0290
0.5	0	0.9130	0.2330	0.2451	*	1.7030	*	0.6200	0.0212
1	0	5.8750	4.6080	0.2029	0.0998	1.7240	0.0135	0.6042	0.0222
2	0	0.6810	0.1480	0.1092	0.0846	1.6700	0.0041	0.6700	0.0943
4	0	1.1250	0.1770	0.2396	0.0230	1.6680	0.0337	0.6587	0.0505
0	1	2.7770	1.3040	0.3517	0.0494	1.6500	*	0.6259	0.0467
0.5	1	*	*	*	*	*	*	*	*
1	1	*	*	*	*	*	*	*	*
2	1	*	*	*	*	*	*	*	*
4	1	1.5290	*	0.0641	*	1.7060	*	0.5771	*

Chapter 3: Shade, nutrients and grass competition are important for tree sapling establishment in a humid savanna

Table 1. Mean growth, with standard error (SE), of *Acacia karroo* saplings under the influence of various treatment combinations. We measured final-initial relative growth rate of stem length (RGR_L in $\text{mm mm}^{-1} \text{ week}^{-1}$), final-initial relative growth rate in stem basal diameter (RGR_D in $\text{mm mm}^{-1} \text{ week}^{-1}$), final proportion of leaf to sapling dry biomass (LP) and final \log_{10} transformed sapling dry biomass (SDB in mg). Two levels of each treatment provided are indicated as ‘0’ (control) and ‘1’ (treatment). Water: rainfall (0) and regular watering (1), Shade: no shade (0) and 80 % shade (1), Nutrients: without fertilizer (0) and with fertilizer (1) and Grass: absence (0) and presence (1). See **Methods** for further details

Treatment combination				RGR_L		RGR_D		LP		SDB	
Water	Shade	Nutrients	Grass	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0	0	0	0.0329	0.0124	0.0547	0.0074	0.4926	0.0284	3.9370	0.1774
0	0	1	0	0.0635	0.0090	0.0837	0.0066	0.3746	0.0166	4.9650	0.0464
0	1	0	0	0.0404	0.0082	0.0459	0.0053	0.4427	0.0665	3.9920	0.1422
0	1	1	0	0.0309	0.0047	0.0512	0.0071	0.3151	0.0261	3.9000	0.1461
1	0	0	0	0.0474	0.0097	0.0581	0.0091	0.4340	0.0197	4.0250	0.1742
1	0	1	0	0.0450	0.0098	0.0784	0.0070	0.3754	0.0364	4.5740	0.4794
1	1	0	0	0.0342	0.0088	0.0510	0.0064	0.3292	0.0516	3.8860	0.1654
1	1	1	0	0.0569	0.0066	0.0536	0.0045	0.3197	0.0324	4.0930	0.1947
0	0	0	1	0.0005	0.0073	0.0242	0.0056	0.1909	0.0567	3.1430	0.2451
0	0	1	1	0.0223	0.0133	0.0527	0.0118	0.3830	0.0633	3.6210	0.4016
0	1	0	1	0.0468	0.0052	0.0369	0.0071	0.3100	0.0501	3.6070	0.0863
0	1	1	1	0.0473	0.0089	0.0464	0.0071	0.3834	0.0517	4.0000	0.1576
1	0	0	1	0.0178	0.0090	0.0300	0.0033	0.3465	0.0410	3.3390	0.2762
1	0	1	1	0.0548	0.0130	0.0586	0.0086	0.3279	0.0823	3.7760	0.4433
1	1	0	1	0.0472	0.0149	0.0540	0.0069	0.4227	0.0254	3.8610	0.0809
1	1	1	1	0.0436	0.0060	0.0519	0.0054	0.3932	0.0230	3.9300	0.1949

Table 2. Mean growth, with standard error (SE), of *Acacia sieberiana* saplings under the influence of various treatment combinations. We measured final-initial relative growth rate of stem length (RGR_L in $\text{mm mm}^{-1} \text{ week}^{-1}$), final-initial relative growth rate in stem basal diameter (RGR_D in $\text{mm mm}^{-1} \text{ week}^{-1}$), final proportion of leaf to sapling dry biomass (LP) and final \log_{10} transformed sapling dry biomass (SDB in mg). Two levels of each treatment provided are indicated as ‘0’ (control) and ‘1’ (treatment). Water: rainfall (0) and regular watering (1), Shade: no shade (0) and 80 % shade (1), Nutrients: without fertilizer (0) and with fertilizer (1) and Grass: absence (0) and presence (1). See **Methods** for further details

Treatment combination				RGR_L		RGR_D		LP		SDB	
Water	Shade	Nutrients	Grass	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0	0	0	0.0143	0.0134	0.0327	0.0085	0.5921	0.0332	3.5840	0.1576
0	0	1	0	0.0403	0.0138	0.0595	0.0095	0.4890	0.0392	4.0450	0.3282
0	1	0	0	0.0161	0.0075	0.0275	0.0061	0.4546	0.0367	3.5160	0.0899
0	1	1	0	0.0205	0.0107	0.0345	0.0032	0.3870	0.0679	3.6250	0.0898
1	0	0	0	0.0344	0.0059	0.0543	0.0077	0.4236	0.0418	4.3110	0.1322
1	0	1	0	0.0585	0.0077	0.0939	0.0089	0.3722	0.0155	5.0080	0.1374
1	1	0	0	0.0282	0.0082	0.0291	0.0101	0.4703	0.0597	3.5290	0.1595
1	1	1	0	0.0256	0.0066	0.0376	0.0055	0.4719	0.0785	3.8540	0.2239
0	0	0	1	0.0135	0.0057	0.0294	0.0058	0.4958	0.0476	3.3300	0.1809
0	0	1	1	0.0143	0.0034	0.0239	0.0109	0.3289	0.0413	2.9780	0.3006
0	1	0	1	0.0139	0.0113	0.0320	0.0071	0.3581	0.0531	3.1030	0.2911
0	1	1	1	0.0261	0.0079	0.0364	0.0061	0.3758	0.0617	3.7570	0.1796
1	0	0	1	0.0140	0.0106	0.0272	0.0085	0.2929	0.0803	3.2200	0.3558
1	0	1	1	0.0483	0.0147	0.0623	0.0154	0.2755	0.0706	3.7420	0.3779
1	1	0	1	0.0323	0.0119	0.0378	0.0076	0.4600	0.0716	3.1710	0.1221
1	1	1	1	0.0186	0.0095	0.0309	0.0057	0.3783	0.0417	3.6120	0.1129

Table 3. Mean growth, with standard error (SE), of *Schotia brachypetala* saplings under the influence of various treatment combinations. We measured final-initial relative growth rate of stem length (RGR_L in $\text{mm mm}^{-1} \text{ week}^{-1}$), final-initial relative growth rate in stem basal diameter (RGR_D in $\text{mm mm}^{-1} \text{ week}^{-1}$), final proportion of leaf to sapling dry biomass (LP) and final \log_{10} transformed sapling dry biomass (SDB in mg). Two levels of each treatment provided are indicated as ‘0’ (control) and ‘1’ (treatment). Water: rainfall (0) and regular watering (1), Shade: no shade (0) and 80 % shade (1), Nutrients: without fertilizer (0) and with fertilizer (1) and Grass: absence (0) and presence (1). See **Methods** for further details

Treatment combination				RGR_L		RGR_D		LP		SDB	
Water	Shade	Nutrients	Grass	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0	0	0	0.0147	0.0137	0.0308	0.0026	0.5607	0.0441	3.1630	0.1527
0	0	1	0	0.0318	0.0135	0.0435	0.0053	0.5886	0.0288	3.4460	0.0771
0	1	0	0	0.0285	0.0047	0.0157	0.0080	0.5805	0.0475	2.8270	0.1012
0	1	1	0	0.0168	0.0051	0.0269	0.0035	0.4042	0.0454	2.8880	0.1108
1	0	0	0	0.0129	0.0061	0.0347	0.0060	0.5281	0.0796	3.1600	0.1059
1	0	1	0	0.0339	0.0063	0.0423	0.0072	0.4830	0.0535	3.4730	0.0926
1	1	0	0	0.0297	0.0032	0.0265	0.0077	0.6021	0.0203	3.0090	0.0664
1	1	1	0	0.0467	0.0149	0.0382	0.0117	0.5664	0.0276	3.0240	0.0502
0	0	0	1	0.0027	0.0027	0.0064	0.0019	0.3498	0.0771	2.7930	0.2748
0	0	1	1	0.0197	0.0082	0.0219	0.0133	0.4045	0.1188	2.9100	0.4633
0	1	0	1	0.0241	0.0054	0.0140	0.0043	0.5511	0.0567	2.8150	0.0543
0	1	1	1	0.0115	0.0045	0.0176	0.0048	0.3722	0.0414	2.9370	0.0967
1	0	0	1	-0.0029	0.0028	0.0073	0.0056	0.3851	0.0609	2.5620	0.0646
1	0	1	1	0.0224	0.0031	0.0226	0.0017	0.5129	0.0086	2.9390	0.1358
1	1	0	1	0.0261	0.0066	0.0166	0.0038	0.5624	0.0491	2.9570	0.0341
1	1	1	1	0.0190	0.0049	0.0167	0.0049	0.5356	0.0191	2.8980	0.1171

Table 4. Mean growth, with standard error (SE), of *Strychnos spinosa* saplings under the influence of various treatment combinations. We measured final-initial relative growth rate of stem length (RGR_L in $\text{mm mm}^{-1} \text{ week}^{-1}$), final-initial relative growth rate in stem basal diameter (RGR_D in $\text{mm mm}^{-1} \text{ week}^{-1}$), final proportion of leaf to sapling dry biomass (LP) and final \log_{10} transformed sapling dry biomass (SDB in mg). Two levels of each treatment provided are indicated as ‘0’ (control) and ‘1’ (treatment). Water: rainfall (0) and regular watering (1), Shade: no shade (0) and 80 % shade (1), Nutrients: without fertilizer (0) and with fertilizer (1) and Grass: absence (0) and presence (1). See **Methods** for further details

Treatment combination				RGR_L		RGR_D		LP		SDB	
Water	Shade	Nutrients	Grass	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0	0	0	-0.0153	0.0144	0.0149	0.0074	0.4804	0.0765	2.8280	0.2297
0	0	1	0	-0.0174	0.0148	0.0077	0.0039	0.4012	0.0367	2.6750	0.1325
0	1	0	0	0.0099	0.0133	0.0284	0.0083	0.3934	0.1163	2.5540	0.0923
0	1	1	0	-0.0264	0.0146	0.0315	0.0195	0.2933	0.0799	2.4930	0.2490
1	0	0	0	-0.0119	0.0113	0.0284	0.0078	0.3863	0.0818	2.7810	0.1746
1	0	1	0	0.0214	0.0054	0.0314	0.0030	0.5535	0.0098	3.0780	0.1624
1	1	0	0	-0.0069	0.0152	0.0171	0.0075	0.4726	0.0979	2.6120	0.0860
1	1	1	0	0.0143	0.0053	0.0233	0.0055	0.4821	0.0675	2.9810	0.1278
0	0	0	1	-0.0417	0.0113	0.0019	0.0087	0.2683	0.1020	2.2670	0.1681
0	0	1	1	-0.0118	0.0070	0.0024	0.0026	0.4306	0.0294	2.4000	0.1010
0	1	0	1	-0.0112	0.0076	0.0227	0.0155	0.4030	0.0765	2.5790	0.0726
0	1	1	1	-0.0163	0.0126	0.0181	0.0165	0.3732	0.1118	2.4890	0.1266
1	0	0	1	-0.0145	0.0053	0.0140	0.0066	0.4400	0.0345	2.5890	0.1991
1	0	1	1	0.0144	0.0050	0.0103	0.0108	0.4959	0.0548	2.5860	0.0914
1	1	0	1	0.0006	0.0105	0.0166	0.0041	0.4376	0.0701	2.4560	0.1827
1	1	1	1	0.0021	0.0128	0.0132	0.0072	0.4524	0.1026	2.6740	0.1128

Table 5. Mean growth, with standard error (SE), of *Acacia nigrescens* saplings under the influence of various treatment combinations. We measured final-initial relative growth rate of stem length (RGR_L in $\text{mm mm}^{-1} \text{ week}^{-1}$), final-initial relative growth rate in stem basal diameter (RGR_D in $\text{mm mm}^{-1} \text{ week}^{-1}$), final proportion of leaf to sapling dry biomass (LP) and final \log_{10} transformed sapling dry biomass (SDB in mg). Two levels of each treatment provided are indicated as ‘0’ (control) and ‘1’ (treatment). Water: rainfall (0) and regular watering (1), Shade: no shade (0) and 80 % shade (1), Nutrients: without fertilizer (0) and with fertilizer (1) and Grass: absence (0) and presence (1). See **Methods** for further details

Treatment combination				RGR_L		RGR_D		LP		SDB	
Water	Shade	Nutrients	Grass	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0	0	0	0.0120	0.0050	0.0357	0.0023	0.1592	0.0787	3.8100	0.1656
0	0	1	0	0.0245	0.0013	0.0625	0.0095	0.2983	0.0231	4.0530	0.6521
0	1	0	0	0.0121	0.0106	0.0373	0.0095	0.3871	0.0122	3.3080	0.0973
0	1	1	0	0.0121	0.0081	0.0386	0.0046	0.2492	0.0748	3.5300	0.1058
1	0	0	0	0.0004	0.0045	0.0436	0.0160	0.2430	0.0350	3.1110	0.4628
1	0	1	0	0.0344	0.0108	0.0615	0.0016	0.3261	0.0235	4.1300	0.2241
1	1	0	0	0.0220	0.0116	0.0493	0.0091	0.3701	0.0276	3.4590	0.2068
1	1	1	0	0.0214	0.0073	0.0486	0.0034	0.3992	0.0282	3.7180	0.1610
0	0	0	1	-0.0225	*	0.0074	*	0.0000	*	2.2790	*
0	0	1	1	*	*	*	*	*	*	*	*
0	1	0	1	0.0093	0.0067	0.0353	0.0084	0.3299	0.0344	3.3540	0.1806
0	1	1	1	0.0340	0.0119	0.0378	0.0031	0.2434	0.0349	3.3780	0.1523
1	0	0	1	0.0063	0.0137	0.0137	0.0120	0.1865	0.0924	2.2910	0.1151
1	0	1	1	0.0250	0.0109	0.0332	0.0054	0.3246	0.0600	2.8290	0.2772
1	1	0	1	0.0188	0.0026	0.0334	0.0094	0.3814	0.0413	3.1160	0.1475
1	1	1	1	0.0264	0.0141	0.0458	0.0088	0.3214	0.0487	3.4000	0.2769

Table 6. Mean growth, with standard error (SE), of *Acacia tortilis* saplings under the influence of various treatment combinations. We measured final-initial relative growth rate of stem length (RGR_L in $\text{mm mm}^{-1} \text{ week}^{-1}$), final-initial relative growth rate in stem basal diameter (RGR_D in $\text{mm mm}^{-1} \text{ week}^{-1}$), final proportion of leaf to sapling dry biomass (LP) and final \log_{10} transformed sapling dry biomass (SDB in mg). Two levels of each treatment provided are indicated as ‘0’ (control) and ‘1’ (treatment). Water: rainfall (0) and regular watering (1), Shade: no shade (0) and 80 % shade (1), Nutrients: without fertilizer (0) and with fertilizer (1) and Grass: absence (0) and presence (1). See **Methods** for further details

Treatment combination				RGR_L		RGR_D		LP		SDB	
Water	Shade	Nutrients	Grass	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0	0	0	0.0379	0.0262	0.0432	0.0153	0.4825	0.0291	2.9490	0.4159
0	0	1	0	0.0667	0.0154	0.0641	0.0080	0.4242	0.0286	4.1200	0.4949
0	1	0	0	0.0163	0.0106	0.0197	0.0057	0.3086	0.0474	2.5260	0.1942
0	1	1	0	0.0071	0.0146	0.0117	0.0123	0.2759	0.0550	2.6780	0.3320
1	0	0	0	0.0220	0.0084	0.0528	0.0053	0.3962	0.0447	3.5040	0.2401
1	0	1	0	0.0616	0.0070	0.0748	0.0094	0.3723	0.0297	4.0790	0.2618
1	1	0	0	0.0287	0.0081	0.0167	0.0124	0.3891	0.0800	2.4750	0.3292
1	1	1	0	0.0150	0.0068	0.0284	0.0080	0.3081	0.0674	2.7490	0.1366
0	0	0	1	-0.0039	0.0064	0.0106	0.0092	0.2133	0.0656	2.0760	0.1906
0	0	1	1	0.0183	0.0138	0.0089	0.0152	0.2612	0.0824	2.4500	0.1864
0	1	0	1	0.0121	0.0091	-0.0016	0.0088	0.1236	0.0398	2.1200	0.1490
0	1	1	1	0.0019	0.0089	0.0197	0.0054	0.3165	0.0816	2.3700	0.2131
1	0	0	1	0.0005	0.0051	-0.0024	0.0127	0.2014	0.0641	2.2640	0.0926
1	0	1	1	0.0474	0.0158	0.0372	0.0049	0.2177	0.0266	2.9440	0.2244
1	1	0	1	0.0263	0.0084	0.0195	0.0056	0.3731	0.0482	2.5890	0.1083
1	1	1	1	0.0312	0.0110	0.0214	0.0095	0.2845	0.0703	2.5970	0.1021

Table 7. Mean growth, with standard error (SE), of *Colophospermum mopane* saplings under the influence of various treatment combinations. We measured final-initial relative growth rate of stem length (RGR_L in $\text{mm mm}^{-1} \text{ week}^{-1}$), final-initial relative growth rate in stem basal diameter (RGR_D in $\text{mm mm}^{-1} \text{ week}^{-1}$), final proportion of leaf to sapling dry biomass (LP) and final \log_{10} transformed sapling dry biomass (SDB in mg). Two levels of each treatment provided are indicated as ‘0’ (control) and ‘1’ (treatment). Water: rainfall (0) and regular watering (1), Shade: no shade (0) and 80 % shade (1), Nutrients: without fertilizer (0) and with fertilizer (1) and Grass: absence (0) and presence (1). See **Methods** for further details

Treatment combination				RGR_L		RGR_D		LP		SDB	
Water	Shade	Nutrients	Grass	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0	0	0	0.0137	0.0068	0.0329	0.0040	0.6749	0.0325	3.2800	0.1252
0	0	1	0	0.0285	0.0112	0.0518	0.0069	0.6912	0.0486	3.6200	0.1806
0	1	0	0	0.0034	0.0153	0.0132	0.0087	0.4995	0.0322	2.9040	0.1401
0	1	1	0	0.0168	0.0055	0.0237	0.0035	0.4513	0.0477	3.0730	0.1084
1	0	0	0	0.0212	0.0092	0.0456	0.0072	0.6249	0.0287	3.3400	0.1653
1	0	1	0	0.0348	0.0089	0.0490	0.0073	0.5951	0.0247	3.6800	0.0307
1	1	0	0	0.0137	0.0077	0.0328	0.0105	0.5614	0.0271	3.0980	0.0926
1	1	1	0	0.0011	0.0075	0.0203	0.0065	0.5319	0.0372	3.0330	0.0318
0	0	0	1	-0.0054	0.0040	0.0133	0.0060	0.5408	0.0734	2.8760	0.1442
0	0	1	1	0.0463	0.0152	0.0216	0.0193	0.4373	0.0454	3.2790	0.2063
0	1	0	1	0.0033	0.0059	0.0262	0.0037	0.4927	0.0735	3.0190	0.1051
0	1	1	1	0.0133	0.0043	0.0278	0.0042	0.4208	0.0403	2.9060	0.1125
1	0	0	1	-0.0039	0.0016	0.0159	0.0057	0.5930	0.0119	3.0330	0.0696
1	0	1	1	0.0144	0.0053	0.0301	0.0059	0.5777	0.0251	3.2320	0.1802
1	1	0	1	0.0075	0.0073	0.0152	0.0056	0.5197	0.0467	2.8460	0.1244
1	1	1	1	0.0209	0.0058	0.0256	0.0065	0.5064	0.0337	2.9690	0.0677

Table 8. Mean growth, with standard error (SE), of *Combretum apiculatum* saplings under the influence of various treatment combinations. We measured final-initial relative growth rate of stem length (RGR_L in $\text{mm mm}^{-1} \text{ week}^{-1}$), final-initial relative growth rate in stem basal diameter (RGR_D in $\text{mm mm}^{-1} \text{ week}^{-1}$), final proportion of leaf to sapling dry biomass (LP) and final \log_{10} transformed sapling dry biomass (SDB in mg). Two levels of each treatment provided are indicated as ‘0’ (control) and ‘1’ (treatment). Water: rainfall (0) and regular watering (1), Shade: no shade (0) and 80 % shade (1), Nutrients: without fertilizer (0) and with fertilizer (1) and Grass: absence (0) and presence (1). See **Methods** for further details

Treatment combination				RGR_L		RGR_D		LP		SDB	
Water	Shade	Nutrients	Grass	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0	0	0	0.0105	0.0088	0.0387	0.0102	0.6106	0.0376	3.5170	0.1661
0	0	1	0	0.0365	0.0095	0.0686	0.0117	0.5270	0.0215	4.1580	0.1019
0	1	0	0	0.0077	0.0120	0.0268	0.0040	0.5443	0.0305	3.2400	0.0840
0	1	1	0	0.0211	0.0080	0.0340	0.0027	0.4630	0.0669	3.4440	0.2291
1	0	0	0	0.0151	0.0102	0.0598	0.0044	0.5530	0.0252	3.7810	0.1875
1	0	1	0	0.0232	0.0075	0.0668	0.0065	0.5205	0.0322	4.1960	0.1492
1	1	0	0	0.0287	0.0077	0.0401	0.0040	0.5096	0.0348	3.2820	0.1390
1	1	1	0	0.0182	0.0099	0.0311	0.0073	0.3636	0.0573	3.4880	0.1468
0	0	0	1	-0.0027	0.0032	0.0110	0.0094	0.4413	0.0979	2.7950	0.2138
0	0	1	1	0.0384	0.0195	0.0260	0.0152	0.5072	0.0491	3.3090	0.1322
0	1	0	1	0.0271	0.0068	0.0333	0.0037	0.5285	0.0343	3.2410	0.0929
0	1	1	1	0.0114	0.0058	0.0314	0.0054	0.4815	0.0449	3.2340	0.1390
1	0	0	1	-0.0026	0.0074	0.0140	0.0051	0.4874	0.0848	2.6860	0.1513
1	0	1	1	0.0183	0.0059	0.0541	0.0145	0.5179	0.0255	3.6980	0.1961
1	1	0	1	0.0280	0.0136	0.0269	0.0105	0.5117	0.0471	3.1130	0.1400
1	1	1	1	0.0177	0.0073	0.0324	0.0092	0.4880	0.0652	3.3360	0.1412

Chapter 4: Herbivory effects on saplings are influenced by nutrients and grass competition in a humid South African savanna

Table 1. Mean growth, with standard error (SE), of *Acacia karroo* saplings under the influence of various treatment combinations, after clipping. We measured final-initial relative growth rate of stem length (RGR_L in $\text{mm mm}^{-1} \text{ week}^{-1}$), final-initial relative growth rate in stem basal diameter (RGR_D in $\text{mm mm}^{-1} \text{ week}^{-1}$) after clipping in April 2010. The final proportion of leaf to sapling dry biomass (LP) and final \log_{10} transformed sapling dry biomass (SDB in mg) at the end of the experiment were measured. Two levels of each treatment provided are indicated as ‘0’ (control) and ‘1’ (treatment). Water: rainfall (0) and regular watering (1), Shade: no shade (0) and 80 % shade (1), Nutrients: without fertilizer (0) and with fertilizer (1) and Grass: absence (0) and presence (1). See **Methods** for further details

Treatment combination			RGR_L		RGR_D		LP		SDB	
Clipping	Nutrients	Grass	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0	0	0.0043	0.0038	0.0339	0.0028	0.4340	0.0197	4.0250	0.1742
0	1	0	0.0097	0.0086	0.0308	0.0045	0.3754	0.0364	4.5740	0.4794
1	0	0	0.1736	0.0126	0.0122	0.0013	0.3054	0.0543	3.1690	0.0809
1	1	0	0.1877	0.0125	0.0168	0.0028	0.3472	0.0576	3.7600	0.0882
0	0	1	0.0102	0.0060	0.0172	0.0036	0.3465	0.0410	3.3390	0.2762
0	1	1	-0.0034	0.0108	0.0253	0.0024	0.3279	0.0823	3.7760	0.4433
1	0	1	0.1277	0.0145	0.0110	0.0056	0.1951	0.0591	2.8080	0.1756
1	1	1	0.1714	0.0132	0.0071	0.0027	0.2756	0.0819	2.8730	0.1313

Table 2. Mean growth, with standard error (SE), of *Acacia sieberiana* saplings under the influence of various treatment combinations, after clipping. We measured final-initial relative growth rate of stem length (RGR_L in $\text{mm mm}^{-1} \text{ week}^{-1}$), final-initial relative growth rate in stem basal diameter (RGR_D in $\text{mm mm}^{-1} \text{ week}^{-1}$) after clipping in April 2010. The final proportion of leaf to sapling dry biomass (LP) and final \log_{10} transformed sapling dry biomass (SDB in mg) at the end of the experiment were measured. Two levels of each treatment provided are indicated as ‘0’ (control) and ‘1’ (treatment). Water: rainfall (0) and regular watering (1), Shade: no shade (0) and 80 % shade (1), Nutrients: without fertilizer (0) and with fertilizer (1) and Grass: absence (0) and presence (1). See **Methods** for further details

Treatment combination			RGR_L		RGR_D		LP		SDB	
Clipping	Nutrients	Grass	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0	0	0.0023	0.0031	0.0341	0.0044	0.4236	0.0418	4.3110	0.1322
0	1	0	0.0046	0.0023	0.0406	0.0061	0.3722	0.0155	5.0080	0.1374
1	0	0	0.1912	0.0135	0.0117	0.0031	0.2634	0.0675	3.2690	0.1414
1	1	0	0.2311	0.0126	0.0127	0.0013	0.2865	0.0519	3.6130	0.1453
0	0	1	0.0081	0.0146	0.0185	0.0061	0.2929	0.0803	3.2200	0.3558
0	1	1	-0.0004	0.0062	0.0274	0.0073	0.2755	0.0706	3.7420	0.3779
1	0	1	0.1774	0.0158	0.0093	0.0054	0.2490	0.1032	2.9490	0.1877
1	1	1	0.1491	0.0140	0.0091	0.0035	0.2886	0.0773	2.7820	0.1640

Table 3. Mean growth, with standard error (SE), of *Schotia brachypetala* saplings under the influence of various treatment combinations, after clipping. We measured final-initial relative growth rate of stem length (RGR_L in $\text{mm mm}^{-1} \text{ week}^{-1}$), final-initial relative growth rate in stem basal diameter (RGR_D in $\text{mm mm}^{-1} \text{ week}^{-1}$) after clipping in April 2010. The final proportion of leaf to sapling dry biomass (LP) and final \log_{10} transformed sapling dry biomass (SDB in mg) at the end of the experiment were measured. Two levels of each treatment provided are indicated as ‘0’ (control) and ‘1’ (treatment). Water: rainfall (0) and regular watering (1), Shade: no shade (0) and 80 % shade (1), Nutrients: without fertilizer (0) and with fertilizer (1) and Grass: absence (0) and presence (1). See **Methods** for further details

Treatment combination			RGR_L		RGR_D		LP		SDB	
Clipping	Nutrients	Grass	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0	0	0.0042	0.0047	0.0199	0.0041	0.5281	0.0796	3.1600	0.1059
0	1	0	0.0105	0.0048	0.0169	0.0026	0.4830	0.0535	3.4730	0.0926
1	0	0	0.1217	0.0095	0.0059	0.0043	0.3459	0.0808	2.7120	0.1370
1	1	0	0.1431	0.0075	0.0121	0.0029	0.3708	0.0862	2.8110	0.1077
0	0	1	0.0109	0.0140	-0.0061	0.0069	0.3851	0.0609	2.5620	0.0646
0	1	1	0.0007	0.0043	0.0077	0.0033	0.5129	0.0086	2.9390	0.1358
1	0	1	0.1089	0.0086	0.0070	0.0014	0.2551	0.0693	2.4450	0.0574
1	1	1	0.1144	0.0176	0.0063	0.0007	0.2205	0.0511	2.4710	0.1656

Table 4. Mean growth, with standard error (SE), of *Strychnos spinosa* saplings under the influence of various treatment combinations, after clipping. We measured final-initial relative growth rate of stem length (RGR_L in $\text{mm mm}^{-1} \text{ week}^{-1}$), final-initial relative growth rate in stem basal diameter (RGR_D in $\text{mm mm}^{-1} \text{ week}^{-1}$) after clipping in April 2010. The final proportion of leaf to sapling dry biomass (LP) and final \log_{10} transformed sapling dry biomass (SDB in mg) at the end of the experiment were measured. Two levels of each treatment provided are indicated as ‘0’ (control) and ‘1’ (treatment). Water: rainfall (0) and regular watering (1), Shade: no shade (0) and 80 % shade (1), Nutrients: without fertilizer (0) and with fertilizer (1) and Grass: absence (0) and presence (1). See **Methods** for further details

Treatment combination			RGR_L		RGR_D		LP		SDB	
Clipping	Nutrients	Grass	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0	0	-0.0220	0.0141	0.0465	0.0155	0.3863	0.0818	2.7810	0.1746
0	1	0	0.0102	0.0043	0.0307	0.0083	0.5535	0.0098	3.0780	0.1624
1	0	0	0.1463	0.0089	0.0245	0.0070	0.3553	0.1040	2.5190	0.0818
1	1	0	0.1566	0.0157	0.0219	0.0027	0.3291	0.1076	2.6120	0.1253
0	0	1	-0.0099	0.0032	0.0396	0.0089	0.4400	0.0345	2.5890	0.1991
0	1	1	0.0018	0.0050	0.0302	0.0055	0.4959	0.0548	2.5860	0.0914
1	0	1	0.1605	0.0116	0.0258	0.0123	0.2622	0.0846	2.3140	0.0762
1	1	1	0.1097	0.0153	0.0250	0.0085	0.2613	0.0994	2.1990	0.1343

Table 5. Mean growth, with standard error (SE), of *Acacia nigrescens* saplings under the influence of various treatment combinations, after clipping. We measured final-initial relative growth rate of stem length (RGR_L in $\text{mm mm}^{-1} \text{ week}^{-1}$), final-initial relative growth rate in stem basal diameter (RGR_D in $\text{mm mm}^{-1} \text{ week}^{-1}$) after clipping in April 2010. The final proportion of leaf to sapling dry biomass (LP) and final \log_{10} transformed sapling dry biomass (SDB in mg) at the end of the experiment were measured. Two levels of each treatment provided are indicated as ‘0’ (control) and ‘1’ (treatment). Water: rainfall (0) and regular watering (1), Shade: no shade (0) and 80 % shade (1), Nutrients: without fertilizer (0) and with fertilizer (1) and Grass: absence (0) and presence (1). See **Methods** for further details

Treatment combination			RGR_L		RGR_D		LP		SDB	
Clipping	Nutrients	Grass	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0	0	-0.0062	0.0042	0.0098	0.0035	0.2481	0.0276	3.0310	0.3672
0	1	0	0.0022	0.0026	0.0203	0.0067	0.3272	0.0183	3.8360	0.3418
1	0	0	0.1973	0.0119	0.0217	0.0094	0.3860	0.0502	3.2960	0.1882
1	1	0	0.2159	0.0088	0.0204	0.0032	0.3028	0.0450	3.4710	0.2121
0	0	1	0.0073	0.0099	0.0029	0.0086	0.2038	0.0737	2.2950	0.0893
0	1	1	-0.0021	0.0027	0.0091	0.0067	0.3246	0.0600	2.8290	0.2772
1	0	1	0.1841	0.0066	0.0176	0.0034	0.2717	0.0867	2.6480	0.0763
1	1	1	0.1846	0.0258	0.0114	0.0025	0.2665	0.0888	2.7610	0.1375

Table 6. Mean growth, with standard error (SE), of *Acacia tortilis* saplings under the influence of various treatment combinations, after clipping. We measured final-initial relative growth rate of stem length (RGR_L in mm mm⁻¹ week⁻¹), final-initial relative growth rate in stem basal diameter (RGR_D in mm mm⁻¹ week⁻¹) after clipping in April 2010. The final proportion of leaf to sapling dry biomass (LP) and final log₁₀ transformed sapling dry biomass (SDB in mg) at the end of the experiment were measured. Two levels of each treatment provided are indicated as ‘0’ (control) and ‘1’ (treatment). Water: rainfall (0) and regular watering (1), Shade: no shade (0) and 80 % shade (1), Nutrients: without fertilizer (0) and with fertilizer (1) and Grass: absence (0) and presence (1). See **Methods** for further details

Treatment combination			RGRL		RGRD		LP		SDB	
Clipping	Nutrients	Grass	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0	0	0.0053	0.0064	0.0184	0.0034	0.3962	0.0447	3.5040	0.2401
0	1	0	0.0197	0.0030	0.0269	0.0023	0.3723	0.0297	4.0790	0.2618
1	0	0	0.1313	0.0127	0.0099	0.0048	0.2755	0.0526	2.5280	0.1245
1	1	0	0.2373	0.0340	0.0077	0.0066	0.4022	0.0540	3.1450	0.3410
0	0	1	-0.0027	0.0057	0.0007	0.0113	0.2014	0.0641	2.2640	0.0926
0	1	1	-0.0080	0.0057	0.0036	0.0048	0.2177	0.0266	2.9440	0.2244
1	0	1	0.1387	0.0128	0.0062	0.0023	0.2606	0.0500	2.3590	0.0954
1	1	1	0.1547	0.0058	0.0031	0.0087	0.1417	0.0712	2.0230	0.1015

Table 7. Mean growth, with standard error (SE), of *Colophospermum mopane* saplings under the influence of various treatment combinations, after clipping. We measured final-initial relative growth rate of stem length (RGR_L in $\text{mm mm}^{-1} \text{ week}^{-1}$), final-initial relative growth rate in stem basal diameter (RGR_D in $\text{mm mm}^{-1} \text{ week}^{-1}$) after clipping in April 2010. The final proportion of leaf to sapling dry biomass (LP) and final \log_{10} transformed sapling dry biomass (SDB in mg) at the end of the experiment were measured. Two levels of each treatment provided are indicated as ‘0’ (control) and ‘1’ (treatment). Water: rainfall (0) and regular watering (1), Shade: no shade (0) and 80 % shade (1), Nutrients: without fertilizer (0) and with fertilizer (1) and Grass: absence (0) and presence (1). See **Methods** for further details

Treatment combination			RGRL		RGRD		LP		SDB	
Clipping	Nutrients	Grass	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0	0	0.0074	0.0053	0.0228	0.0050	0.6249	0.0287	3.3400	0.1653
0	1	0	0.0091	0.0018	0.0209	0.0028	0.5951	0.0247	3.6800	0.0307
1	0	0	0.1463	0.0084	0.0110	0.0045	0.4525	0.0657	2.8340	0.1173
1	1	0	0.2015	0.0079	0.0130	0.0005	0.4391	0.1058	2.8250	0.1063
0	0	1	-0.0049	0.0024	0.0034	0.0056	0.5930	0.0119	3.0330	0.0696
0	1	1	0.0007	0.0040	0.0080	0.0022	0.5777	0.0251	3.2320	0.1802
1	0	1	0.1264	0.0072	0.0167	0.0031	0.3919	0.1002	2.6090	0.1329
1	1	1	0.1434	0.0098	0.0010	0.0020	0.3859	0.0935	2.6540	0.1049

Table 8. Mean growth, with standard error (SE), of *Combretum apiculatum* saplings under the influence of various treatment combinations, after clipping. We measured final-initial relative growth rate of stem length (RGR_L in $\text{mm mm}^{-1} \text{ week}^{-1}$), final-initial relative growth rate in stem basal diameter (RGR_D in $\text{mm mm}^{-1} \text{ week}^{-1}$) after clipping in April 2010. The final proportion of leaf to sapling dry biomass (LP) and final \log_{10} transformed sapling dry biomass (SDB in mg) at the end of the experiment were measured. Two levels of each treatment provided are indicated as ‘0’ (control) and ‘1’ (treatment). Water: rainfall (0) and regular watering (1), Shade: no shade (0) and 80 % shade (1), Nutrients: without fertilizer (0) and with fertilizer (1) and Grass: absence (0) and presence (1). See **Methods** for further details

Treatment combination			RGRL		RGRD		LP		SDB	
Clipping	Nutrients	Grass	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0	0	-0.0063	0.0072	0.0199	0.0054	0.5530	0.0252	3.7810	0.1875
0	1	0	0.0053	0.0053	0.0249	0.0026	0.5205	0.0322	4.1960	0.1492
1	0	0	0.1947	0.0119	0.0154	0.0046	0.4802	0.0917	3.3650	0.1481
1	1	0	0.2633	0.0091	0.0201	0.0022	0.5756	0.0076	3.9550	0.0578
0	0	1	-0.0053	0.0088	0.0050	0.0101	0.4874	0.0848	2.6860	0.1513
0	1	1	0.0051	0.0091	0.0203	0.0084	0.5179	0.0255	3.6980	0.1961
1	0	1	0.1232	0.0148	0.0085	0.0052	0.3259	0.0775	2.4600	0.1867
1	1	1	0.1518	0.0149	0.0082	0.0047	0.3995	0.0802	2.6980	0.2193

Appendix II: Tables of correlation matrices

Chapter 2: Fire and nutrient gradient effects on the sapling ecology of four *Acacia* species

in the presence of grass competition

Species-wise correlation matrix for study variables – values provided in **bold** are significant ($p < 0.05$)

<i>Acacia karroo</i>					
	RGR _h	RGR _d	Root biomass	Shoot biomass	CT conc.
RGR _h	1.0000				
RGR _d	0.4817	1.0000			
Root biomass	0.4995	0.5435	1.0000		
Shoot biomass	0.5745	0.6317	0.9084	1.0000	
CT conc.	-0.2138	-0.0294	0.2392	0.0434	1.0000
Thorn length	-0.3399	-0.4769	-0.7428	-0.8688	-0.1583

<i>Acacia sieberiana</i>					
	RGR _h	RGR _d	Root biomass	Shoot biomass	CT conc.
RGR _h	1.0000				
RGR _d	0.8122	1.0000			
Root biomass	-0.2756	0.0809	1.0000		
Shoot biomass	-0.2764	0.0983	0.9503	1.0000	
CT conc.	0.1999	0.1037	-0.0232	-0.0368	1.0000
Thorn length	0.1504	-0.2064	-0.8618	-0.9094	0.0887

<i>Acacia nigrescens</i>					
	RGR _h	RGR _d	Root biomass	Shoot biomass	CT conc.
RGR _h	1.0000				
RGR _d	0.1358	1.0000			
Root biomass	-0.1297	0.5269	1.0000		
Shoot biomass	-0.1363	0.5457	0.9103	1.0000	
CT conc.	0.0489	-0.4275	-0.0373	-0.0594	1.0000
Thorn length	0.2794	-0.4934	-0.8955	-0.865	-0.1049

Acacia tortilis

	RGR _h	RGR _d	Root biomass	Shoot biomass	CT conc.
RGR _h	1.0000				
RGR _d	0.9147	1.0000			
Root biomass	0.3953	0.4217	1.0000		
Shoot biomass	0.5193	0.5824	0.8323	1.0000	
CT conc.	0.1992	0.1264	0.3959	0.5279	1.0000
Thorn length	-0.3101	-0.3284	-0.6934	-0.4383	0.0492

We analysed the ratio of final-initial relative growth rate of stem height (RGR_h) to final-initial relative growth rate in stem basal diameter (RGR_d) measured in mm mm⁻¹ week⁻¹, log₁₀ transformed root/shoot dry biomass ratio measured in mg, log₁₀ transformed leaf condensed tannin (CT) concentration (in mg mL⁻¹ quebracho equivalents (QE)) and power (-0.3) transformed mean thorn length (in mm).

Chapter 3: Shade, nutrients and grass competition are important for tree sapling establishment in a humid savanna

Species-wise correlation matrix for study variables – values provided in **bold** are significant ($p < 0.05$)

<i>Acacia karroo</i>			
	RGR _L	RGR _D	LP
RGR _L	1.0000		
RGR _D	0.6031	1.0000	
LP	0.3172	0.2314	1.0000
SDB	0.6185	0.7852	0.3471

<i>Acacia nigrescens</i>			
	RGR _L	RGR _D	LP
RGR _L	1.0000		
RGR _D	0.5493	1.0000	
LP	0.4336	0.3626	1.0000
SDB	0.3628	0.7876	0.3055

<i>Acacia sieberiana</i>			
	RGR _L	RGR _D	LP
RGR _L	1.0000		
RGR _D	0.6597	1.0000	
LP	0.1109	0.1183	1.0000
SDB	0.5886	0.7572	0.2032

<i>Acacia tortilis</i>			
	RGR _L	RGR _D	LP
RGR _L	1.0000		
RGR _D	0.5351	1.0000	
LP	0.3654	0.3811	1.0000
SDB	0.7165	0.7652	0.4276

<i>Schotia brachypetala</i>			
	RGR _L	RGR _D	LP
RGR _L	1.0000		
RGR _D	0.4229	1.0000	
LP	0.3235	0.2503	1.0000
SDB	0.3783	0.5867	0.3798

<i>Colophospermum mopane</i>			
	RGR _L	RGR _D	LP
RGR _L	1.0000		
RGR _D	0.5395	1.0000	
LP	0.2104	0.2995	1.0000
SDB	0.6492	0.6786	0.5169

<i>Strychnos spinosa</i>			
	RGR _L	RGR _D	LP
RGR _L	1.0000		
RGR _D	0.0128	1.0000	
LP	0.6841	0.0188	1.0000
SDB	0.6085	0.2207	0.5835

<i>Combretum apiculatum</i>			
	RGR _L	RGR _D	LP
RGR _L	1.0000		
RGR _D	0.4417	1.0000	
LP	0.2331	0.3189	1.0000
SDB	0.4879	0.7504	0.4048

We analyzed final-initial relative growth rate of stem length (RGR_L in mm mm⁻¹ week⁻¹), final-initial relative growth rate in stem basal diameter (RGR_D in mm mm⁻¹ week⁻¹), final proportion of leaf to sapling dry biomass (LP), and final log₁₀ transformed sapling dry biomass (SDB in mg).

**Chapter 4: Herbivory effects on saplings are influenced by nutrients and grass competition
in a humid South African savanna**

Species-wise correlation matrix for study variables – values provided in **bold** are significant ($p < 0.05$)

<i>Acacia karroo</i>				<i>Acacia nigrescens</i>			
	RGR _L	RGR _D	LP		RGR _L	RGR _D	LP
RGR _L	1.0000			RGR _L	1.0000		
RGR _D	-0.548	1.0000		RGR _D	0.2321	1.0000	
LP	-0.0805	0.5206	1.0000	LP	0.2	0.1598	1.0000
SDB	-0.3238	0.6773	0.5245	SDB	0.0798	0.3774	0.2764

<i>Acacia sieberiana</i>				<i>Acacia tortilis</i>			
	RGR _L	RGR _D	LP		RGR _L	RGR _D	LP
RGR _L	1.0000			RGR _L	1.0000		
RGR _D	-0.5287	1.0000		RGR _D	-0.2378	1.0000	
LP	-0.1176	0.3897	1.0000	LP	0.0108	0.4991	1.0000
SDB	-0.4273	0.7022	0.4623	SDB	-0.2175	0.5372	0.5272

<i>Schotia brachypetala</i>				<i>Colophospermum mopane</i>			
	RGR _L	RGR _D	LP		RGR _L	RGR _D	LP
RGR _L	1.0000			RGR _L	1.0000		
RGR _D	-0.0963	1.0000		RGR _D	-0.0899	1.0000	
LP	-0.4867	0.4746	1.0000	LP	-0.4376	0.1876	1.0000
SDB	-0.3956	0.5473	0.6376	SDB	-0.5593	0.4029	0.6553

<i>Strychnos spinosa</i>				<i>Combretum apiculatum</i>			
	RGR _L	RGR _D	LP		RGR _L	RGR _D	LP
RGR _L	1.0000			RGR _L	1.0000		
RGR _D	-0.3538	1.0000		RGR _D	-0.0709	1.0000	
LP	-0.2236	-0.027	1.0000	LP	-0.0638	0.435	1.0000
SDB	-0.2843	-0.0103	0.6619	SDB	-0.0381	0.5005	0.5868

We analysed final - initial relative growth rate of stem length (RGR_L in mm mm⁻¹ week⁻¹) and final - initial relative growth rate in stem basal diameter (RGR_D in mm mm⁻¹ week⁻¹), after clipping in April 2010. The final proportion of leaf to sapling dry biomass (LP) and final log₁₀ transformed sapling dry biomass (SDB in mg), taken in June 2010, were also analyzed.

