Surviving and growing amidst others
the effect of environmental factors on
germination and establishment of savanna trees

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Chapter 1

General introduction

Eduardo R. M. Barbosa

General introduction

Savannas are tropical or near-tropical ecosystems that are characterized by a continuous herbaceous layer dominated by grasses and herbs and occasionally interrupted by trees and shrubs (Scholes &Walker 1993; Sims 1988; McPherson 1997; Davis et al. 1998; Sankaran et al. 2005). These ecosystems are geographically extensive (Scholes & Archer 1997) and are found on almost all tropical habitable continents of the world (Stibig & Beuchle 2003), across substantial rainfall gradients ranging from arid (Scholes 1997; Jeltsch et al. 2000) to humid conditions (Puyravaud et al. 2003) or even very wet (Pinheiro et al. 2010), and from coastal lowlands (Christensen 1988) to sub-alpine zones (Ball et al. 1997). Regardless of the particularities of each region's environmental conditions, savannas are by definition habitats in which trees and grasses coexist (Scholes & Archer 1997) at all scales of consideration and which have high seasonal climatic variation (Kanegae et al. 2000; Russell-Smith et al. 2003; Venter et al. 2003; Schwinning & Sala 2004; Kottek et al. 2006; Staver et al. 2009). As a result of a complex set of environmental drivers and the tree species independent evolution of adaptations to the constraints of savanna environments (Hoffman & Franco 2003), savanna tree communities throughout the world largely differ in their floristic composition (Cole 1986).

In Africa, *ca.* 40% of the land surface is savanna and is inhabited by fast-growing human and livestock populations (Solbrig & Young 1993). As natural vegetation is generally the only source of food for livestock, high numbers of livestock may lead to overgrazing of the pastures. The clearance of the vegetation will increase the chances of soil erosion and invasions (e.g., wood plant encroachment) potentially leading to habitat degradation (Kelly & Walker 1976; Van Auken & Bush 1989; Skarpe 1990; Ward 2005; Balfour & Midgley 2008). Such ongoing anthropogenic pressure combined with the expected global climactic change is a growing concern in these areas since they negatively affect the vegetation structure and composition in savannas (Sala et al. 2000). One of the strongest indications of savanna degradation (Kreuter et al. 1999; Ward 2005) is the invasion of woody plants in tropical grasslands and savannas, often labelled as "bush encroachment" in the literature. Most invasive woody species are often unpalatable to domestic and game animals (Rohner & Ward 1997; Masocha et al. 2011). Consequently, bush encroachment reduces sustained stocking rates for grazing and browsing animals (Dean & Macdonald 1994), and threatens the livelihood of people depending on commercial and communal game (Condon 1986). In arid and semi-arid areas where other forms of agriculture are not feasible due to the low rainfall, the increase in woodland cover raises socio-economic concerns (Kraaij & Ward 2006).

Although germination and tree seedling establishment are critical stages in the life cycle of plants (Grubb 1977) and can influence community composition (Higgins et al. 2000; Wiegand et al. 2006; Van Langevelde et al. 2011), the complex mechanisms that affect woody plant establishment in savannas are still unclear (Sankaran et al. 2004), being studied in a fairly limited range of species and habitats (e.g., Kambatuku et al. 2011; Ward & Esler 2011). Hence, a better understand the factors influencing species recruitment and the ecology of seedlings are needed to improve the ability to predict changes in community composition and eventually to manage and conserve this important biome.

The importance of tree seedling establishment in savannas

Different types of explanatory models have been proposed for the tree and grass co-occurrence in savannas, such as the equilibrium models, the non-equilibrium models and the disequilibrium models (see Box 1). The equilibrium models of savanna dynamics emphasise the role of ecological niches separation on the savanna coexistence between tree and grass life-forms in mediating (Walker & Noy-Meir 1982). Over the last two decades there has also been increasing agreement that disturbances (e.g., fire, herbivory, drought) substantially contribute to the stability and dynamics of savanna systems largely through their influence on the demographics of seedlings and juvenile trees (Bond et al. 2005; Wiegand et al. 2006). However, seedlings are more susceptible to disturbances and competition from neighbouring plants than older, established plants. As such, seedlings establishment represent the most serious bottleneck to successful tree recruitment in tropical grasslands and savannas (Prins & Van der Jeugd 1993; Higgins et al. 2000; Loth et al. 2005; Wiegand et al. 2006; Van der Waal et al 2009; van Langevelde et al. 2011). However, it is unclear how important competitive interactions between tree seedlings and grass are for savanna long-term dynamics (see reviews of Sankaran et al. 2004; Van Langevelde et al. 2011; Van der Waal et al. 2011).

While non-equilibrium models (Higgins et al. 2000;Van Wijk & Rodriguez-Iturbe 2002) assume that tree recruitment is controlled only by disturbance (such as fire) and environmental productivity (drought), disequilibrium models (Menaut et al. 1990; Jeltsch et al. 2000) assume competitive interactions between trees and grasses also control successful recruitment. However, almost all of these arguments are based on theoretical or modelling studies. Few comparative investigations have been done on seedlings of savanna tree species either within or across communities (Ward et al. in prep). This lack of information on inter-specific variation critically limits the ability to understand the dynamics of savannas, which are multi-species systems. Consistent and repeated changes in species composition across topographic variation (Cole 1986) and community differences across adjacent geologies (Cole 1986; Pickett et al. 2003; Venter et al. 2003) give reason to believe that many of these large-scale patterns might be a result of divergent seedling strategies (Prior 2005).

Resources and disturbances influencing tree seedling establishment and growth

Several interacting environmental factors are thought to determine vegetation structure and composition in savannas, such as water (resource), soil nutrients (resource), solar radiance (resource), fire (disturbance), herbivory (disturbance) (Walker 1987; Scholes & Archer 1997; Higgins et al. 2000; Jeltsch et al. 2000; Sankaran et al. 2005), and atmospheric CO_2 levels (resource) (Bond & Midgley 2000; Ward 2010). Grasses also strongly affect tree seedling recruitment, either through direct competition or through effects on disturbance intensity (e.g., fuel load for fires). The level to which grasses inhibit the growth of tree seedlings is variable, with evidence that grass impact ranges from positive (Anthelme & Michalet 2009), through negligible (O'Connor 1995; Brown & Archer 1989), to negative (Loth 1999; Cramer et al. 2007; Van der Waal et al. 2009; Grellier et al. 2012).

Water

Water stress is thought to be the greatest constraint to seedling survival in savanna systems. Many studies suggest that tree seedling recruitment and survival are mostly limited by rainfall variability (Loth et al. 2005, Kraaij & Ward 2006; Moustakas et al. 2006; Meyer et al. 2007).While other resources can be stored (Schutz et al. 2009; Wigley et al. 2009), few African savanna tree species show the propensity to store water in large quantities (e.g., Baobab - *Adansonia digitata*). Consequently, extended dry periods during the wet season and the dry season drought are critical to seedling survival (Ward & Esler 2011; Grellier et al. 2012); most seedlings die if accessible soil water pressure falls below a minimum they can withstand (Prins 1988; Meyer et al. 2007). Differences in annual rainfall and intra-annual rainfall distribution can hence lead to different tree growth strategies. For example, plants may adopt recruitment strategies to best utilize rainfall that is largely unpredictable in highly variable climates (Ward 2009), such as increasing the seed production to enhance seedling recruitment (Venable 2007). Soil physical properties also determine water availability (Frost et al. 1986; Ward & Esler 2011; Schleicher et al. 2011), influencing the general physiognomy and primary productivity of tropical savannas (Knoop & Walker 1985, Burke et al. 1990; Van Langevelde et al. 2003).

Box 1: *The different models on tree and grass interactions in savannas*

Equilibrium models: These models emphasize the fundamental role of competitive interactions in fostering co-existence between trees and grasses. Moreover, these models suggest a stable tree-grass co-existence. Two different, but potentially related, competition-based models currently dominate the savanna literature: the root niche separation model (e.g., Walter 1971) and the phenological niche separation model (Sala et al. 1997; Scholes & Archer 1997; House et al. 2003).

Non-equilibrium models: These models invoke the trade-off between competitive ability and colonization potential (Levins & Culver 1971; Levine & Rees 2002; Amarasekare 2003; Kneitel & Chase 2004), with the added caveat that the competitive rankings of trees and grasses and their colonization potential change in response to fluctuations in soil water stress caused by inter-annual rainfall variability (Fernandez-Illescas& Rodriguez-Iturbe 2003).

Disequilibrium models: These models argue that the impacts of climatic variability and disturbances differ between life-history stages of trees. Furthermore, the models assume that competitive interactions between trees and grasses occur at all life stages. In this model, the grasses have superior competitiveness over the seedlings and juveniles trees, however, such competiveness advantage changes toward the trees as they reach adulthood. Moreover, local conditions determine the competitive advantage of each life form and disturbance prevents long-term general dominance by one life form (Hochberg et al. 1994; Jeltsch et al. 1996; Higgins et al. 2000; Van Wijk& Rodriguez-Iturbe 2002).

Nutrients

Many external factors such as fire suppression, soil fertilization and high herbivore density can rapidly change the amount of local soil nutrients. These factors increase the organic content in the upper soil layers and lead to a higher availability of nitrogen and other nutrients (Bilbao & Medina 1991). Studies conducted indicate that an increase in soil fertility leads to more constrained seedling establishment (Scholes 1990; Debain et al.2005; Van der Wall et al. 2009, 2011). However, the mechanism underlying constrained tree seedling recruitment in fertile environments might be due to the increase of competition with grass for soil resources (Cohn et al. 1989; Kraaij & Ward 2006; Sankaran et al. 2008). That said, little is known about the effects of changes in soil nutrient availability on the nutrient concentrations in the leaves of savanna trees (but see Breman et al. 1982; Prins & Olff 1998; Van der Waal et al. 2011).While unlikely to kill plants directly, low nutrient content can limit plant growth rate, resulting in an increased risk that seedlings die during stress periods (Hermans et al. 2006). Moreover, a close relationship has been suggested between water availability and soil nutrient increase (Van der Wall et al. 2009), where increases in nutrient availability may induce water stress that probably translates into lower growth performance (Alam 1999; Hu & Schmidhalter 2005).Therefore, to fully understand the drivers of savanna dynamics, it is important to consider the effects of nutrients on tree recruitment.

Light

Light is important for plant carbohydrate production (photosynthesis) and for plant water balance (evapotranspiration rates). Light, or the lack thereof, can have important consequences for the manner in which tree species occupy ecological niches in the savanna landscapes. For example, growing under established adult trees may have some advantages, allowing tree seedlings to have access to soil moisture for longer periods (Belsky 1994; Holmgren et al. 1997; Phillips & Barnes 2002) due to reduced temperature and evapotranspiration (Bernhard-Reversat 1982) or hydraulic lift (Ludwig et al. 2004).

Fire

Fire has influenced savannas for millions of years (Bond & Van Wilgen 1996; Bond & Keeley 2005). The frequency and intensity of fire can prevents the rapid reversion of savanna into forest (Hoffmann et al. 2004; Sankaran et al. 2005; Bond & Parr 2010; Staver et al. 2011) due to its assumed strong influence on early stages of tree establishment (e.g., Stock et al. 1997; Van de Vijver 1999; Bond & Keeley 2005). While fire reduces tree juveniles and seedlings to ground level (Bond & Keeley 2005), many individual trees are able to recover by accumulating growth reserves underground in swollen roots , where they are beyond the reach of fires (Van Langevelde et al. 2003; Schutz et al. 2009; Wigley et al. 2009; Bond & Parr 2010; Tomlinson et al. 2012). The reserves storage in roots may allow trees seedlings to regrow following shoot removal by fires (Bell et al. 1996; Hoffmann et al. 2004; Schutz et al. 2009; Wigley et al. 2009). However, in semi-arid savannas natural fires are rare events (Meyer et al. 2005; Seymour & Huyser 2008), seedlings of tree species may possess few adaptations to survive or avoid fire damage, decreasing their chances of survival after intensive fires.

Herbivory

Herbivory by vertebrate (Prins & Van der Jeugd 1993; Dublin 1995; Sinclair 1995; Asner et al. 2009) and invertebrates (Meiners et al. 2000) can affect savanna structure. Herbivory can be divided into grazing, affecting grasses and other herbs, and browsing, affecting shrubs and trees. Savanna tree species have strategies to tolerate or avoid herbivory, such as spinescence or chemicals compounds (Lundberg & Astrom 1990; Rohner & Ward 1997). Tree species are most severely affected by browsing events during early stages of development, when browsing directly affects the success of tree seedling establishment (Pastor et al. 1988; Campa et al. 1992). Therefore strategies to tolerate or avoid herbivory are likely a result of selective pressures during early tree development stages (Midgley et al. 2010). On the other hand, grazing may have an indirect facilitative effect on woody plants by severely suppressing grass and thereby reducing its competitive effect on tree seedlings (Pandey & Singh 1992; Van Auken & Bush 1997; Ward & Esler 2011; Grellier et al. 2012), and opening space for seedlings to occupy (Van Auken & Bush 1989). However, it is still unclear if the impacts of herbivory are influenced by other environmental factors, such as water or nutrient availability.

As herbivory is thought to have an important role in maintaining tree density low in savannas, several management measures to prevent expansion of woody species involve the increase of browsers density. However, compensatory growth of plants due to herbivory has been described for grasses, and also for some dicotyledonous plants (McNaughton 1983; Prins et al. 1980). Such response could mean that increasing browsing would end up having an effect opposite to the expected by conservation practitioners. Further studies on the effect of herbivory on several stages of the establishment of multiple savanna tree species are hence necessary.

Tree seedling and grass competition

Grasses can substantially suppress seedling growth of certain tree species (e.g., Van der Waal et al. 2009; Kambatuku et al. 2011), likely due to depletion of water or nutrient availability. In depleting water or nutrients, grasses reduce the period of suitable growing conditions for tree seedlings (Sankaran et al. 2004). While most explanations for co-existence of grasses and tree seedlings relate to below-ground competition with grasses for water (equilibrium models and disequilibrium models) (Bush &Van Auken 1991; Danielsen & Halvorson 1991), some studies suggest that seedling growth can be independent of grass competition (non-equilibrium models) (O'Connor 1995; Brown & Archer 1999). Unfortunately, few empirical studies have followed seedling growth and survival from emergence through the first wet and dry season with and without grass competition. Therefore, to date, it is unclear under which conditions tree seedlings are suppressed or facilitated by grasses and how these effects vary across moisture gradients. For instances, during the dry season tree seedling mortality caused by resource competition with grass may be greater than during the wet season (e.g., Chirara et al. 1999). Conversely, during the dry season grass competition may play a minor (lower competition due low resource availability), or even a positive role (due shading and decreasing soil water evaporation rate) on tree seedling establishment success. To explain these apparently contradictory results, field experiments are needed to define the conditions when competition with grasses determines tree recruitment in savannas.

Study aim and layout of the thesis

Savannas are characterized by large regional and temporal variability in water resources (Eamus et al. 1999; Kanegae et al. 2000; Kottek et al. 2006), in nutrient resources (due to pulses of nutrient availability, Venter et al. 2003; Schwinning & Sala 2004), and disturbances by fire (Barbosa et al. 1999; Russell-Smith et al. 2003) and herbivory (Van Langevelde et al. 2003; Staver et al. 2009). As several studies suggest that water shortage (Midgley & Bond 2001; Sankaran et al. 2005; Kraaij & Ward 2006; Meyer et al. 2007), nutrient shortage (Bardgett & Wardle 2003; Van der Waal et al. 2009), and shade (Gerhardt 1996) may limit seedling survival and establishment in dry systems, it is possible that, savanna tree species have lower productivity under low resource supply conditions (e.g., infrequent water supply and low soil nutrient), particularly if competing with grasses. Indeed, most theoretical studies on savanna vegetation dynamics assume similar responses among species (e.g., Jeltsch et al. 1996; Higgins et al. 2000; Wiegand et al. 2006). However, tropical savannas are characterised by high species diversity (Solbrig et al. 1996), and plants species are thought to respond differently to environmental pressures (Grubb 1977), each occupying a specific ecological nice, and hence being able to co-exist. Differences in species functional traits may provide a mechanistic explanation for why species perform differently across resource and disturbance gradients (Chapin et al. 1993; Violle et al. 2007).

While there are many experimental studies evaluating the growth of single tree species in response to resource supply and disturbance with and without grass competitors (e.g., Kraaij & Ward 2006; Van der Waal et al. 2009, 2011; Kambatuku et al. 2011; Ward & Esler 2011), there are few comparative investigations on seedlings of savanna tree species either within or across communities. This is a critical limitation on the ability to understand the dynamics of savannas, which are multi-species systems, species competing or facilitating each other. Therefore, it is unclear to what extent variation in responses of savanna tree seedlings to differences in local environmental conditions can explain their dominance in different environments, and how such variation in responses relates to species functional traits.

The research presented in my thesis aims to evaluate how variable are the responses of savanna tree seedlings to differences of local environmental conditions (water, nutrients, light), hence tree community composition and dynamics. This study is one of the few multi-species experiments on the effect of environmental factors on germination success, survival and above and belowground growth of savanna tree seedlings. I examined several common savanna trees (16 species, see Table 1.1), which are presumably the best adapted to local conditions (hence their general high abundance) and assessed inter-specific variation in savanna seedling response to difference levels of resources and in their ability to compete with grasses for the different resources, and to tolerate defoliation. Given their high abundance, differences in the establishment of the studied tree species may substantially affect the local savanna vegetation, and the associated animal diversity.

Table 1.1 - The 16 savanna tree species used during the different experiments.

Species	Family	Chapter
Acacia exuvialis Verd.	Fabaceae	2
Acacia gerrardii Benth	Fabaceae	2
Acacia karroo Hayne	Fabaceae	2, 3, 4 and 5
Acacia nigrescens Oliv.	Fabaceae	2, 3, 4 and 5
Acacia nilotica (L.) Willd.	Fabaceae	2, 3 and 4
Acacia sieberiana Keay& Brenan	Fabaceae	2, 3 and 4
Acacia tortilis Hayne	Fabaceae	2, 3 and 4
Acacia xanthophloea Benth.	Fabaceae	2
Colophospermum mopane (Benth.) J. Léonard	Caesalpiniaceae	2, 3, 4 and 5
Combretum apiculatum Sond.	Combretaceae	2
Combretum hereroense Schinz	Combretaceae	2, 3 and 4
Dichrostachys cinerea (L.) Wight &Arn.	Mimosaceae	2, 3 and 4
Euclea divinorum Hiern	Ebenaceae	2
Peltophorum africanum Sond.	Caesalpiniaceae	2, 3 and 4
Philenoptera violacea (Klotzsch) Schrire	Fabaceae	2
Schotia brachypetala Sond.	Caesalpiniaceae	2, 3 and 4

Throughout this thesis the influence of environmental factors was assessed for several stages of establishment, from seed to 18months old seedlings (Figure.1.1). As increased resource availability is suggested to enhance tree establishment (Bardgett & Wardle 2003; Sankaran et al. 2005; Meyer et al. 2007), I expected an increase in growth and survival in the treatments with frequent water supply, fertilization and under shade. Moreover, I hypothesized that the greatest growth of tree seedlings occurs when both aboveground (shade) and belowground (competition for resources) interferences from grass are absent. Given the negative effects of herbivory on foliar surface and photosynthetic rates (Pinkard et al. 2006), which, in turn, may affect biomass allocation and therefore shoot and/or root growth rate (Van Langevelde et al. 2003; Hoffmann et al 2004; Schutz et al. 2011), I expected that herbivory events (in this study simulated by manual defoliation) would increase shoot growth rate due to compensatory growth (McNaughton 1983; Prins et al. 1980), with higher allocation of resources to shoot biomass at the expense of root growth.

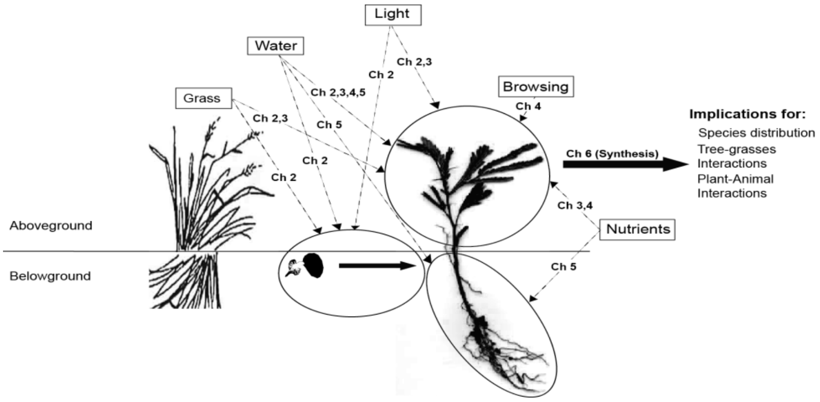

Figure 1.1 – Schematic outline illustrating the different thesis chapters (Ch).

In Chapter 2, I test how germination and early survival of several tree seedling species growing in a semi-arid savanna differ in response under different temporal patterns of water supply (simulating even or uneven rainfall patterns), light and grass presence, and whether these different responses could be explained by differences in species seed characteristics (e.g., seed biomass and seed mineral concentration).

In Chapter 3, I investigate whether tree seedlings of four common fine-leafed tree species and four common broad-leafed tree species differed in their responses to variation in the resource distribution (water, nutrient and light supply) during their first growing season, and whether they differed in their response to competition from a C_4 grass species (*Digitaria eriantha*) for these resources.

Chapter 4 shows the result of an experiment that evaluates the effects of soil nutrient (low vs. high) and water availability (uneven vs. even supply) on leaf nutrient concentrations and aboveground biomass production of seedlings of tree species from different climate types (semi-arid vs. humid savannas) and areas with different soil fertility levels (low vs. high). I focus on the foliar concentration of nitrogen (N), phosphorus (P) and potassium (K) because these nutrients are important in many plant metabolism processes, such as photosynthesis, respiration and enzymes activation (Kuiper et al. 1989; Marschner 1995; Bednarz & Oosterhuis 1999), and in the diets of herbivores (Prins &Van Langevelde 2008; Olff et al. 2002; Ahrestani et al. 2011).

Chapter 5 describes a field experiment that test the effects of variations in water and nutrient availability as well as browsing events on the aboveground and belowground growth of three abundant savannas tree species: *Acacia karoo*, *A. nigrescens* and *Colophospermum mopane*. According to the functional equilibrium theory (Brouwer 1962, 1963) and the subsequent optimal partitioning theory (OPT) (Bloom et al. 1985), plants should allocate additional biomass to the organ that takes up the resource that most limits growth. Therefore I expected investment in root growth during drought periods or when nutrient resources are scarce.

In the Chapter 6, I synthetize my results and discuss how the differences in the responses to environmental factors could influence the establishment of tree species, and consequently plant community composition and dynamics. I then relate my findings to possible changes in the functional structure of savannas, and to the on-going debates on both tree-grass interaction and the vegetation dynamics shifts predictions due to climate changes. Lastly, I discuss the applications of my findings to savanna management and conservation.

The study area

The research was conducted in Timbavati Private Game Reserve (Fig. 1.2 and 1.3), Limpopo Province, South Africa (24°15’20.23”S, 31°23’23.63”E) from 2008 to 2010, in a fenced area that excluded large herbivores. The climate of the study area is semi-arid (Kottek et al. 2006) with a dry season from April to October (winter), and a wet season from November to March (summer). In the wet season, rainfall ranges between 450–550 mm with a mean temperature of 33.7 °C, while in the dry season rainfall ranges between 0–80 mm with a mean temperature of 9.4°C (Venter et al. 2003). The average climate of the area is classified as semi-arid under the Köppen-Geiger System (Kottek et al. 2006). The area is included in the Granite Lowveld region (Mucina & Rutherford 2006). The geological substrata are granite and gneiss with numerous dolerite intrusions that never exceed 10 metres in thickness (Gertenbach 1983). As the soil of the experimental plots originates from granite rocks, this soil is shallow and nutrient poor (Venter et al. 2003). This study area is representative of the conditions of a larger area that is crucial for conservation (e.g., Kruger Natural Park), for tourism (e.g., game farms & Kruger NP) and for the subsistence of a large human population.

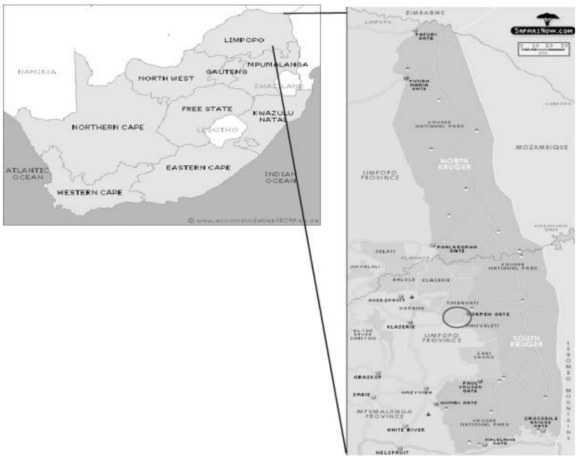

Figure 1.2 – Map indicating the location of the study area in the north-eastern parts of South Africa, bordering Kruger National Park. The insert layout shows Kruger National Park, and the field site is indicated by the marked area.

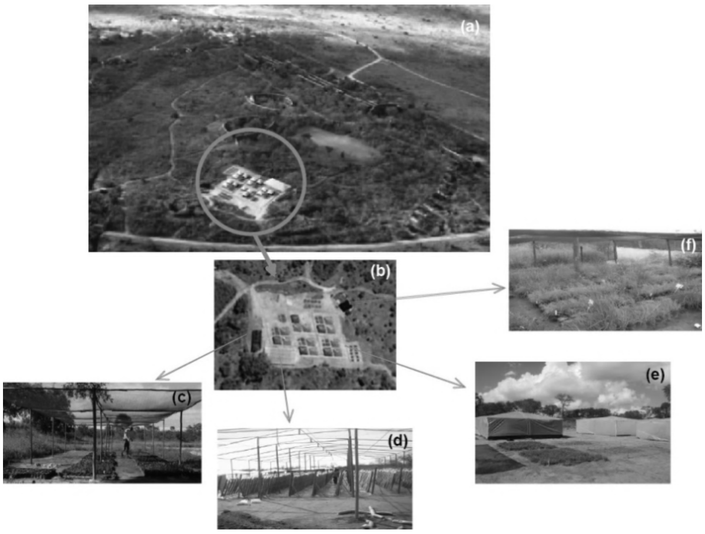

Figure 1.3 – The experimental site that was set up during the first year of the PhD research project to conduct all experiments described in this thesis. (a) bird's eye view of the Southern African Wildlife College, Limpopo, South Africa, (b) close look at the experimental field sites, (c) the nursery: where all the seedlings used in the experiments were sowed and kept, (d) the "big-bags experiment" described in Chapter 5, (e) the field experiment on seedling growth and establishment, the picture shows the sheds used to simulate the studied environment factors (Chapter 3 and 4) and (f) the germination and early survival experiment (Chapter 2).

Chapter 2

Inter-specific variability in germination and early survival of savanna trees in response to environmental variation

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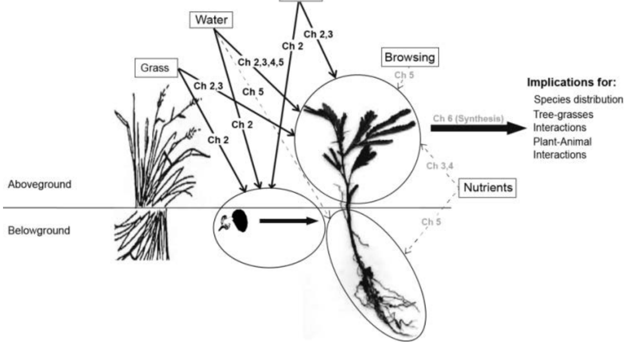
Submitted for publication



Summary

1. Although plant species are expected to respond differently to environmental pressures (regeneration niche theory), there is still a lack of empirical comparative studies on how plant community composition might change under environmental conditions. Here, we evaluate how variable is germination and early seedling survival of a diverse set of common savanna tree species under different environmental conditions, and how differences in species' seed characteristics can explain such inter-specific variation.
2. A fully crossed field experiment evaluated the influence of soil moisture availability, grass presence and sun exposure on the establishment of 16 common savanna tree species. A subsample of the seeds collected for the experiment was analysed to get information on species' seed characteristics (dry mass, water content and nutrient contents).
3. Species responded differently to the treatments, frequent water supply and shade (conditions that enhance soil moisture) only enhancing germination of some species. Seed characteristics explained part of this variation. Under low soil moisture conditions (infrequent water supply and full sun), species with seeds with higher water content and magnesium concentration had higher germination rates, while species with higher calcium concentration had lower germination rate. Under high soil moisture conditions seed with higher phosphorus concentration had a better germination. The effect of grass on germination and early survival also differed among species (neutral, positive or negative). The seed traits did not significantly contribute to explain the variation in seedling survival.
4. *Synthesis*: Our findings confirm a large difference in the species responses to environmental variation during the tree species early recruitment phases (germination and early seedling survival), and show the importance of seed traits for the explanation of such variability. Seed characteristics of a given species may vary from region to region or from year to year. Nevertheless, it is clear that considering inter-specific variation among tree species, and seed traits can improve our ability to manage woody cover invasion and predict impacts of environmental changes.

Introduction

Savannas are ecosystems, found on all continents, where plant communities are dominated by trees and grasses (Sarmiento 1983; Cole 1986). They are characterised by spatial and temporal variability in water resources (Eamus et al. 1999; Kanegae et al. 2000; Kottek et al. 2006) and in nutrient resources (Venter et al. 2003; Schwinning & Sala 2004), as well as by frequent disturbances by fire (Barbosa et al. 1999; Russell-Smith et al. 2003) and herbivory (Van Langevelde et al. 2003; Staver et al. 2009). Savannas can have high species diversity (Sarmiento 1983; Cole 1986), but the causes of such high diversity remane unclear. While a wealth of information has been collected on the life histories and environmental responses of individual species within these communities (see reviews by Van Auken 2000; Bond 2008), yet few studies have compared the performance of multiple savanna species (Bond 2008). Consequently, our understanding of the processes driving species diversity, coexistence and turnover along environmental gradients in savannas is poor. Possible explanations for the high diversity of communities within savannas include niche differentiation of species along resource gradients (Hutchinson 1957; Cole 1986; Venter et al. 2003; Tomlinson et al. 2012) and differential responses of species to disturbance by fire or herbivory (Grime 1973; Staver et al. 2012). In this paper we focus on the role of differential responses of species to disturbance, looking particularly at how differences in the regeneration strategyof species promote species coexistence in savannas (Grubb 1977).

The Regeneration Niche Theory proposes that plant species may co-exist within a community because they respond differently to spatial and temporal -temporal variability in environmental space during their recruitment phase (Grubb 1977). Seed germination and juvenile growth are critical stages in the regeneration of plants and consequently in plant community dynamics (Higgins et al. 2000; Bond 2008). In this study we investigated whether seed germination and seedling early survival of 16 common tree species growing in a semi-arid savanna in South Africa differ in response to variation in environmental conditions (water stress, light and grass competition), and whether these differences could be explained by differences in species seed traits (seed biomass, water content and nutrient concentration).

Although exposure to high temperatures (e.g., fires) or animal consumption (e.g., passed through herbivores digestive tracts) may stimulate tree seed germination (Goor & Barney 1976; Mbalo & Witkowski 1997), seed germination is thought to depend on a steady supply of water, provided either by frequent rainfall events (Baskin & Baskin 2001) or on low evaporative demand. Consequently, germination and early growth may be higher in shaded environments created by other plants that protect the seeds from direct irradiation (Uhl 1987) and maintain moisture availability for longer periods (Holmgren et al. 1997; Anthelme & Michalet 2009). Seedling establishment is influenced by multiple environmental conditions, including availability of water, nutrients and light (Radford et al. 2001). When resources are abundant, seedling establishment may be high across all species (Higgins et al. 2000; Wiegand et al. 2006), but as abundance of resources is rare in Savannas, community species composition might depend on differences in ability to tolerate low resource conditions. Difficulties in accessing these resources can be exacerbated by competition with grasses (Bush & Van Auken 1990), further constraining seedling establishment (Davis et al. 1999), despite of any earlier facilitative benefits for germination (Holmgren et al. 1997).

Differences in functional traits may explain why species differ in their performance across resource and disturbance gradients (Chapin et al. 1993; Violle et al. 2007). For example, seed mass may aid seedling survival in savannas with high fire frequencies (Lahoreau et al. 2006) by providing the seeds with sufficient resources to ensure that they are large enough to withstand aerial shoot removal by fires. Similarly, seed nutrient content may aid germination and establishment under environmental constraints by allowing seedlings to grow independently of any nutrient supply from external sources for an extended period (Moussavi-Nik et al. 1997; Turner et al. 2008).

In this study we investigated whether seed germination and seedling early survival of several (16) common tree species that grow in semi-arid savanna in South Africa differs in response to variation in environmental conditions (water stress, light and grass competition), and whether these differences could be explained by differences in species seed traits (seed biomass, water content and nutrient concentration). Water is a limiting factor for germination and establishment, tree recruitment being more likely to occur during wet seasons with more regular rainfall events (Higgins et al. 2000; Sankaran et al. 2004; Wiegand et al. 2006; Van Langevelde et al. 2011). Therefore, we expected that all species would germinate and establish best under higher soil moisture conditions (frequent water supply and shade), while under stress conditions (i.e. low soil moisture due to low water and full sun; and in the presence of grasses), species will differ in their performance (Hypothesis 1). As high moisture conditions are rare in semi-arid environments, shade will create a niche richer in humidity which might be favourable for early stages of tree establishment. However, inter-specific differences in performance may provide an explanation for plant diversity within savannas (Grubb 1977) As reserves free seeds from dependence on environmental resources (soil water and nutrients availability) (Lahoreau et al. 2006), we expected that species with lager seeds, water percentage and higher nutrient contents would perform better under low water conditions (Hypothesis 2) .

Methods

Study area

This study was conducted in a savanna region, at Timbavati Private Game Reserve, Limpopo Province, South Africa (24°15’20.23”S, 31°23’23.63”E) during the wet season of 2009-2010 (December to March), in a fenced area that excluded large herbivores. The long term mean rainfall (2000-2010) is about 438.2 mm (Hans Hoheisen Research Facility station 10 km NE). The mean maximum temperature during January (hottest month) is 33.7 ºC and the mean minimum temperature for June (coolest month) is 9.4 ºC (Venter et al. 2003). The average climate of the area is classified as semi-arid under the Köppen-Geiger System (Kottek et al. 2006). The area is included in the Granite Lowveld region (Mucina & Rutherford 2006). As the soil of the experimental plots are originated from granite rocks, this soil are shallow and nutrient poor (Venter et al. 2003). The upland soils are sandy (70-84 %) with a percentage of clay between 6 and 15%, (Gertenbach 1983). The experimental site was cleared of plants prior to the start of the experiment.

Species

To test whether different savanna tree species differed in their germination and survival when exposed to changes in environmental conditions, 16 species from savanna tree species were selected (see Table 2.1). All species selected are locally abundant, though associated with different soil types (Venter et al. 2003). Since these species represent a substantial portion of the tree biomass in the local savanna, their responses to different environmental conditions will have substantial effects on the local vegetation structure.

Experimental design

All species were subjected to a fully crossed combination of treatments of water availability (infrequent water supply W0 *vs.* frequent water supply W1), shade (natural light S0 *vs.* shade S1) and grass presence (grass G1 *vs.* no grass G0). A total of 240 seeds per species were collected from wild trees and seeded (30 seeds per treatment combination). Each of these seeds were seeded in individual deep nursery bags (150 mm of diameters and 350 mm in depth, to reduce restrictions to root growth capacity) filled with soil from the study site and placed under each of the treatment combinations. To evaluate whether and how the seasonal variation in the distribution of water affected seed germination and survival and different tree species, we set up two water treatments that differed in the regularity with which they supplied water to plants. For the regular water treatment (W1), we supplied water every two days, resulting in a constantly wet environment for the seeds and seedlings. To simulate a temporal water stress, in the irregular water treatment (W0) we supplied water only every six days. All bags (W0 and W1) were subject to natural rainfall (257 mm), and at the end of the experiment, all experimental bags (W0 and W1) received additional 400 mm of water.

Table 2.1 – Seed characteristics of the 16 savanna tree species used during the experiment. Mean dry mass (in g), water and nutrient concentration (in %) per species, and the percentage of seeds that germinated in all treatments after 20 weeks (TG).

Species	Family	Dry mass	Water	Nutrient concentration						TG
				N	P	K	Ca	Mg	Na	
Acacia exuvialis Verd.	Fabaceae	-	-	-	-	-	-	-	-	18.3
Acacia gerrardii Benth	Fabaceae	0.04	5.12	3.94	0.31	0.97	0.94	0.25	0.01	40.8
Acacia karroo Hayne	Fabaceae	0.11	4.5	3.62	0.37	1.23	0.58	0.25	0.08	17.0
Acacia nigrescens Oliv.	Fabaceae	0.16	7.4	5.46	0.46	1.31	0.79	0.31	0.01	33.3
Acacia nilotica (L.) Willd.	Fabaceae	0.11	7.5	2.96	0.32	1.04	0.85	0.26	0.01	50.8
Acacia sieberiana Keay & Brenan	Fabaceae	0.22	4.0	3.61	0.32	0.94	0.69	0.33	0.00	22.5
Acacia tortilis Hayne	Fabaceae	0.05	4.4	4.76	0.40	1.26	0.69	0.25	0.01	10
Acacia xanthophloea Benth.	Fabaceae	0.02	7.1	2.93	0.37	1.15	0.54	0.35	0.04	15.8
Colophospermum mopane (Benth.) J. Léonard	Caesalpiniaceae	0.36	5.7	5.19	0.57	1.91	0.81	0.31	0.02	39.5
*Combretum apiculatum*_Sond.	Combretaceae	0.10	10.1	3.72	0.40	0.80	0.43	0.41	0.01	25.8
Combretum hereroense Schinz	Combretaceae	0.09	9.1	3.20	0.44	0.88	0.58	0.25	0.02	13.3
Dichrostachys cinerea (L.) Wight & Arn.	Mimosaceae	0.02	19.9	4.78	0.40	1.35	0.40	0.24	0.00	15.8
Euclea divinorum Hiern	Ebenaceae	0.06	8.00	5.01	0.56	1.89	0.10	0.30	0.00	16.2
*Peltophorum africanum*_Sond.	Caesalpiniaceae	0.08	6.1	4.06	0.33	0.95	0.78	0.28	0.01	16.2
Philenoptera violacea (Klotzsch) Schrire	Fabaceae	0.17	17.22	6.12	0.32	1.07	0.63	0.16	0.00	55.8
*Schotia brachypetala*_Sond.	Caesalpiniaceae	0.54	0.4	1.82	0.13	0.80	0.29	0.12	0.00	61.6

To evaluate whether species differed in their germination success and ability to tolerate shade, we included a low light treatment (S1) that aimed to mimic the level of shading found under closed tree canopies, by covering the seedlings with a 2 m high shade net, consisting of one layer of polyurethane shade cloth that blocked 80% of incident solar radiation. Alternatively, seeds were seeded in full sunshine (high light treatment, S0). Moreover, to test the effect of grass presence in tree seed germination and subsequently survival, we used the C_4 grass species (*Digitaria eriantha*) a common and important local species. This species was chosen due its great tolerance to shade and drought, so that when present, grass abundance is kept constant throughout the different treatments. The seeds were seeded in bags with already established grass (with similar grass biomass) (G1), and bags without (G0) grass. During the experimental time, all bags were exposed to natural seasonal temperature.

Measurements

Individual seed germination and early seedling survival was monitored weekly for 20 weeks (December 2009 to March 2010). A sub-sample of the seeds was collected to be used in the experiment was used to obtain the species seed traits. Species' seed characteristics were measured in the laboratory (except *Acacia exuvialis*) using at least 20 seeds of each species, randomly selected from the set of seeds used in this study. Mean fresh and dry seed mass (g) was calculated by weighing the seeds of each species before and after 2 days of drying at 70°C. Seed water content (%) was calculated as the percentage weight loss between mean fresh and dry seed masses. The dry seeds were grounded and analysed for nutrient concentrations (%) following the method described by Temminghoff et al. (2000). Mineral nutrients measured in the analysis were nitrogen (N), phosphorus (P), magnesium (Mg), sodium (Na), calcium (Ca) and potassium (K).

Data analysis

To test for differences in the germination percentage and tree seedling survival among the treatments, we applied generalized linear models (GZLMs using a binomial distribution with probit link function), using water, shade, grass and time as explanatory variables. Given the complexity of our experiment and the important role of multiple interactions between explanatory variables, we selected the most parsimonious model, using stepwise model selection (forward and backward variable selection) based on the Akaike Information Criterion (AIC) (Burnham & Anderson 2002; Crawley et al. 2007). We used Sidak test for pairwise comparisons between the treatments.

We used general linear models (GLMs) to test the effect of dry weight, water content and concentrations of N, P, Mg, Na, Ca and K on the germination and survival percentage in the expected low moisture conditions (W0S0) and in the expected high moisture conditions (W1S1). For this test we only used the 15 study species for which we managed to collect sufficient seeds for chemical analyses (i.e. not including *Acacia exuvialis*, see Table 2.1). Again, we used forward and backward stepwise model selection and AIC to select the select the most parsimonious model.

Results

Inter-specific variability of plant responses (germination and early seedling survival) was high not only under stress conditions, but also under perceived favourable conditions. Seed traits (water content and some mineral concentrations) explained part of this variation in germination but not of seedling survival.

Seed germination success

Germination patterns were diverse among species (Tables 2.1 and Fig. 2.1). While we expected that under conditions that maximize soil moisture (S1W1) species would achieve their best performance (Hypothesis 1), this was only the case for three species (*Acacia nigrescens*, *Combretum hereroense*, *Acacia xanthophloea*). Five other species germinated best in W1 independently of shade (*Euclea divinorum*, *Acacia nilotica*, *Philonoptera violaceae*, *Schotia brachypelata*, *Combretum apiculatum*) and for two of these independently of grass (*E. divinorum* and *S. brachypelata*) (Fig. 2.1). For one species (*Acacia exuvialis*) even water supply and shade even had a negative effect on germination, particularly when grass was present, while the germination of another (*Acacia tortilis*) was not significantly affected by water regime or shade. For other species water regularity could have a positive or negative effect depending on the specific micro-climatic conditions. For example, in S1G1 frequent water supply enhanced germination of *Peltophorum africanum*, and reduced germination of *Colophospermum mopane* and *Dichrostachys cinerea*. Under S0G1germination of *P. africanum* was supressed while germination success of *C. mopane* and *D. cinerea* was enhanced. Shade affected tree species under specific conditions (Fig. 2.1). We found a positive effect of shade on germination of *P. violacea* (in W0G1), *A. nigrescens* and *A. xanthophloea* (both in W0G0), *C. hereroense* (in W1G0), *E. divinorum* (in W1G1). The germination of *Acacia gerrardii* and *S. brachypelata* was negatively affected (both in W1G1). In *C. mopane* and *P. africanum,* we found both positive and negative effects of shade. The presence of shade did not influence the germination of *A. exuvialis*, *Acacia karroo, A. nilotica*, *Acacia sieberiana*, *A. tortilis*, *C. apiculatum* and *D. cinerea* (Fig. 2.1).

The effect of grass presence on the germination patterns was also diverse between species (Tables 2.1 and 2.2, Fig. 2.1).The beneficial effect of high moisture conditions detected for some species (e.g., *A. nigrescens*, *C. hereroense*, *A. xanthophloea*) was significantly supressed by grass presence. Grass also had a negative effect on *C. apiculatum* and *D. cinerea* but only under irregular water supply (W0). However, grass had a positive effect on five tree species: *A. exuvialis* and *A. nilotica* (W0)*, A. tortilis* (W1S1), *C. mopane* (in W1S1 and W1S0) and *S. brachypelata*. The germination of *A. karroo* was also significantly enhanced by grass presence when exposed to the infrequent water supply and shade, and negatively affected by the frequent water supply and full sun. In *P. violacea* germination was positively affected by grass with the infrequent water supply and shade, and negatively affected in frequent water supply. The presence of grass did not influence the germination of *A. sieberiana, P. africanum* and *E. divinorum* (Fig. 2.1).

Early seedling survival

Seedling survival patterns also varied among species (Table 2.2 and Fig. 2.2). Seedling survival patterns also varied among species (Table 2.2), frequent water supply only affected the survival of five species (A. *gerrardii*, *A. nilotica*, *A. sieberiana*, *S. brachypelata* and *P. africanum*) in the bags without grass and full sun. Shading increased the survival of *P. africanum* (only for W0G0), whereas it had a negative effect on *A. nilotica* (only for W1G0), *A. gerrardii* (only for W1G1) and *C. mopane* (only for W0G0) (Fig. 2.1). For the other species, we did not find an effect of shade. The presence of grass negatively affected seedling survival of *A. nigrescens*, *A. nilotica*, *A. gerrardii* (in W1), *A. sieberiana* and *P. africanum* (in W1S0), and *D. cinerea* (in W0) (Fig. 2.2). It positively affected the survival of *S. brachypelata* (W0S1). The presence of grass did not influence *A. exuvialis*, *A. karroo*, *A. tortilis, A. xanthophloea*, *C. apiculatum*, *C. hereroense*, *E. divinorum*, *P. africanum* and *P. violacea* survival (Fig. 2.2).

Effect of seed characteristics on germination and early seedling survival

The selected tree species showed a large variation in the seed characteristics (seed dry mass, water content and nutrient concentrations) (see Table 2.1). Species' germination rate under low moisture conditions (infrequent water supply and full sun) was positively related with percentage seed water content and magnesium concentration and negatively related with calcium concentration (Table 2.3). Species' germination rate under high moisture conditions (frequent water supply and shade) was positively related with phosphorus concentration (Table 2.3). Species 'seedling survival was not related to any of the measured seed traits (Table 2.3).

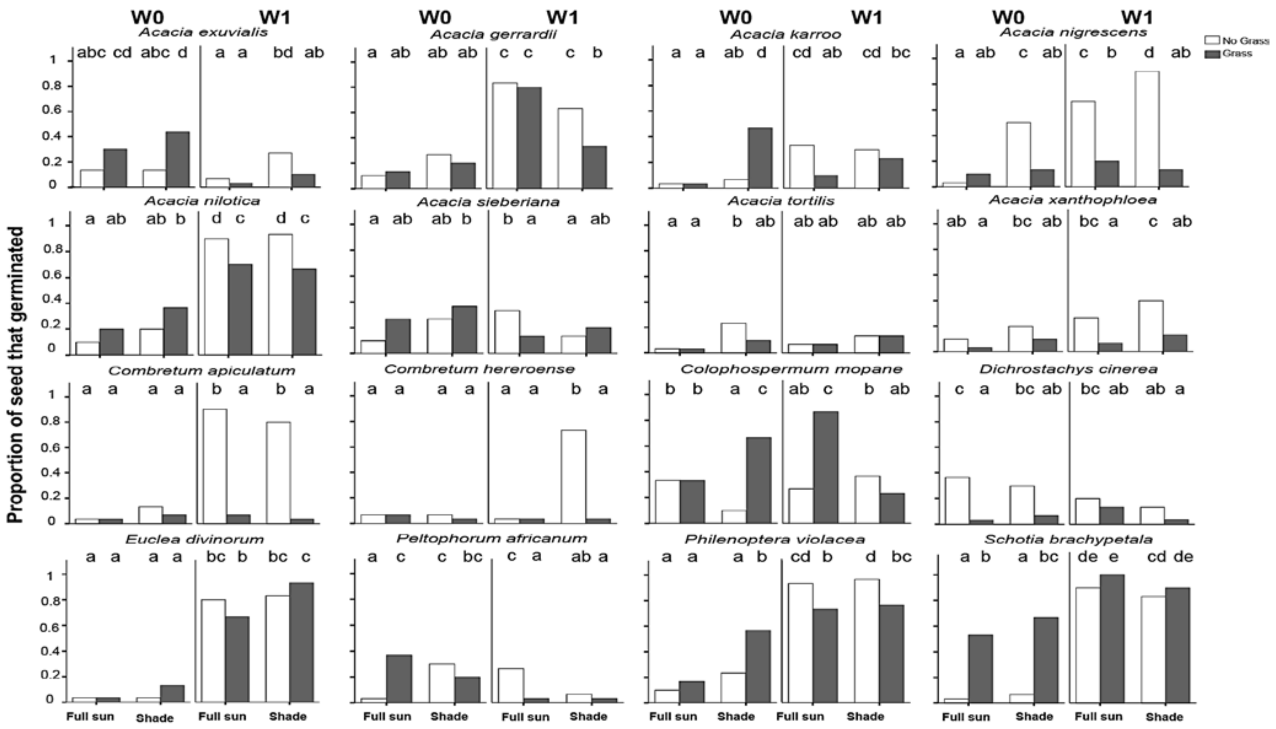


Figure 2.1 – Proportion of seeds that germinated under frequent (W1) and infrequent water supply (W0), shade, full sun, and in the presence (light bars) and absence of grass (dark grey bars). The letters in black represent the differences in germination between treatments.

Discussion

Despite the many studies that focused on the establishment and survival of savanna trees (e.g., Higgins et al. 2000; Schutz et al. 2002), there is a lack of comparative knowledge on the effects of the main environmental conditions on the germination and early survival of different savanna trees (Bond 2008). By setting up an experiment involving a wide variety of savanna tree species subjected to standardized treatments of several factors that are considered important drivers of seed and seedling ecology, namely water, light and grass competition, we demonstrated how diverse the modulating factors are among the different tree species and how seed characteristics can explain part of that variation.

Seed germination success

As an adequate amount of water has to be available for imbibition and subsequent germination (Baskin & Baskin 2001; Bunker & Carson 2005; Kraaij & Ward 2006), we expected that a more frequent soil moisture would lead to a higher germination rate in all study species (Hypothesis 1). Although frequent soil moisture availability did lead to higher germination of ten of the study species, some species were negatively affected by frequent water supply while for others the positive effect of water was depended on the presence of grass and shade (Fig. 2.1).Seeds used in the experiment were collected directly from trees, and no pre-treatment to release seeds from dormancy (e.g., simulation of fire or seed passage through an animal's gut, see Mbalo & Witkowski 1997; Bodmer & Ward 2006) was applied in the seed before seeded in the bags. Seeds could therefore be less responsive to frequent water supply. However, seed passage through an animal's gut does not always lead to enhanced germination (Coe & Coe 1987), and frequent water supply had a positive effect on some species with hard seed coats (e.g., *A. nigrescens*), while it did not have a clear effect on other species with hard coats (e.g., *A. sieberiana*) or without hard coats (e.g., *C. mopane*), suggesting that differences in the responses to frequent water supply are not related with coat-imposed dormancy. Seed germination could potentially be higher when seeds are deposited in dung which provides moisture and nutrients (Traveset et al. 2007). However, dung can also inhibit these processes because it dries out quickly under hot conditions, thereby impeding seed imbibition and seedling emergence (Brown & Archer 1989). The lack of a positive effect of water on the germination of some species is, therefore, unlikely related to the absence of dung or treatment to break the dormancy of the seed. The fact that some of our hard coated species (e.g., *A. exuvialis*) were negatively affected by frequent water supply could be related to fungal attack (personal observations).

Table 2.2 – The effects of the presence of grass, shade and water supply on the germination percentage and recruitment percentage of the 16 studied savanna species (30 seedlings per species). W: water treatment; S: shade; G: grass. The results obtained for the most parsimonious models (lowest AIC) are provided for each subset. P-values were obtained from a likelihood ratio test in which deviances with and without that term in the model were compared n.s., P > 0.05. The symbol "-" represents a variable not included in the model (Illustrated in Figure 2.1).

Species	W	S	G	W*G	W*S	G*S	W*G*S
Germination							
A. exuvialis	ns (0.6)	0.039	0.004	0.002	-	-	-
A. gerrardii	<0.0001	ns(0.09)	ns(0.08)	-	<0.0001	-	-
A. karroo	0.001	ns(0.16)	ns(0.40)	0.003	ns(0.08)	ns(0.07)	-
A. nigrescens	<0.0001	<0.0001	ns(0.40)	0.001	ns(0.11)	0.003	-
A. nilotica	<0.0001	-	ns(0.08)	<0.0001	-	-	-
A. sieberiana	0.037	ns(0.10)	ns(0.10)	0.017	0.016	ns(0.43)	ns(0.07)
A. tortilis	-	0.013	-	-	-	-	-
A. xanthophloea	0.021	0.049	0.001	-	-	-	-
C. apiculatum	<0.0001	ns(0.16)	ns(0.46)	<0.0001	ns(0.06)	-	-
C. hereroense	ns(0.56)	ns(1.00)	ns(1.00)	ns(1.00)	0.004	ns(0.65)	ns(0.13)
C. mopane	ns(0.57)	ns(1.00)	0.037	0.001	0.031	0.001	0.000
D. cinerea	0.038	-	0.001	ns(0.09)	-	-	-
E. divinorium	<0.0001	ns(0.77)	ns(0.27)	-	-	0.041	-
P. africanum	0.032	0.021	0.009	0.001	0.003	0.007	0.016
P. violacea	<0.0001	0.001	0.011	<0.0001	ns(0.07)	-	-
S. brachypetala	<0.0001	ns(0.23)	0.000	0.017	0.051	-	-
Recruitment							
A. exuvialis	ns(0.56)	ns(0.56)	ns(0.39)	ns(0.39)	ns(0.39)	ns(0.98)	ns(0.98)
A. gerrardii	ns(0.98)	ns(0.98)	ns(0.23)	0.055	ns(0.98)	0.030	-
A. karroo	ns(0.99)	-	-	-	-	-	-
A. nigrescens	ns(0.98)	ns(0.98)	<0.0001	-	ns(0.98)	-	-
A. nilotica	<0.0001	0.030	<0.0001	-	-	-	-
A. sieberiana	ns(0.08)	ns(0.64)	ns(0.64)	ns(0.07)	ns(0.23)	ns(0.51)	0.056
A. tortilis		ns(0.99)	ns(0.99)	-	-	-	-
A. xanthophloea	0.010	ns(0.58)	ns(0.99)	-	-	ns(0.99)	-
C. apiculatum	ns(0.99)	ns(0.99)	ns(0.99)	-	ns(0.99)	-	-
C. hereroense	ns(0.99)	ns(0.99)	ns(0.99)	-	-	-	-
C. mopane	ns(0.24)	0.019	ns(0.99)	ns(0.99)	ns(0.31)	ns(0.31)	ns(0.99)
D. cinerea	ns(0.06)	-	<0.0001	-	-	-	-
E. divinorium	ns(0.98)	ns(0.78)	ns(0.99)	ns(0.99)	-	ns(0.09)	-
P. africanum	0.040	0.040	ns(0.19)	0.021)	0.009	ns(0.10)	ns(0.09)
P. violacea	ns(0.98)	ns(0.99)	ns(0.99)	ns(0.99)	ns(0.99)	-	-
S. brachypetala	<0.0001	-	0.006	ns(0.08)	-	-	-

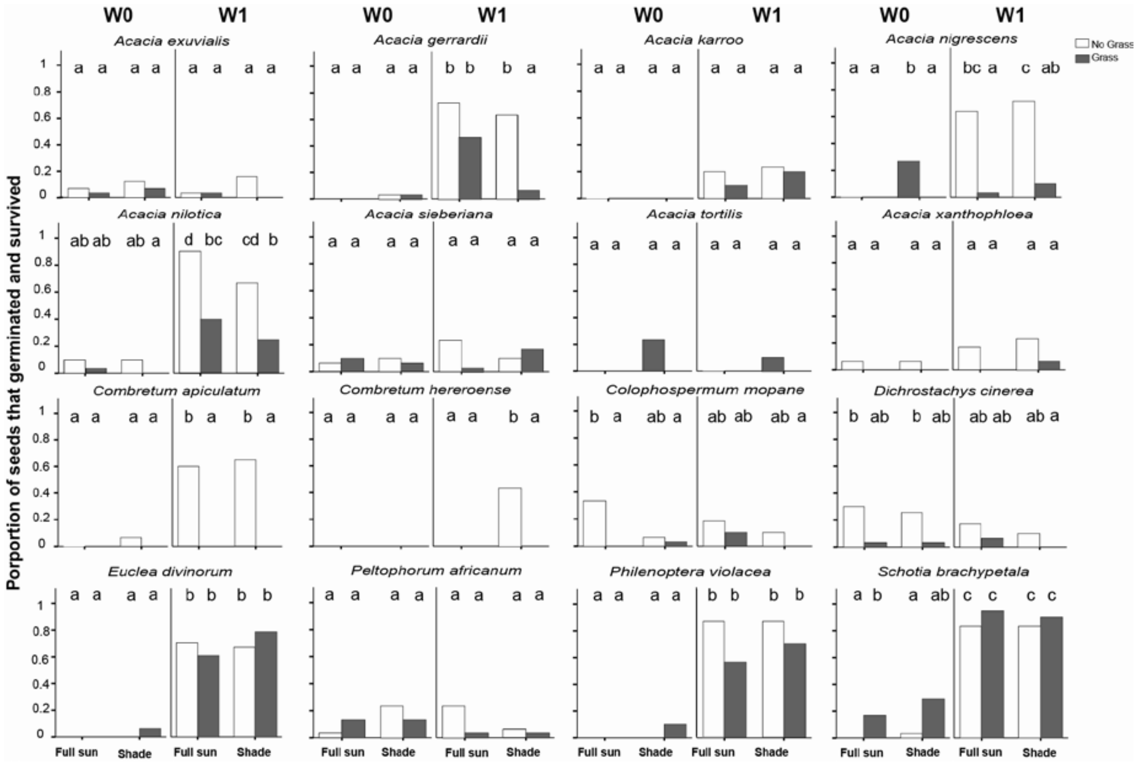


Figure 2.2 –Proportion of seeds that germinated and survived until the end of the experiment (20 weeks) under frequent (W1) and infrequent water supply (W0), shade, full sun, and in the presence (light bars) and absence of grass (dark grey bars).The letters in black represent the differences in survival between treatments.

Table 2.3 – Effect of seed characteristics of savanna species on the germination and survival percentage under the different treatments. DW, dry weight of biomass (mg) (log-transformed); WC, water content; [N], nitrogen concentration; [P], phosphorous concentration; [K], potassium concentration; [Ca], calcium concentration; [Na], sodium concentration; [Mg], magnesium concentration. Treatments: W0S0: Natural Rainfall/Full sun; W1S1: Even water treatment/Shade. All plots considered for this analysis had no grass (G0). Estimates indicate the magnitude and direction of the effect of each variable. The results obtained for the most parsimonious models (lowest AIC) are provided for each subset. P-values were obtained from a likelihood ratio test in which deviances with and without that term in the model were compared. n.s. P > 0.05. N= 15 (the 15studied species). The symbol "-"represents a variable not included in the model. Interactions between explanatory variables did not contribute significantly to any of the models.

	Germination				*Survival*			
	W0S0		*W1S1*		*W0S0*		*W1S1*	
Variables	Estimate	P-value	Estimate	P-value	Estimate	P-value	Estimate	P-value
DW	0.025	ns(0.20)	0.138	ns(0.23)	-	-	-	-
WC	0.021	0.002	0.043	ns(0.17)	-	-	-	-
[N]	-	-	-0.263	ns(0.08)	-	-	-0.150	ns(0.07)
[P]	-0.341	ns(0.11)	3.628	0.034				
[K]	-	-	-	-	-0.116	ns(0.18)	-	-
[Ca]	-0.199	0.044	0.773	ns(0.18)	-	-	-	-
[Na]	-	-	-	-	-	-	-5.823	ns(0.10)
[Mg]	0.911	0.006	-1.535	ns(0.31)	0.778	ns(0.058)	-	-

Shade might provide protection from irradiation (Uhl 1987), higher soil moisture (Vieira et al. 1994), and decreased root competition with grasses (Nepstad 1989; Holmgren et al. 1997), increasing water availability to the seeds. Therefore, we expected that shade would enhance seed germination. However, our results show that the responses of savanna tree species to shade vary markedly among species, and not all species being positively affected by shade (Fig. 2.1). Different results can be found in the literature regarding effects of shading from parental trees. Some studies suggested that shade negatively influence seed germination (Loth et al. 2005) or limit seed from dormancy release (i.e. changing light spectrum, see Fankhauser & Chory 1997). The high variation in the species response to light conditions might also be related to the high variability of the effect of water. Plots with even water supply and shade might have a level of water moisture that is too high for the germination of some species, intermediate moisture (W1S0 or W0S1) possibly being preferable for the germination of some species.

Previous studies revealed that the presence of grass can severely limit tree germination as it may decrease the amount of water available for the seed imbibition (Sun & Dickinson 1996; Hooper 2002). Therefore, we expected that the presence of grass would negatively affect seed germination (Hypothesis 2). Our results demonstrated that the grass presence was an important factor for savanna tree seed germination, with 13 out of 16 species being affected by the presence of grass. However, only six species were negatively affected by grass presence, other four species were positively affected by grass (Fig. 2.1). The negative effect of grass on seedling establishment is also found by others (Ward & Esler 2011), possibly caused by belowground competition for water and nutrients (Kraaij & Ward 2006). However, in extremely dry conditions (under full sun light and low water availability), grass may help to retain soil moisture, providing seeds with a better opportunity for germination (e.g., Anthelme & Michalet 2009).

Early seedling survival

Similar to the results obtained for germination, our study shows that the effect of frequent soil moisture on seedling survival varied among the tree species. As water stress is suggested to be an important cause of savanna tree seedling mortality (Midgley & Bond 2001; Sankaran et al. 2005), we expected that frequent water availability would enhance survival of most species (Hypothesis 1). However, only eight species had a positive response to frequent water supply. The lack of effect of frequent water supply in certain species may be due to the fact that some species are more sensible to fungal infestation (e.g., Toledo et al. 2010, Barbosa et al. unpublished data).

In contrast to the results obtained for germination and contradicting our expectation (Hypothesis 1), early survival of the seedlings of most species was not affected by shade, some species even being negatively affected by shade, which suggests that their establishment is favoured by direct sunlight during the wet season (see Gerhardt 1996). Furthermore, as we expected, grass presence negatively affected most species (except *S. brachypelata*). Indeed, numerous empirical and theoretical studies hypothesized that competition with herbaceous vegetation for soil water during the young plant stage can limit, or even totally prevent, the establishment of woody plants (Davis et al. 1999), particularly in dry environments (Van der Waal et al. 2009; Ward & Esler 2011). The fact that *S. brachypelata* was positively affected by the presence of grass, could be related with the fact that within semi-arid savannas this species is only common in regions with high soil humidity (e.g., in riversides, personal observation), being hence more dependent on the ability of grasses to improve local microclimatic conditions (e.g., water availability) (Maestre et al. 2003).

Effect of seed dry weight, nutrient concentration and water content

The response to changes in environmental factors is quite variable among the most common species. Our results partly confirmed our expectations (Hypothesis 2), as we found that seeds with higher water percentage germinated better under stressful conditions (low soil water moisture) (Table 2.3). Under high moisture conditions, seed germination was independent of water content.

Contrary to our expectations, high seed concentration of most nutrients (N, P, Na, Ca and K) did not promote germination and survival under stressful conditions (low soil moisture) (Table 2.3). Our results only found a positive effect of seed magnesium concentration, and a negative effect of seed calcium concentration on seed germination. The positive effect of Mg content for germination is likely related with the fact that magnesium (Mg-chelatase) is as an ABA (abscisic acid) receptor residing in chloroplasts and mediating ABA responses in seed germination and post-germination growth (Shen et al. 2006). The negative effect of seed calcium (Ca) concentration on germination rate could be related with the fact that calcium serves as a critical messenger in many adaptation and developmental processes, playing a crucial role in the regulation of almost all biological processes including the response to abiotic stresses in plants (Batistic & Kudla 2009). Increases in the levels of Ca concentrations have a combined positive effect on gibberellin acid (GA) and a negative effect on phytohormone abscisic acid (ABA) (Pandey et al. 2008). As ABA and GA are counteracting with each other during germination (Groot & Karssen 1992), the increase of calcium concentration in the seed may destabilize the ABA-GA balance, reducing seed germination.

It is suggested that high P concentration in seed compensates for stress conditions (low water and nutrient availability), increasing the chances of plant establishment (Berezkin et al. 1984). However, only under high soil moisture conditions (frequent water supply and shade) species with higher seed phosphorus (P) concentration had a better germination performance (Table 2.3). Most of the P in seed is in the form of phytic acid (Shi et al. 2003), and low concentration of phytic acid in the seeds can result in negative effects on both seed germination and early seedling growth (Welch & House 1984; Feil & Fossati 1997). It is possible that low moisture conditions reduce the levels of phytic acid (or their ability of being utilized by seeds), reducing the benefits of P concentration. Further tests would be needed to understand why differences in water availability lead to differences of the nutrients influencing seed germination.

Although seedlings resulting from larger seeds (i.e. with more reserves) are expected to be more successful in drier environments such as our study area (Muller-Landau 2009), our results showed that seed dry mass did not influence early stages semi-arid savanna tree species early tree establishment (Table 2.3, see also Hou et al. 2010). It is suggested that smaller seeds may have an advantage in stressful conditions because of a shorter germination time (e.g., Murali 1997). It is therefore possible, that for our study species the advantages compensated the disadvantages, leading to a neutral effect of size. Further studies are needed to test how later stages of tree establishment are affected by these environmental conditions, but the older the seedling the less likely its survival is to be affected by the characteristics of the seed.

Implications of results to the study and conservation of savanna plant community

Our results indicated in early tree recruitment stages (germination and survival) the responses of savanna tree species differ markedly under environmental conditions variation. Water supply and shade not always enhanced germination and seedling survival, and grass not always had a negative effect, each species being adapted to only dominate very specific micro-habitats found in the savanna biomes. Indeed, the germination and early survival was only strongly negative affect by presence of grass in some tree species, and under very specific under a different combination of conditions (e.g., when water is scarcer and there no protection from sun light). As an example, under low moisture conditions while niches with high grass abundance are likely to be mostly utilized by *S. brachypetala*, *Combretum* species and *A. nigrescens* are only likely to have a competitive advantage over other tree species in the absence of grass. Also, under infrequent water supply and full sun only two species had germination and survival above 30%, *C. mopane* and *D. cinerea*, both being, however, significantly suppressed by the presence of grass. These species have hence an ecological advantage over other tree species under low moisture conditions, typical from semi-arid systems, but such advantage would be lost if water availability becomes more regular, or under shaded conditions (Figs. 2.1 and 2.2).

Our results clearly support the idea that a diversity of ecological niches will support a diversity of species. Such patterns have important implications for the dynamics of savannas. If all species had a common response to drivers (assumption made in some predictive models of vegetation dynamics, e.g., Jeltsch et al. 1996; Higgins et al. 2000; Wiegand et al. 2006), environmental changes could lead to faster changes in the overall vegetation structure, while the demonstrated diversity of responses will likely have a strong buffering effect, enhancing resilience of savanna habitats. Hence environment variability that characterizes savannas (e.g., accentuated seasonality of rain patterns, and fire regimes) is likely essential to maintain the overall vegetation structure within savanna systems i.e. competitive advantage of species is strongly confined to particular niches, reducing the chances of a vast expansion of only one species. Recent increase in the human intervention (Solbrig & Young 1993) may, however, homogenize environmental conditions (e.g., due to changes in fire regimes or intensification of herbivory by cattle) may reduce the variability of niches available may benefiting specific tree species (e.g., leading to bush encroachment).

While it is challenging to include such diversity of patterns in predictive models, the seed trait analyses indicates that some general rules to model germination of trees may be extracted from the characteristics of the seeds (e.g., under low moisture conditions water content and Mg concentration enhanced germination while Ca reduced germination, and under high moisture conditions P concentration enhanced germination). Including such information on inter-specific variability will hence improve the predictive ability of ecological models, hence their applicability in conservation management.

Concluding remarks

While it is recognized that the high diversity in tropical savannas and grasslands can help these ecosystems to better face changing conditions (Seghieri et al. 1995), lack of empirical information on different savanna tree species led to predictive models based on only few species. These models assume a similar response of seedlings of tree species to water availability and grass competition (Wiegand et al. 2006), hence relying on the assumption that demographic stochasticity is similar for all species and is the main regulator of community composition (neutral theory, see Hubbell 2006). Our study clearly shows that a high variation in the species responses to environmental changes and, such variability being likely essential to maintain the overall vegetation structure within savanna systems. To draw general conclusions about the functioning of savannas, this high inter-specific variability should be taken into account when studying dynamics of savannas vegetation or suggesting management options.

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Chapter 3

Responses of savanna tree seedlings to grass competition and resource variation depend on species functional traits

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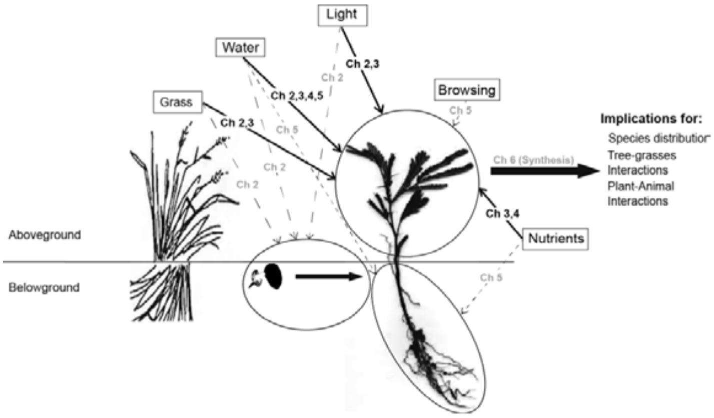

Summary

1. The regeneration niche theory presumes that plant species respond differently to environmental pressures, allowing plant species to coexist, and explaining their uneven distributions across resource and disturbance gradients. However, due to lack of empirical comparative studies, predictive models on vegetation dynamics assume similar responses among species. To cover this gap of knowledge it is essential to better understand how seedling performance of different species in a community varies under different environmental conditions, and how species functional traits influence this performance.
2. By setting up a large multi-species experiment in an African semi-arid savanna, we investigated whether species with different functional traits differ in their response to variation in resource availability, and to competition with grass. We selected four species with a syndrome of small leaves, spines and N-fixing associations (hereafter termed ‘fine-leafed species’), and four with a syndrome of broad leaves, no spines, and lacking N-fixing associations (hereafter termed ‘broad-leafed species’).
3. Our results show that even in favourable resource conditions seedling growth and establishment of several abundant tree species are severely affected by grass competition. However, recruitment strategies varied among species functional groups, particularly under high stress conditions (water or light deprivation). While in the absence of grass, light deprivation (shade) reduced seedling growth of all species, shade also reduced the negative effects of the presence of grass on the growth of broad-leafed species. Moreover, while the increase in nutrient availability did not enhance seedling stem growth production per se, it reduced the negative effect of grasses on the seedlings. However, this reduction differed between the species groups, affecting only broad-leafed species under natural rainfall, and only fine-leafed species under even watering treatment.
4. *Synthesis*. Given the existent high variation in micro-climatic conditions within savannas, the variability of recruitment strategies among species revealed by our study is likely to maintain high tree species diversity, a given species only having advantage over others under specific environmental conditions. Our findings hence support the regeneration niche theory. Including information on variation of responses among functional groups in future predictive studies might improve our ability to predict vegetation structure changes in savannas.

Introduction

Due to continue increase of human population and its consequent changes in land use, African savannas are considered to be one of the most susceptible habitats to climate changes (IPCC 2007). Consequently, there is an increasing concern on the consequences of such changes on the vegetation dynamics of this ecosystem. The regeneration niche theory (Grubb 1977) proposes that plant species within a community respond differently to environmental pressures, which allows for their coexistence, and explains their uneven distributions across resource and disturbance gradients. However, due to lack of empirical comparative studies involving several plant species, most predictive models on vegetation dynamics assume that, in general, species respond similarly to environmental changes (e.g., Higgins et al. 2000; Wiegand et al. 2006). Seedling establishment and juvenile growth are critical stages in the regeneration of plants and overall plant population dynamics (Higgins et al. 2000; Radford et al. 2001; Wiegand et al. 2006). Therefore, better understanding of how seedlings of different species in a community perform under different environmental conditions relative to themselves and to one another, will help to explain the composition of plant communities and how they might develop under changed environmental conditions (Davis et al. 2000; Davis & Pelsor 2001).

Tropical savannas are characterised by large regional and temporal variability in water resources (Eamus et al. 1999; Kanegae et al. 2000; Kottek et al. 2006) and in nutrient resources (Venter et al. 2003; Schwinning & Sala 2004), and disturbances by fire (Barbosa et al. 1999; Russell-Smith et al. 2003) and herbivory (Van Langevelde et al. 2003; Staver et al. 2009), resulting in large variation in plant species occurrence and abundance. Competition with grasses for soil resources may be important for limiting tree recruitment, but this remains a matter of debate (Higgins et al. 2000; Jeltsch et al. 2000; Sankaran et al. 2004; Van der Waal et al. 2009; Van Langevelde et al. 2011). To date, a wealth of information has accumulated on the life histories and environmental responses of individual savanna tree species (see reviews by Van Auken 2000; Bond 2008), providing suitable data for modelling and predicting shifts in the tree and grass cover in savannas (e.g., Higgins et al. 2000; Wiegand et al. 2006). However, comparative studies on the performance of multiple savanna tree species are lacking (Bond 2008), limiting our ability to explain and predict changes in the species composition of savannas under different environmental conditions (Van Langevelde et al. 2011).

Differences in functional traits can mechanistically explain why species differ in their performance when exposed to variations of abiotic (e.g., stability, fertility, drought) and biotic (e.g., competition, herbivory) factors (Chapin et al. 1993; Violle et al. 2007). Qualitative trait differences, associated with nutrient and water gradients, between species have been recognised (Cole 1986; Scholes 1997; Pennington et al. 2000). Notably, within African savannas, dystrophic or humid savannas are dominated by non-spinescent, broad-leafed, non-N-fixing species, while eutrophic or arid savannas are dominated by fine-leafed species which may additionally be spinescent or N-fixing (Scholes 1997; Venter et al. 2003; Mucina & Rutherford 2006; Kottek et al. 2006). These two groups can also be distinguished on the basis of their leaf chemistry and physiology and their biomass allocation traits (Tomlinson et al. submitted a). Therefore there is reason to believe that seedlings of these species respond differently to changes in supply rates of resources, and that these differences may in part explain why they dominate in different environments.

We set up a multi-species experiment in an African semi-arid savanna to investigate whether juveniles of four locally abundant fine-leafed tree species and four common broad-leafed tree species (Table 3.1) differ in their responses to variation in water, nutrient and light supply during their first growing season, and whether they differed in their response to competition from a C_4 grass species (*Digitaria eriantha*) for these resources. To test whether and how the seasonal variation in the resource distribution affected survival and growth of tree seedlings of different species, we measured aboveground growth parameters (stem biomass, leaf biomass and stem length) as proxies for seedling performance under the different treatment conditions. As several studies suggest that water stress (Midgley & Bond 2001; Sankaran et al. 2005; Kraaij & Ward 2006; Meyer et al. 2007), nutrient shortage (Bardgett & Wardle 2003; Van der Waal et al. 2009), and shade (Gerhardt 1996) may limit seedling survival and establishment in dry systems, we expected that all species respond positively to regular water availability, increased nutrient supply and full light (Hypothesis 1). Secondly, because fine-leafed species have greater leaf nutrient concentrations than broad-leafed species (Tomlinson et al. submitted a), and as broad-leafed species are commonly associated with infertile soils in semi-arid savannas (Cole 1986; Frost 1996; Venter et al. 2003), we expected that fine-leafed species respond more positively to added nutrients than broad-leafed species (Hypothesis 2). Thirdly, we expected that grass competition for water and nutrients suppresses tree seedlings under high light conditions (Hypothesis 3). However, as C_4 grasses are suppressed by low light conditions (Pulido & Díaz 2005; Smit et al. 2008), and broad-leafed species are suggested to be more tolerant to shade than fine-leafed species (Smith & Goodman 1986; 1987), we expected seedlings to have a better growth performance in shaded environments, particularly for broad-leafed species (Hypothesis 4).

Methods

Study area

This study was conducted in a savanna region, at Timbavati Private Game Reserve, Limpopo Province, South Africa (24°15’20.23”S, 31°23’23.63”E) during the wet season of 2009-2010 (November – May), in a fenced area that excluded large herbivores. The mean rainfall of the previous ten years (2000-2010) was *ca.* 438 mm (Hans Hoheisen Research Facility station 10 km NE). The mean maximum temperature during January (hottest month) is 33.7 ºC and the mean minimum temperature for June (coolest month) is 9.4 ºC (Venter et al. 2003).The average climate of the area is classified as semi-arid under the Köppen-Geiger System (Kottek et al. 2006). The area is included in the Granite Lowveld region (Mucina & Rutherford 2006). As the soil of the experimental plots originated from granite rocks, they are nutrient poor (Venter et al. 2003) and are also shallow (ca. 1.5m depth). The upland soils are sandy (70-84 %) with a percentage of clay between 6 and 15%, (Gertenbach 1983). The experimental site was cleared of plants prior to the start of the experiment, by removing vegetation aboveground, and breaking and turning the soil to remove the roots.

Species

To test whether various savanna tree species differed in their response to environmental conditions, eight locally abundant tree species that make up a large proportion of vegetation cover in savannas were selected. The species belonged to two different functional trait groups: four with characteristic small leaves, spines, and N-fixing associations (hereafter termed ‘fine-leafed species’), and four with characteristic broad leaves, no spines, and lacking N-fixing associations (hereafter termed ‘broad-leafed species’) (see Table 3.1). Since these species are abundant in the savannas of Southern African region, changes in their populations due to varying environmental conditions will likely have substantial effects on the local vegetation structure.

Experimental design

The experiment layout involved five treatment blocks within the study area. Each block was divided in 12 plots ($4m^2$), giving a total of 60 plots. A total of 240 seedlings per species were planted, 20 seedlings for each treatment combination (five replication blocks, each with four seedlings per plot). Different treatments involving variation in grass (with, G1, and without, G0), water (regular supply, W1, and natural rainfall W0), shade (with, L1, and without, L0) and nutrients (with, N1, and without, N0) were then applied to each of the 12 plots within a block. Due to logistic constrains it was not possible to have natural rainfall and shade in the same plot (the shade cloth would stop most of the rainfall to reach the plot soil), an incomplete treatment design was used where light and water were not fully crossed (all combinations of W0×L0 were absent). For plots with grass a single local common grass species *Digitaria eriantha* was seeded and established. To examine whether and how the seasonal variation in the distribution of rainfall affected survival and growth of tree seedlings of different species, we set up two water treatments that differed in the regularity with which they supplied water to plants. For the regular water treatment (W1), natural rainfall was excluded from the treatments by rainout shelters and we supplied a fixed amount of 46.3 mm (185 L per 2x2 m plots) of water to the seedlings every two weeks for the 6 months of the experiment (November 2009 to May 2010), yielding a total of 556 mm water over the experiment. This amount of water was based on the water deficit rules as defined in the Köppen-Geiger climate classification (Kottek et al. 2006). The irregular, natural rainfall treatment (W0) received 623 mm during the period of the experiment, which was higher than the long-term mean for the area (438 mm/season). The 2009-2010 wet season rainfall distribution had two major rain peaks in November-December 2009 (320 mm) and April-May 2010 (202 mm), with an intermittent 3-month dry period (January to March) (Fig. 3.1)

As we were interested in whether species differed in their ability to tolerate shade and whether shade affected competitive effects of grass on seedlings, we included a low light treatment (L0) that aimed to mimic the level of shading found under closed tree canopies, by covering the seedlings with a 2 m high shade net, consisting of one layer of polyurethane shade cloth that blocked 80% of incident solar radiation. Alternatively, plants were grown in full sunshine (high light treatment, L1).

To test whether seedlings of different species differed in their growth response to increased soil nutrient supply and whether this affected the competitive effect of grasses on tree seedling growth, we included two nutrient treatments: no nutrients added (N0) and nutrients added (N1). Granular slow-release 3:1:2 NPK inorganic fertilizer (Osmocote Exact Standard 15:9:11, Scotts International, The Netherlands) was added to the N1 treatment plots once at the start of the experiment at a rate of 4 g N m^{-2} per plot (Kraaij and Ward 2006).

Each seedling was monitored for 6 months from November 2009 - May 2010. At the end of the experiment, the number of surviving seedlings per species per treatment combination was recorded. The final longest stem length (from ground level to tip) was measured on each individual seedling. After that, the shoot of each seedling was harvested, separated into leaf and stem, dried at 70°C for at least 48 hours and the leaf biomass and stem biomass were weighed separately.

Data analysis

An incomplete block design was used where water and light treatments were not fully crossed. Therefore, we first used the data from plots where light was always maximized (L1 - full sun) and analysed the effect of species functional trait group (fine-leafed, broad-leafed), water (regular, irregular), grass (presence, absence) and nutrients (natural, nutrients added) and their interactions on seedling survival, and stem height and leaf and stem biomass of seedlings (referred to as WGN analysis). Subsequently, we repeated the analyses using the data from plots where water was always evenly applied (W1) and tested the effects of species functional trait group, light (shade, full sun), grass and nutrients and their interactions (referred to as LGN analysis). The treatment W1L1G0N1 (Even water supply-Full sun–No grass-Nutrient addition) was chosen as control treatment due the fact that this treatment was common to both analyses, and also because it was the treatment where the best growth performance in all study species was expected, as it represents the treatment where resources are most abundant in space and time.

To evaluate the effects of the treatment in seedling survival we used generalized linear mixed models (GLMM), with binomial distribution (using maximum likelihood), and species as a random factor. As growth data are naturally exponentially distributed, to normalise the residuals, we first log-transformed the raw data. For each species j and each trait, we calculated the relative performance of each individual i of that species for a given trait, $R_{i,j}$, as the trait value for the individual, $T_{i,j}$, divided by the average value for the trait in the control treatment, $\overline{C_j}$ (W1L1G0N1).

$$R_{i,j} = \frac{T_{i,j}}{\overline{C_j}}$$

This procedure was used in all growth variables. We modelled the data using GLMMs (using maximum likelihood), with species treated as a random factor. We applied a Sidak comparison test for pairwise comparisons between the species groups in each treatment. All statistical analyses were done using the software SPSS, version 20 (IBM SPSS Statistics; Chicago, IL, USA).

Results

The seedling survival rate did not differ significantly between species groups (fine-leafed species and broad-leafed species), nor was it significantly affected by the different treatments. However, seedling growth parameters (leaf and stem biomass and stem length) were significantly affected by the treatments (Figs. 3.1 and 3.2).

In most of the cases the species groups showed similar responses to the different treatments, grass presence being the most important factor strongly affecting growth (Figs. 3.1 and 3.2). Indeed, the negative effect of presence of presence of grass on seedling performance occurred for all species (Table 3.2), reducing plant growth (biomass and length) under all water (W0 and W1), light (L0 and L1) and nutrient (N0 and N1) treatments (Figs. 3.1 and 3.2). Shade also had similar effect in both species groups (fine-leafed and broad-leafed species), negatively affecting all growth parameters (leaf and stem biomass and stem length), accentuating the negative effect of grass on growth. However, seedling growth in shaded and grassed plots is greater than that in grassed plots in full sun (Table 3.2 and Fig. 3.2). Under high stress conditions (grass competition or light deprivation), the species groups responded significantly differently to the environmental pressures (i.e., significant interactions were detected between functional group (F), and environmental variables such as water supply, W, and shade, S; see Table 3.2).

Table 3.1 - Functional trait data for tree species used in the experiment. Sources of data are indicated in postscripts.

Species	Family	N_2-fixing‡	Relative growth rate† ($g\ g^{-1}\ d^{-1}$)	Leaf type‡‡	Leaf size†† (cm^2)	Leaf N content†† ($mg\ N\ g^{-1}$)	Spinescence††
Fine-leafed species							
Acacia nigrescens Oliv.	Mimosoideae	Yes	0.031	Bipinnate	16.055	30.589	Yes
Acacia nilotica Willd.	Mimosoideae	Yes	0.035	Bipinnate	12.077	31.475	Yes
Acacia tortilis Hayne	Mimosoideae	Yes	0.042	Bipinnate	12.567	32.651	Yes
Dichrostachys cinerea Wight &Arn	Mimosoideae	Yes	0.041	Bipinnate	31.710	28.796	Yes
Broad-leafed species							
Colophospermum mopane J. Léonard	Detarieae	No	0.027	Simple	47.212	21.895	No
Combretum apiculatum Sond.	Combretaceae	No	0.039	Simple	25.397	26.959	No
Schotia brachypetala Sond.	Detarieae	No	0.036	Pinnate	42.664	26.409	No
Peltophorum africanum Sond.	Caesalpinioideae	No	0.034	Bipinnate	99.549	25.878	No

‡ Schulze et al (1991); Treydte et al. (2007); Cramer et al. (2010).

† Tomlinson et al. (2012), species grown under common greenhouse conditions. Relative growth rate calculated over 5 to 20 weeks growth.

‡‡ Coates-Palgrave (2002).

†† Tomlinson et al. (submitted b), species grown under common greenhouse conditions. Leaf size and leaf N content measured on 20 week-old plants.

Table 3.2 - Effect of even water supply (WNG model) and light (LGN model), presence of grass and nutrient addition on the performance of savanna tree seedlings of different species: significance values based on general linear mixed models. The independent factors are: species functional trait group (F), water (W), grass (G) and nutrients (N) and their interaction terms.

	Leaf Biomass		**Stem Biomass**		**Stem Length**	
	F	p	f	p	f	p
WGN						
F	2.99	0.123	0.74	0.415	2.28	0.170
W	7.07	0.008	2.09	0.149	1.37	0.242
G	1457.99	<0.001	818.47	<0.001	513.7	<0.00
N	1.53	0.216	6.34	0.012	5.67	0.017
F *W	0.73	0.394	0.93	0.334	15.16	<0.00
F *G	4.88	0.027	0.49	0.482	1.54	0.215
F *N	0.83	0.363	0.58	0.446	2.73	0.099
W*G	0.37	0.541	0.94	0.333	4.59	0.032
W*N	0.23	0.629	2.47	0.116	0.15	0.695
G*N	0.25	0.617	0.35	0.553	1.56	0.211
F	1.10	0.294	0.26	0.612	0.02	0.886
F	0.81	0.367	0.52	0.472	1.76	0.185
F *G*	1.85	0.174	0.00	0.983	0.28	0.598
W*G*N	0.36	0.549	0.26	0.609	0.16	0.692
F	0.08	0.773	0.62	0.431	6.74	0.010
LGN						
F	9.75	0.014	2.56	0.148	3.74	0.089
L	21.82	<0.001	74.73	<0.001	4.49	0.034
G	925.87	<0.001	634.36	<0.001	317.4	<0.00
N	0.06	0.800	0.23	0.630	12.89	<0.00
F *L	27.90	<0.001	13.81	<0.001	33.97	<0.00
F *G	9.00	0.003	3.27	0.071	0.39	0.533
F *N	0.02	0.896	0.00	0.956	0.35	0.553
L*G	54.77	<0.001	71.63	<0.001	4.01	0.045
L*N	0.26	0.607	0.45	0.502	2.77	0.096
G*N	0.36	0.551	0.01	0.922	2.06	0.152
F *L*G	0.07	0.791	2.86	0.091	3.56	0.059
F *L*N	0.03	0.864	0.00	0.967	0.07	0.794
F *G*	2.23	0.136	0.15	0.701	0.04	0.842
L*G*N	0.38	0.540	0.02	0.899	0.08	0.775
F*L*G*	0.13	0.715	0.22	0.639	2.90	0.089

For the fine-leafed species the negative effect of grass (G) in the stem length growth of the seedlings was less accentuated under the even water supply treatment, but only when nutrients (N) were added (i.e. significant interaction F*W*G*N, see Fig. 3.1 For the broad-leafed species the negative effect of grass (G) on leaf biomass and stem length was less accentuated under natural rainfall plots (Fig. 3.1). Furthermore, shade, also reduced the negative effects of grass on stem length growth, this effect being more accentuated for broad-leafed species than for fine-leafed species (Fig. 3.2).

The responses of seedling biomass to the simulated environmental changes also differed between functional groups. The even water supply treatment did not significantly affect seedling leaf and stem biomass of broad-leafed species seedlings (Table 3.2).

Discussion

Although the regeneration niche theory suggests diverse responses of plant species within a community to environmental variations (Grubb 1977), the majority of the current models on vegetation dynamics assume a similar response of species' seedlings to increased water availability and decreased grass competition (e.g., Higgins et al. 2000; Wiegand et al. 2006). Here our results indicate that depending on their functional traits, savanna tree species may perform differently under competition with grass and under environmental variations. We discuss the implications of these findings to conservation and predictive studies.

Effect on tree seedling survival rate

Our study shows that the different environmental conditions (here simulated by the treatments) did not substantially change the survival rate of savanna species. This result contradicts our initial expectations of lower seedling survival in less favourable conditions (grass presence, full sun and natural (erratic) rainfall plots). We thus reject Hypothesis 1. Several studies suggest that water stress and grass competition are important factors causing savanna tree seedling failure to survive (e.g., Flyman1999; Midgley & Bond 2001; Van der Waal et al. 2009; Ward & Esler 2011). However, this study focuses on water variation during a rainy season, whereas most of the other studies compare low and high water supply.

Although, our study site is characterized by water scarcity (438.18 mm/season) and unbalanced rainfall distribution during the raining season (mostly in the beginning of the wet season, November to January), the 2009-2010 wet season rainfall distribution had two major rain peaks in November-December 2009 (320 mm) and April-May 2010 (202 mm), with an intermittent 3-month dry period (January to March). This particular rainfall distribution created a more balanced water distribution during the wet season, reducing the effect of wet season droughts on seedling survival. Furthermore, it is also possible that the effect of water limitation takes place only at an earlier stage of recruitment (e.g., germination) or during the subsequent dry season (see Schupp et al. 1990).

Effect on tree seedling performance

Although grass competition did not affect seedling survival during the wet season, it negatively affected seedling growth (stem growth and stem and leaf aboveground biomass) for all species under all resource treatments (Figs. 3.1 and 3.2). This result is in agreement with our expectations (Hypothesis 3), suggesting that belowground competition for resources is playing an important role in seedling establishment (see also Kambatuku et al. 2011), possibly reducing the period of suitable growing conditions for tree seedlings (Danielsen & Halvorson 1991; D'Antonio et al. 1998).

Despite the suggestion that water stress may limit seedling survival and establishment in dry systems (Sankaran et al. 2005; Kraaij & Ward 2006), the seedling recruitment of most species was not affected by the differences in water regime. Even water supply only had a positive influence on stem length of the fine-leafed species (Fig. 3.1).As mention before, the pattern of rainfall during the 2009/2010 wet season was higher and more continuous than normal, which may have brought favourable conditions to seedlings under the natural rainfall treatment (556 evenly distributed in W1 vs. 623 mm unevenly distributed in W0). However, there was still a drought period between the two major rain peaks in 2009-2010 raining season (Fig. S3.1). Our results show that the differences in these two water regimes were not large enough to have an effect on the seedling survival and performance, but it remains unclear if under more extreme droughts seedling establishment would be limited.

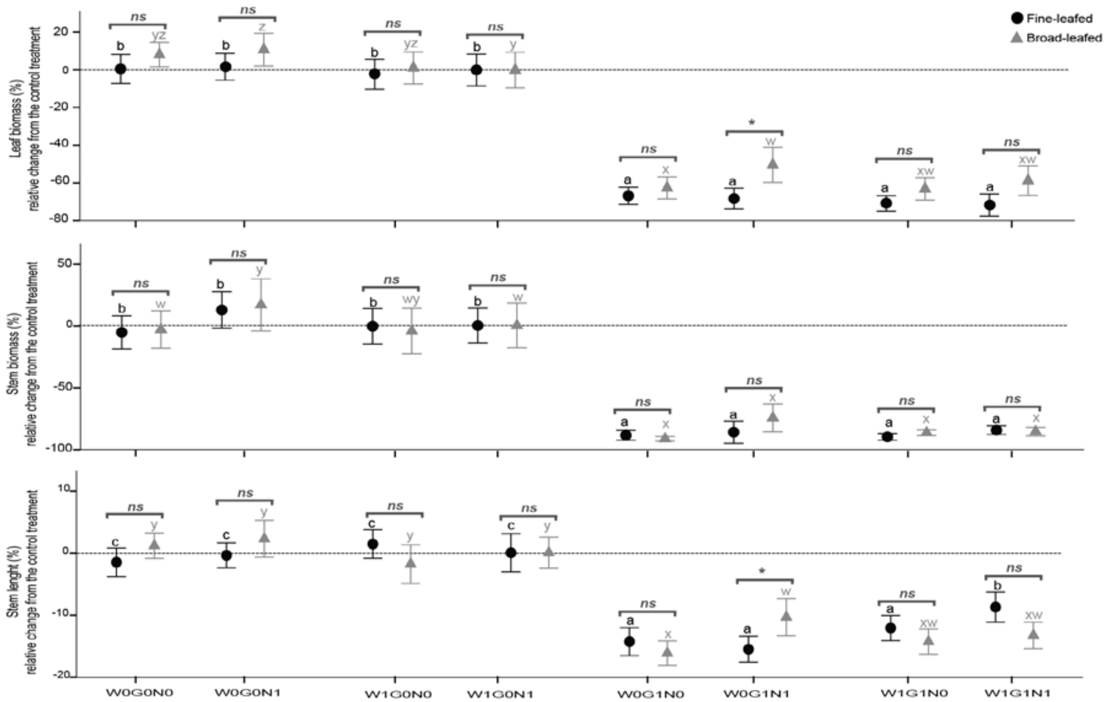

Figure 3.1 - Effect of water supply, nutrient addition and grass presence on leaf biomass, stem biomass, and stem length of savanna tree seedlings of different species functional trait groups compared to the control treatment (WGN model).The x-axis represents the different treatment in the experiment: W0-even water supply, W1-natural rainfall, G0- no grass, G1-grass, N0-no nutrient addition and N1- nutrient addition. The dashed line represents the control treatment (even watering treatment, full sun, no grass and nutrient addition). Black circles represent fine-leafed species and the grey triangles represent broad-leafed species. The error bars represent 95% confidence intervals for each mean. The letters indicate significant differences among treatments per species functional group (Table 3.2).Results of pairwise comparisons between the species groups within a treatment combination are indicated by the brackets(*: p <0.05; ns: non-significant).

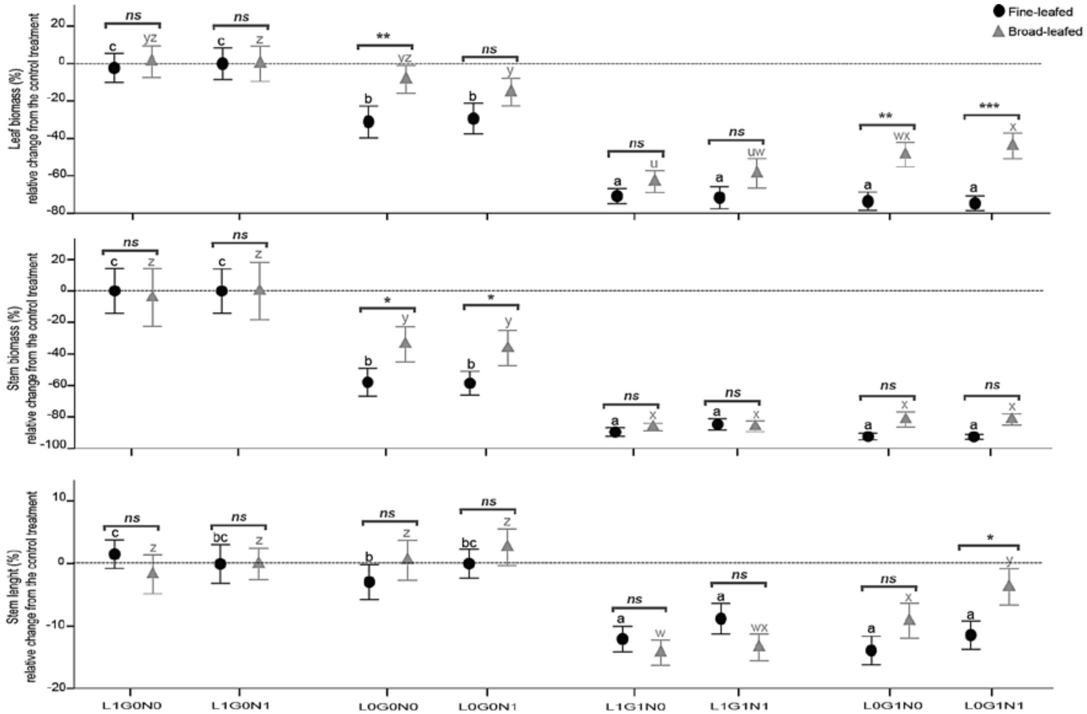

Figure 3.2 - Effect of shade, nutrient addition and grass presence on leaf biomass, stem biomass, and stem length of savanna tree seedlings of different species functional trait groups (LGN model). The x-axis represents the different treatments in the experiment: L1-full sun, L0-shade, G0- no grass, G1-grass, N0-no nutrient addition and N1- nutrient addition. The dashed line represents the control treatment (even watering treatment, full sun, no grass and nutrient addition). Black circles represent fine-leafed species and the grey triangles represent broad-leafed species. The error bars represent 95% confidence intervals for each mean. The letters indicate significant differences among treatments per species functional group (Table 3.2).Results of pairwise comparisons between the species groups within a treatment combination are indicated by the brackets (*: p <0.05; **: p <0.01; ***: p <0.001; ns: non-significant).

Contrary to our expectations (part of Hypothesis 1), nutrient addition did not enhance seedling biomass production *per se*, independently of species trait groups (contradicting Hypothesis 2, see Fig. 3.1), although it did reduce the negative effects of grass on leaf biomass and stem length. Under even water supply this beneficial effect of fertilization was most accentuated for fine-leafed species (which have N-fixing nodes), which were expected to perform better under nutrient rich soils, where they are more common than broad leafed species (Cole 1986; Frost 1996; Venter et al. 2003). However, under natural rainfall (W0G1N1) this beneficial effect of fertilization was only significant for broad-leafed species. These are surprising results, as broad-leafed species are commonly associated with infertile soils in semi-arid savannas (Cole 1986; Frost 1996; Venter et al. 2003). Therefore these species were expected to perform worse than fine-leaf species in nutrient rich conditions. Heavy rainfall events are common during the wet season in the study area (personal observations), consequently, the soil of W0G1 plots (natural rainfall and grass), which were not protected against heavy rainfall, may have had increased losses of minerals due to water run-over (Dos Santos 1987). Such conditions could hence explain the lower performance of species which are poorly adapted to low nutrient soils (fine-leafed). Moreover, grass presence may sustain water in the soil for longer time (Blanco-Canqui et al. 2004) helping create a locally humid condition. Savanna regions with such conditions (infertile soils in humid areas) are usually dominated by non N-fixing modulated species (Huntley 1982), and in our study broad-leafed species were also non N-fixing modulated species (see Table 3.1).

Underneath trees, light availability is highly reduced, and savanna tree seedling growth can be considerably reduced (Loth 1999; Lewis & Tanner 2000; Hoffmann et al. 2004). Indeed, our results show that most of the tree species were negatively affected by shade in terms of biomass (Hypothesis 1). Lower light intensity found under parental trees might limits carbohydrate accumulation in seedlings (Wright & Van Schaik 1994; Mulkey et al. 1996), slowing their growth rates and prolonging their existence as juvenile plants. However, this negative influence of shade (especially on stem biomass and length) was significantly more accentuated in the non-grass plots (the interaction between shade and grass was significant, Table 3.2). Moreover, broad-leafed species showed a better performance (leaf biomass and stem length) in shaded environments than fine-leafed species in the presence of grass and the absence of grass (Fig. 3.2). This result suggests that shade reduces the negative effects of grass on seedling growth. This positive effect of shade is may be due to the suppression of grass growth under low light availability (personal observation, see also Holl 1999; Siemann & Rogers 2003). Moreover, contrasting with our expectations (Hypothesis 4), positive effects of shade were not detected for fine-leafed species (Fig. 3.2). It is suggested that the light compensation point is great in fine-leafed than broad-leaved tree species (Walters & Reich 1999). This suggestion in association with our results gives strength to the idea that fine-leafed species are less tolerant to low light conditions (shade) than the broad-leafed species (Smith & Goodman 1986; 1987).

In summary, grass presence suppressed the growth of all studied species, revealing that savannas can sustain low tree density when grass is present (i.e., a strong resistance of savanna vegetation structure to tree encroachment). Therefore, grass suppression caused by herbivory and fire (frequent events in savannas) could lead to abrupt vegetation type transitions (van Langevelde et al. 2003), particularlydue to stimulation of tree seedling growth in shaded places with high nutrient and water availability.

Concluding remarks

There is a growing concern on consequences of environmental changes on savannas. Consequently, several models that predict how climate changes and alterations of land use will affect the co-existence of savanna trees and grasses have been developed (for a review see Van Langevelde et al. 2011). These models are also used to predict how the balance among different vegetation states (forest, savannas and grasslands) may shift in relation to climate changes (Warman & Moles 2009). Our study demonstrated that, within a large range of recruitment conditions, grass presence is the most limiting factor for savanna seedling growth, even in favourable recruitment conditions (e.g., high water and nutrient availability). These findings suggest that an abrupt transition from grassland to woodland savanna could only occur if grass abundance is severely affected (e.g., via high levels of herbivory and/or changes in fire frequency: Jeltsch et al. 1996, 1998, or droughts: Higgins et al. 2000). As herbivory and fire are frequently featuring in savannas, abrupt vegetation type transitions could occur (van Langevelde et al. 2003). Moreover, our study showed that under high stress conditions (grass competition or light deprivation) the species with different functional traits responded differently to the environmental pressures. These findings support the regeneration niche theory of variation between plant species responses within a community to variations in environmental conditions (Grubb 1977), allowing plant species coexistence and explaining the high diversity of plant species in savannas. As the functional groups here considered explained part of the variation in responses, information on species traits can be used to improve our ability to predict vegetation structure changes in savannas.

Acknowledgements

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Supplementary Information

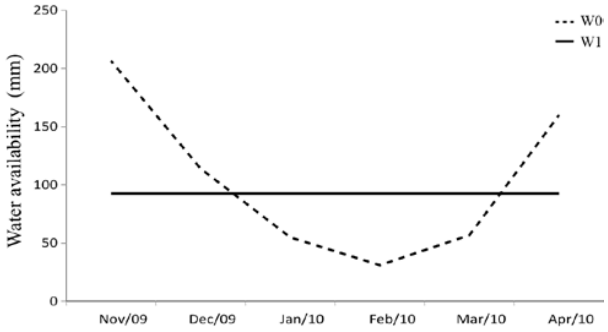

Figure S 3.1 – Water availability in the even water supply treatment (W1) and in the natural rainfall treatment (W0) during the experiment. The rainfall data is from the Hans Hoheisen Research Facility weather station.

Chapter 4

Effect of nutrient deposition and rainfall distribution on the forage quality of savanna tree species

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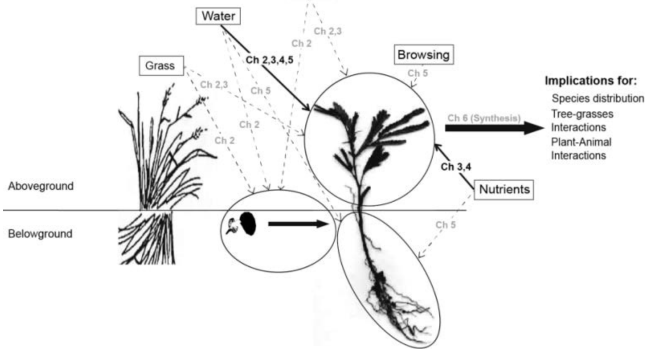
Submitted for publication



Summary

1. Increased atmospheric nitrogen deposition, intensification of agricultural fertilizer use and large herbivore management interventions are on-going processes that increase soil nutrient levels in many savannas. Uncertainty about how these changes affect abundance and quality of savanna tree cover limits the development of adequate environmental policies and management plans.
2. By setting up a large controlled experiment within one of the largest savanna regions (Southern Africa), we tested how water and nutrient availability affected leaf nutrient concentrations and aboveground biomass production of seedlings from10 dominant savanna tree species from different climate types (semi-arid vs. humid) and different soil fertility levels (low vs. high).
3. Contrary to expectations, neither changes in water availability (uneven vs. even supply) nor changes in nutrient levels (low vs. high) affected biomass production in any of the study species. Plant quality for herbivores (i.e. leaf nutrient content) did, however, change significantly. Soil nutrient input increased leaf nutrient content, but only in the even water supply treatment, indicating that plant nutrient uptake strongly depends on water availability. Under current irregular rainfall patterns, nutrient input significantly reduced leaf quality.
4. On-going increases in N-deposition and rainfall levels can differentially affect leaf nutrient content of savanna trees, potentially changing the functional structure of savannas. Given that large herbivore populations depend on plant nutrient content for their nutritional requirements, such changes will likely impact herbivore populations and their browsing patterns, altering the functional structure of ecosystems even if overall plant biomass remains unaffected.
5. *Synthesis*: The results of our multi-species experiment alert for the potential of synergistic effects between land-use change (and consequent nutrient changes) and climate changes on savanna vegetation. Under more erratic rainfall patterns, leaf quality of a large number of tree species will likely decline, potentially having a strong negative impact on herbivore population dynamics within this region, and also affecting soil ecological processes (e.g., litter decomposition and consequent soil mineralization). Management plans that aim to preserve biodiversity should hence consider the potential indirect impacts of eutrophication via changes in plant leaf quality.

Introduction

Plant productivity and aboveground biomass can increase with higher soil resource availability (e.g., nitrogen, water, phosphorus) (Tilman 1984; Gower et al. 1992; Albaugh et al 1998; Polis 1999). Changes in soil resources can also affect the level of nutrients in the plant leaves, affecting their quality as food for herbivores (Bryant & Chapin 1986; du Toit et al. 1990; Olff et al. 2002; Ahrestani et al. 2011). Increased atmospheric nitrogen deposition (Dentener et al. 2006; Sheffield & Wood 2008; Miyazaki et al. 2012), intensification of agricultural fertilizer use (Pritchard et al. 2007) and large herbivore management interventions (Augustine et al. 2003; Augustine & McNaughton 2004) are on-going processes that increase nutrient levels in the soil in these savannas. Moreover, changes of the rainfall pattern can affects plant nutrient uptake and storage (Shane et al. 2004; Parks et al. 2000), and may limit plant growth during the growing season (e.g., Kambatuku et al. 2011). As plant species differ widely in their response to differences in soil nutrients (Lambers et al. 2008), changes in soil properties (water and nutrient availability) may hence have an important influence on primary productivity and vegetation structure of tropical savannas (Van Langevelde et al. 2003; Wijesinghe et al. 2005; De Knegt et al. 2008; Groen et al. 2008; Sankaran et al. 2008). Despite the importance of leaf nutrient availability to local species composition (Matson et al. 1999) and to the herbivory communities (Owen-Smith & Cooper 1988; Pretorius et al. 2011), little is known about the effects of changes in soil nutrient and water availability on the leaf nutrient concentration of savanna trees (but see Van der Waal et al. 2011).

This study evaluates the effects of soil nutrient (low vs. high) and water availability (uneven vs. even supply) on leaf nutrient concentrations and aboveground biomass production of seedlings of tree species from different climate zones (semi-arid vs. humid savannas) and areas with different soil fertility levels (low vs. high). We focus on the foliar concentration of nitrogen (N), phosphorus (P) and potassium (K) because these nutrients are important in many plant metabolism processes, such as photosynthesis, respiration and enzymes activation (Marschner 1995; Bednarz & Oosterhuis 1999), and in the diets of herbivores (Prins &Van Langevelde 2008; Olff et al. 2002; Ahrestani et al. 2011).

As increases in soil resources are thought to increase plant productivity (Tilman 1984; Gower et al. 1992; Albaugh et al. 1998; Polis 1999), we expected that increases in soil moisture and soil nutrients would increase aboveground plant biomass (Hypothesis 1). Nutrient concentration in the leaves also depends on soil nutrient availability (Patterson et al. 1997; Prior et al. 1997; Paquin et al. 2000) and soil moisture (Scholes & Walker 1993). Increased amounts of nutrients in the plant leaves might increase their quality as food for herbivores (Bryant & Chapin 1986; du Toit et al. 1990; Olff et al. 2002; Ahrestani et al. 2011), whereas increases in water availability may decrease quality due to dilution of nutrient concentration in the leaves (Olff et al. 2002). Plant species from dystrophic soils tend to accumulate nutrients for longer periods than species from eutrophic soils (Lambers et al. 2008) and hence the species from dystrophic soils likely make more use of soil nutrient availability to increase their leaf nutrient content. Furthermore, increases of soil moisture may decrease plant leaf mineral content, due to "dilution effects" (see Rittenhouse & Roath 1987) of increased plant growth (Breman & DeWit 1983; Olff et al. 2002). Therefore, we expected that leaf nutrient concentration would increase with increasing soil nutrient availability, particularly for tree species from dystrophic soils, and that it would decrease with irregular water supply (Hypothesis 2).

Methods

This manipulative field experiment was conducted in a savanna region, at Timbavati Private Game Reserve, Limpopo Province, South Africa (24°15'20.23"S, 31°23'23.63"E) during the wet season of 2009-2010 (November–May), in a fenced area that excluded large herbivores. The growing season mean rainfall (from October till April) of the previous 10 y (2000-2010) is *ca.* 440 mm (Hans Hoheisen Research Facility station 10 km northeast). The mean maximum temperature during January (hottest month) is 33.7ºC and the mean minimum temperature for June (coolest month) is 9.4ºC (Venter et al. 2003). The average climate of the area is classified as semi-arid under the Köppen-Geiger System (Kottek et al. 2006). The area is included in the Granite Lowveld region (Mucina & Rutherford 2006). The geological substrata are granite and gneiss with numerous dolerite intrusions which never exceed 10 m in thickness (Gertenbach 1983). As the soil of the experimental plots is derived from granite rocks, this soil is shallow and nutrient-poor (Venter *et al.* 2003).

The experimental site was cleared of plants prior to the start of the experiment. Ten locally abundant tree species, and hence likely to be best adapted to the local conditions, were selected (Table 4.1). Since these species represent a substantial portion of the tree biomass in savannas, their responses to different environmental conditions substantially affect the local vegetation structure. All the seeds used in the experiment were collected in areas surrounding the experimental site.

Treatments

The study site was ploughed (about 20 cm deep) to homogenize the soil and to give all treatments the same starting conditions. Five blocks were laid out in in a restricted area (90 x 90 m), the similarity on the soil type was a primary reason to the selection of the experimental area. Inside of each blocks, four 4-m^2 plots, separated by a 2m gaps between the plots. Seedlings were subjected to two different water regimes: one covered with a rain-out shelter (W1 - even water supply) and another exposed to natural rainfall conditions (W0 - natural rainfall or uneven water supply). The nutrient treatment was separated in two different nutrient applications (N0 - no nutrient supply vs. N1 - nutrient supply), leading to a total of 20 experimental plots. The seedling density inside of the plots was lower than the normal early seedling density in savannas (more than 50 seedlings per m2 in the seed/seedling bank (Tefera 2011). Three weeks before the experiment all seeds was sowed in nursery bags (using the same soil of the experimental area), and after germination a total of 20 seedlings per species were raised in similar conditions. After around four weeks, 20 seedlings per species (five replicate blocks, each with four seedlings per plot) were random transplanted in treatment combination plots (W0N0, W0N1, W1N0 and W1N1). Moreover, we indicate that the seedlings position in the plots were random selected.

Plots within the uneven water supply treatment (W0) received 623 mm of water from natural rainfall during the period of the experiment, which was higher than the long-term mean for the area during this period (438 mm). The distribution was naturally uneven during the experiment: 206 mm in November, 114 mm in December, 55 mm in January, 31 mm in February, 57 mm in March and 160 mm in April. To simulate a change in the temporal rainfall distribution, in the even water supply treatment (W1), we supplied a fixed amount of 46.3 mm (185 l per 2 × 2-m plot) of water to the seedlings every 2 weeks for the 6 months of the experiment, yielding a total of 556 mm water and a constantly wet environment for the plants (Figure 1). This amount of water in W1 was based on the water deficit rules as defined in the Köppen-Geiger system (550 mm per season).

To increase the nutrient availability for the tree seedlings (treatment N1), granular slow-release inorganic fertilizer containing nitrogen (N), phosphorus (P) and potassium (K) in the ratio 3:1:2 (Osmocote Exact Standard 15:9:11, Scotts International, The Netherlands) was added once at the start of the experiment at a rate of 160g N m^{-2} (640g per plot), following rates applied by Kraaij & Ward (2006).

Shoot foliar nutrient concentration and biomass

At the end of the experiment (May 2010), the seedlings were harvested and oven-dried at 70^{o}C for 24 h, and their dry weights were measured. To quantify the concentration of the elements N (total), P and K, the leaf material was destructed with a mixture of H_2SO_4, Se and salicylic acid (Novozamsky et al. 1983). The concentrations of N and P in the leaves were measured with a Skalar San-plus auto analyzer, and K was measured with an Atomic Absorption Spectrometer (AAS) from Varian.

Data analysis

To understand how the different treatments affected leaf nutrient concentrations and biomass production, we used General Linear Mixed Models (GLMM) nutrient (addition *vs.* no addition) and watering regime (natural rainfall *vs.* even water supply) as explanatory variables. To account for the variation among species, 'Species' was included as a random factor. The Sidak comparison test was used to compare the differences among the treatments. All analyses were performed using the software R (R Development Core Team 2011, package nlme). The P-values were obtained by testing the model with and without the term in the model, as indicated by Pinheiro & Bates (2000) and Zuur et al. (2009).

Table 4.1 – Tree species used in the experiment for the different climate types and soil fertility of the sites where the seeds were collected (semi-arid and humid). The information regarding climate type was based on Coates-Palgrave (2002) (‡) and regarding soil fetility was based on Grant & Thomas (2001) (‡‡).

Climate type‡	Soil fertility‡‡	Species	Family
Humid	Low	*Acacia karroo* Hayne	Mimosoideae
	High	*Schotia brachypetala* Sond.	Caesalpinioideae
		Acacia nilotica Willd.	Mimosoideae
		Acacia sieberiana DC.	Mimosoideae
Semi-arid	Low	*Combretum apiculatum* Sond.	Combretaceae
		Colophospermum mopane J. Léonard	Caesalpinioideae
		Peltophorum africanum Sond.	Caesalpinioideae
	High	*Acacia nigrescens* Oliv.	Mimosoideae
		Acacia tortilis Hayne	Mimosoideae
		Dichrostachys cinerea Wight & Arn	Mimosoideae

Results

The statistical results of the effect of water and nutrient supply on leaf nutrient concentration are presented in Table 4.2. We expected that increases in soil moisture and soil nutrients would increase aboveground plant biomass (Hypothesis 1). However, changes in soil resource availability (water and nutrient availability) did not affect aboveground biomass production of any of the tree species (Fig. 4.1).

In relation to leaf quality, we expected that leaf nutrient concentration would increase with increasing soil nutrient availability, particularly for tree species from dystrophic soils, and that it would decrease with irregular water supply (Hypothesis 2). However, for the majority of the species, leaf nitrogen (N) concentration did not respond to variations in water and nutrient supply. The only exception was for species from species humid savannas with eutrophic soils which, under irregular water supply, suffered N leaf concentration decreases as a response to NPK fertilization. Phosphorus (P) and potassium (K) leaf concentrations were more affected by changes in the availability of soil resource (water and nutrients) than N leaf concentration. Under irregular water supply, soil nutrient input lowered leaf concentration of these nutrients. However, while for N the effect was more marked in eutrophic species from humid savannas; for P and K the negative effect on leaf quality was particularly accentuated for species from semi-arid savannas from dystrophic soils (Fig. 4.2).

Finally, species from semi-arid savannas with eutrophic soils had an opposite response to nutrient availability, nutrient leaf concentrations (phosphorus, potassium and nitrogen) increasing in fertilized plots.

Discussion

Plant productivity and aboveground biomass are thought to increase with higher soil resource availability (e.g., nitrogen, water, phosphorus) (Tilman 1984; Gower et al. 1992; Albaugh et al. 1998; Polis 1999). However, our results show that aboveground biomass of the species we studied was not affected by such environmental changes. Nevertheless, quality of leaves for herbivores (here measured as nutrient content) strongly varied with nutrient input. The direction of this effect (positive vs. negative) depended on water availability and the magnitude varied from species to species. Here we discuss how the variability of responses of individual tree species found in this study can affect savanna vegetation structure and the implications of such findings for conservation.

Effect of watering regime and nutrient availability on shoot growth and leaf nutrient concentration of tree seedlings

Contrary to our expectations (Hypothesis 1), increased nutrient input and constant water availability did not significantly influence aboveground biomass production in any of the species studied (Fig. 4.1). It is possible that plants allocate more resources to the shoots under frequent water supply and high nutrient availability (Iwasa & Roughgarden 1984; Shipley & Meziane 2002). This lack of effect in aboveground biomass production suggests that these species are well adapted to the high environmental variability of savannas, biomass production of the studied tree species being resistant to variations of soil resources.

Table 4.2 – Effect of the water (even vs. natural rainfall) and nutrient (no addition vs. Nitrogen [N]-Phosphorus [P]-Potassium [K] addition) treatments on leaf nutrient concentration and aboveground biomass for the different climate types (semi-arid or humid) and soil fertility level (low or high). Species nested within soil fertility and climate type was used as a random variable to correct for the variation among the different species.

	Leaf N concentration		*Leaf P concentration*		*Leaf K concentration*		*TotalBiomass*	
	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value
Climate	1.731	0.134	0.674	0.525	1.516	0.180	-0.412	0.695
Soil fertility	2.492	0.047	0.565	0.593	0.295	0.778	0.062	0.953
Water	-2.731	0.007	-1.253	0.211	-3.955	<0.0001	-1.580	0.115
Nutrients	-1.594	0.111	-2.621	0.009	-0.282	0.778	-0.099	0.921
Climate*Soil fertility	-1.434	0.202	-0.334	0.750	-0.996	0.358	0.355	0.735
Climate*Water	0.910	0.363	1.593	0.112	-1.101	0.271	1.328	0.185
Climate*Nutrients	-0.636	0.525	-0.481	0.630	1.171	0.242	-0.046	0.963
Soil fertility*Water	0.803	0.422	0.650	0.516	-0.992	0.322	1.105	0.270
Soil fertility*Nutrients	-0.809	0.419	0.128	0.899	-0.100	0.921	-0.530	0.596
Water*Nutrients	1.140	0.255	3.901	<0.0001	1.393	0.164	0.180	0.858
Climate* Soil fertility*Water	-0.465	0.642	-1.053	0.293	1.051	0.294	-0.185	0.853
Climate*Soil fertility* Nutrients	-0.677	0.499	-0.322	0.748	0.512	0.609	-1.067	0.286
Climate*Water* Nutrients	-0.727	0.467	-1.506	0.133	0.872	0.384	-0.609	0.543
Soil fertility*Water*Nutrients	1.584	0.114	0.555	0.579	0.143	0.887	0.593	0.554
Climate* Soil fertility*Water*Nutrients	-0.260	0.795	0.679	0.497	-0.711	0.478	0.252	0.801

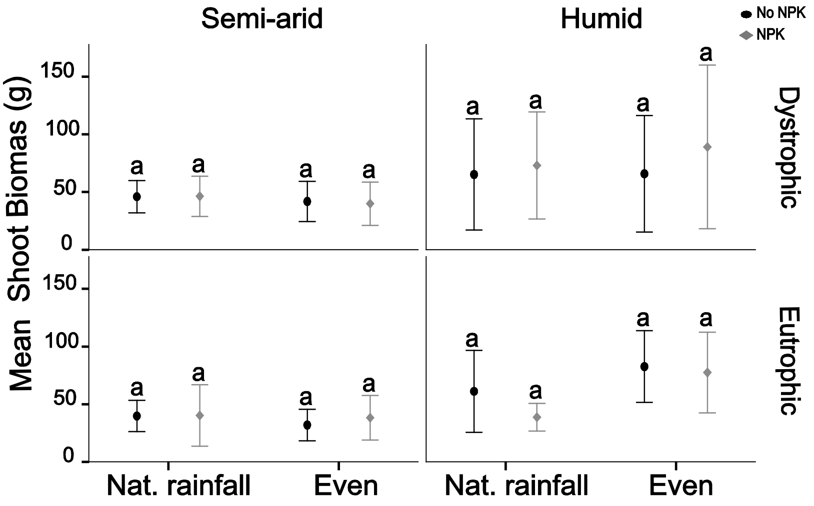


Figure 4.1 – Effect of soil water and nutrient supply on the mean aboveground biomass (g). Bars represent 95% confidence intervals. The grey bars represent the plots where NPK was added, and the black bars represent the plots where no NPK was added. Statistical details are presented in Table 4.2. The letters represent the differences among the species groups and treatments.

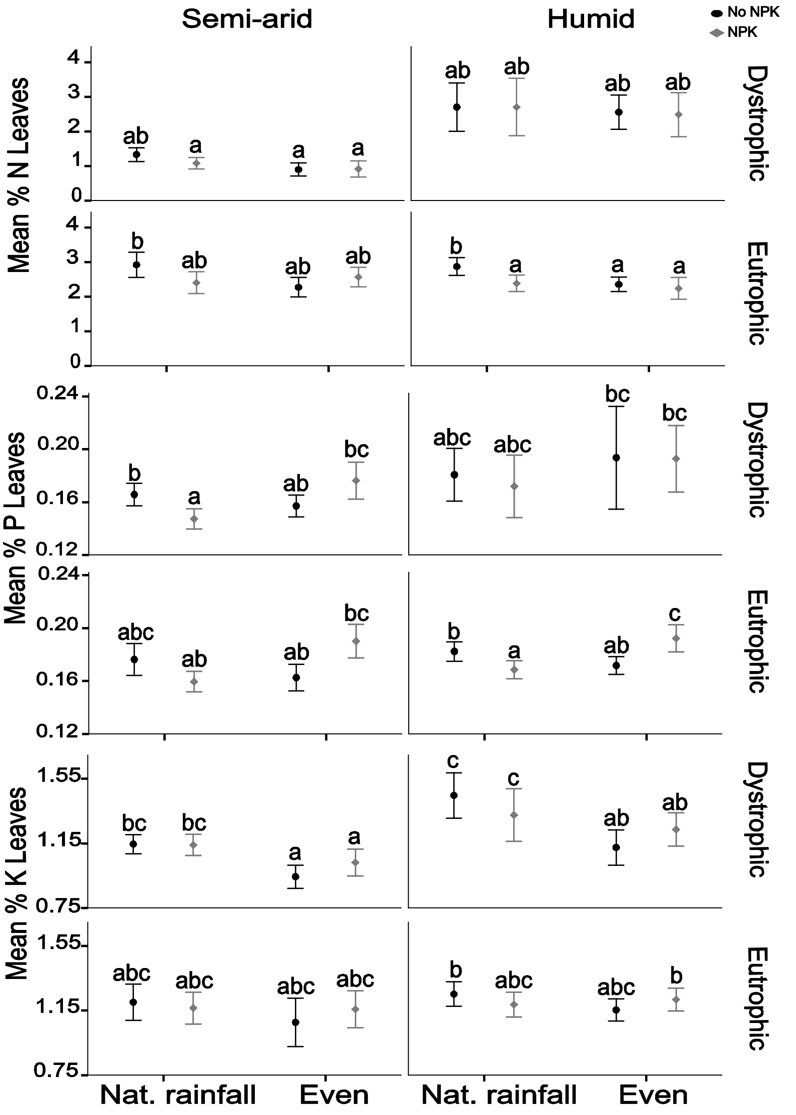


Figure 4.2 – Effect of soil water and nutrient supply on the mean foliar concentration of chemical elements. Bars represent the 95% confidence intervals. The grey dots represent the plots where NPK was added, and the black dots represent the plots without nutrient supply. Statistical details are presented in Table 4.2. The letters represent the differences among the species groups and treatments.

In contrast, the plant quality for herbivores (measured as the nutrient concentration in seedling leafs) did significantly vary with nutrient input and water availability (Fig. 4.2). While a previous study about leaf nutrient concentration of grasses (e.g., Olff et al. 2002) suggests that plant quality (i.e. nutrient content) increases with soil nutrient content and decreases with water availability (similar to our Hypothesis 2), our empirical results with savanna tree seedlings show that nutrient input increased leaf nutrient content only when water input was regular, even leading to decreases in leaf nutrient concentrations when water availability was erratic. Seedling dependence on water for a positive effect of nutrient availability can be explained by the fact that nutrient uptake depends on water movement within plants (Hu & Schmidhalter 2005). Considering this, many authors have suggested that applying nutrient fertilizers mitigates the adverse effects of drought on plant development (Sangakkara et al. 2001; Garget al. 2004). However, it has also been suggested that fertilizers do not increase plant production if sufficient water is not available to the plant, and increasing soil water availability does not increase production without an adequate nutrient supply (Hu & Schmidhalter 2005). With respect to the negative effect of nutrient input under the uneven water supply treatment (natural rainfall), the low leaf-nutrient concentrations found in our study could be due to the allocation of resources to organs, such as roots, that can support growth and survival after soil reserves are exhausted (Grime 1979). Since our measurements were taken at the end of the growing season, this re-allocation of nutrients could explain our findings. Further studies on root production in tree seedlings along soil nutrient and moisture gradients would help to confirm where extra-nutrients are primary allocated.

Implications for nature conservation and herbivore-plant relationship

The mean annual rainfall will probably change in the near future (Voss et al. 2002; Wilby & Wigley 2002; Wehner 2004), together with increases in atmospheric nutrient deposition and the use of fertilizers that may increase nutrient levels of soils (Augustine et al. 2003; Augustine & McNaughton 2004; Pritchard et al. 2007). Uncertainty about how these changes affected tree species (Ward 2005; Smith & Beaulieu 2009) limits the development of adequate environmental policies and management plans (Grace et al. 2002). As tree seedlings are often food sources for herbivores (Prins & van der Jeugd 1983) and the palatability of the leaves depends on their nutrient content that consequently impacts herbivore populations (Grant & Scholes 2006; Prins & van Langevelde 2008), the changes detected in our study may lead to changes in browsing pressures and potentially affect long-term dynamics of plants.

Several herbivore species rely on the nutrient content of tree leaves for their nutritional needs (Owen-Smith & Cooper 1988; Pretorius et al. 2011). Our study shows that, under the irregular water supply that characterizes savanna climate, increase of soil nutrient availability will decrease the quality of tree seedlings as resources for herbivores by changing nutrient content in leaves. Therefore, even when overall food quantity (plant biomass) does not change (Fig. 4.1), increases of soil nutrients can impact herbivores, as food quality (leaf nutrient content) is lower. As some specialist herbivores species are less likely to adapt to changes in vegetation (e.g., Owen-Smith & Cooper 1988; Carvalheiro et al. 2010), these changes in food quality may alter the herbivore communities' composition. Furthermore, such changes in communities' composition can influence the ecosystem services provide by the animals and plant species in savannas, effecting the human populations living in savanna areas that rely on the resources from the savannas for their livelihood.

Concluding remarks

The results of our multi-species experiment show that, even if overall biomass does not change, nutrient input can lead to substantial changes on leaf quality. However, such effect depends on rainfall patterns: leaf quality increases under more regular rainfall and decreases under more erratic water availability. While such changes might not directly affect plant species distribution (but see Van der Waal et al. 2011) consequent changes in browsing pressures may affect overall vegetation structure. Our results hence suggest that, in response to the observed drying trend of about 8 mm/yr in the southern African savanna (IPCC 2007), leaf quality of a large number of trees will reduce, likely negatively affecting herbivore population dynamics (invertebrates and vertebrates), as well as soil ecological processes (e.g., as litter decomposition) and higher levels of the trophic chain. Close monitoring of how vegetation and herbivore communities change in response to climate and land use changes is, therefore, essential both to understanding the full extent of the ecological and social consequences and to contributing to the development of adequate policies and management plans that aim to preserve biodiversity.

Acknowledgements

We are thankful to the staff of Southern African Wildlife College, SANParks, School of Biological and Conservation Sciences (University of KwaZulu-Natal) and members of the Resource Ecology Group of Wageningen University for technical support, especially Luisa Carvalheiro, Gerda Martin, Patricia Meijer, Herman van Oeveren, Anne-Marie van den Driessche, Catherine Yi van Ravenstein, Cradock Mthabine, Promise Mthabine, Lovemore Mthabine, Def Mlambo and Professor Ngomana. Funding and research assistance for this project were provided by The Stichting Shell Research.

Supplementary Information

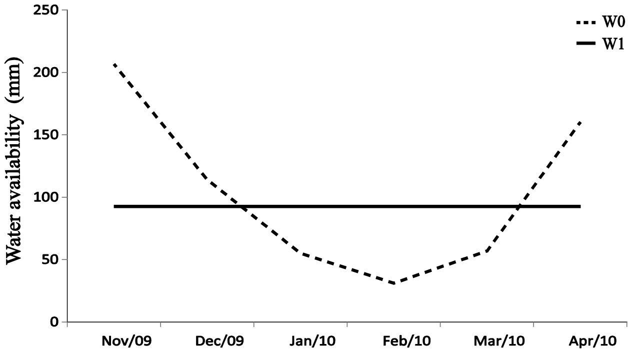


Figure S 4.1 – Water availability in the even treatment (W1) and in the natural rainfall treatment (W0) during the experiment. The rainfall data is from the Hans Hoheisen Research Facility weather station (10 km from the research site).

Chapter 5

Evaluating resistance of trees to environmental variation during early developmental stages – effects on above and belowground growth

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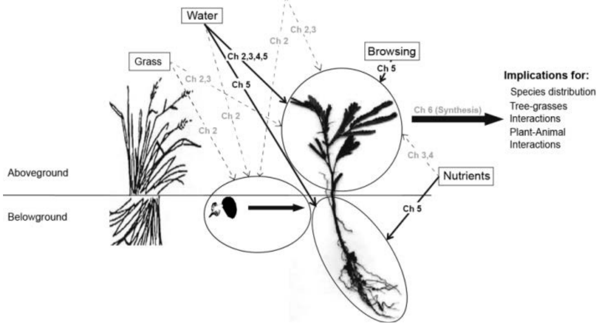

Summary

1. Savannas are subjected to strong recurring environmental variability (e.g., droughts, defoliation from herbivory or fire, fluctuations in resource availability). While it is recognized that inter-specific variation in functional traits influences the variability and long-term stability of plant communities, it is still unclear how frequent fluctuations of environmental conditions influence aboveground and belowground growth of tree seedlings, a critical stage in tree population and community dynamics.
2. Here we studied how juveniles of three savanna tree species (*Acacia karroo*, *A. nigrescens* and *Colophospermum mopane*) with different functional traits (related to N-fixing and spinescence) responded to disturbances (accentuated changes in soil resource availability and herbivory events).
3. Our study disclosed a high resistance of savanna tree seedlings to disturbances. While we expected that low soil resource availability would result in high resource allocation towards roots, simulation of annual, recurring dry events or changes in nutrient availability did not have a clear effect on the seedlings' stem growth rate (SGR) and root measurements (root length fraction, RLF, and root mass fraction, RMF) of juvenile trees. Moreover, contradicting our expectations, our studied species responded similarly to environmental changes (soil resource availability and herbivory events), independently of their traits.
4. Finally, browsing stimulated stem regrowth and root elongation (but not RMF) of savanna tree seedlings, indicating compensatory growth in response to high level herbivory in all tree seedling species.
5. *Synthesis* - The results of this study indicate that savanna tree seedlings (18 months old) are generally able to cope with changes in environmental conditions, droughts and low nutrient supply having little influence on the aboveground and belowground growth patterns. Furthermore, our research also reveals that herbivory stimulated aboveground growth of savanna tree seedlings. Although previous theoretical studies predict that changes in environmental conditions lead to accentuated shifts in savanna community structure, our findings indicate a high resilience of savanna tree seedlings. This result questions the usefulness of herbivory or human land-cleaning in controlling invasive woody species in tropical grasslands and savannas.

Introduction

Tropical savannas are characterised by large regional and temporal variability in water availability (Eamus et al. 1999; Kanegae et al. 2000), nutrient availability (Venter et al. 2003; Schwinning & Sala 2004) and herbivory pressure (Staver et al. 2009). Therefore, savanna plants species may have been selected for functional traits that aid survival in response to large environment variation (Chapin et al. 1993; Violle et al. 2007). The diversity of these traits within a community is thought toinfluence the long-term variability in species composition (Ives et al. 1999) and increase community stability (Ives et al. 2000). However, little empirical evidence exists on how these individual traits influence seedling responses to temporal variability in environmental conditions. As the seedling stage is critical for the population and community dynamics of plants (Grubb 1977; Bond & Van Wilgen 1996; Hean & Ward 2011), evaluating how seedlings respond to environmental conditions and how traits may explain variation in such the responses between plant species can help understand how the distribution and abundance of plants species changes within savannas.

Although aboveground plant traits may influence key ecosystem processes (e.g., productivity and carbon uptake; Westoby 1998; Garnier et al. 2004), the less studied belowground traits also play a crucial role in soil resource uptake, storage and transport (Craine et al. 2003). The traits involved in resource acquisition often show high plasticity to compensate for a limiting environmental resource, such as increased biomass allocation to roots in low-nutrient (e.g., Gedroc et al. 1996). As plastic resource allocation of plants in response to variation on growth conditions might affects dispersal, distribution, resilience and hence plant's competitiveness (Berendse & Elberse 1990; Dong & de Kroon 1994), a better understand in role of seedling aboveground and belowground functional traits on plant establishment patterns. However, there is a lack of information on how the plant species plasticity may modify resource allocation under constant variations in environmental conditions.

In this study, we investigated how in differences in plasticity between species with different life-history traits (Table 5.1) respond to changes in soil resource availability (water and nutrients) and to repeated defoliation. As different savanna types present different growth conditions for tree recruitment (Bond et al. 2003), we selected three species from two savanna types: two species from a semi-arid savanna (*A. nigrescens* and *Colophospermum mopane*) and one species from a humid savanna (*Acacia karroo*) for our study.

Most African savannas have seasonal rainfall with two distinct seasons, a wet summer (September-April) and a dry winter (May-August). In addition to this, savannas are occasionally subjected to multiyear drought cycles (low rainfall during the wet season), followed by years with high rainfall (Charney 1975; Prins & Loth 1988; Nicholson 2000). Rainfall events are an important trigger for the availability of soil nutrients to plants (Prins & Beekman 1989). Soil nutrient concentration might also vary due to dung deposition, agricultural fertiliser use or large-scale atmospheric deposition (Davis et al. 2000; Davis & Pelsor 2001; Peltzer & Kochy 2001). Consequently, in savannas, both water and nutrients are cyclically available in pulses during the year (Birch 1958; Knapp et al. 1998; Schwinning & Sala 2004), these cycles may affect seedling establishment. Tree seedlings, for instance, are found to be particularly vulnerable to water stress (Van der Waal et al. 2009). It is likely that such resource pulses have an important influence on the structure of savannas (Sankaran et al. 2005, 2008). The resource pulses may also influence the degree to which resources are allocated to growth (Grime 1977). According to the functional equilibrium theory (Brouwer 1962, 1963) and the subsequent optimal partitioning theory (OPT) (Bloom et al. 1985), plants should allocate additional biomass to the organ that takes up the resource that most limits growth. As roots are the organs responsible for most water and nutrient uptake (Reynolds & D'Antonio1996; Poorter & Nagel 2000; Wang & Taub 2010), we hypothesize that periods of scarcity of water or nutrients will be associated to a greater root elongation rate and increased allocation to root biomass, and slower aboveground stem extension rate (Hypothesis 1).

However, plants in savannas may have been selected for traits that aid survival in response to large variation in soil resource availability (Tilman 1988; Chapin et al. 1993), and the response to these fluctuations might possibly vary among species (Schenk & Jackson 2002). For instance, root traits such as root elongation rate may especially benefit the recruitment abilities of plant species growing in drier environments (Cornelissen et al. 2003; Tomlinson et al. 2012). Moreover, species with N-fixing traits (mycorrhizal fungi, Wang & Qiu 2006, or *Rhizobium* bacteria in *Acacia* species, Date 1977; Dreyfus & Dommergues 1981) may be better able to exploit high fluxes of nutrient availability to enhance their growth rates (Berger et al. 1995) and leaf nutrient concentration (Prior et al. 1997). This latter trait increases their photosynthetic capacity (Paquin et al. 2000). Therefore, we expected that N-fixing species would vary in their investment in root growth (i.e. higher variation in the root mass fraction, RMF) across nutrient treatments than non N-fixing species (Hypothesis 2).

Herbivory is another important factor affecting savanna vegetation structure because it reduces the photosynthetic capacity of plants (Pinkard et al. 2006). This reduction may affect biomass allocation and therefore shoot and/or root growth rate (Van Langevelde et al. 2003; Schutz et al. 2011). As plants may store reserves belowground to allow regrowth after defoliation events (Van der Meijden et al. 1988; Chapin et al. 1990), we expected that herbivory events would increase allocation of resources to shoot biomass leadind to higher shoot growth rate (compensatory growth, McNaughton 1983), at the expense of roots (lower RMF). However, plant species differ in how they cope with herbivory. Some species have low resistance, mostly with structural defences such as spines and some degree of tolerance for herbivory (McNaughton 1983; Fornara & du Toit 2007). These spinescent species can, however, store reserves belowground (i.e., high RMF) to allow fast regrowth after defoliation events (Chapin et al. 1990; Hean & Ward 2011), particulary because they are typically from productive sites (Mucina & Rutherford 2006; Tomlinson et al. 2012). In opposition, non-spinescent species are commonly slow-growing species, from low fertility soils (reviewed by Myers & Bazely 1991). These species can have high resistance to herbivory due to high production of secondary compounds (Stamp 2003) that efficiently deter herbivores (Milewski et al. 1991; Rohner & Ward 1997). The physical defenses production is more expensive to produce than chemical ones (Skogsmyr & Fagerstrom 1992). As investments in defense come at the expense of investments in growth (see review Herms & Mattson 1992), we expected that non-spinescent species would have a lower compensatory growth rate (Hypothesis 3).

Table 5.1 - Savanna type and functional traits of the studied plant species.

	Acacia karroo	*Acacia nigrescens*	*Colophospermum mopane*
Savanna type ‡‡	Humid	Semi-arid	Semi-arid
Growth rate ‡	High	Low	Low
N_2-fixing ‡‡	Yes	Yes	No
Leaf Nitrogen ††	High	High	Low
Root elongation rate ‡	Low	High	High
Leaf Type ‡‡	Bipinnate	Bipinnate	Simple
Spinescence ‡‡	Yes	Yes	No

† Tomlinson et al. (2012)
†† Tomlinson et al. (submitted b), species grown under common greenhouse conditions. Leaf size and leaf N content measured on 20 week-old plants.
‡‡ Coates-Palgrave (2002)

Methods

Experimental site

This study was conducted in a savanna region, at Timbavati Private Game Reserve, Limpopo Province, South Africa (24°15’20.23”S, 31°23’23.63”E), in a fenced area that excluded large herbivores. The long term mean annual rainfall (2000-2010) is *ca.* 438.18 mm (Hans Hoheisen Research Facility station 10 km NE. The mean maximum temperature during January (hottest month) is 33.7 ºC and the mean minimum temperature for June (coolest month) is 9.4 ºC (Venter *et al.* 2003). The average climate of the area is classified as semi-arid under the Köppen-Geiger System (Kottek et al. 2006). The area is included in the Granite Lowveld region (Mucina & Rutherford 2006). The geological substrata are granite and gneiss with numerous dolerite intrusions that never exceed 10 metres in breadth (Gertenbach 1983). The soils are shallow and nutrient poor (Venter et al. 2003). The upland soils are sandy (70-84 %) with a percentage of clay between 6 and 15% (Gertenbach 1983).

Species

To test whether different savanna tree species were differed in their plastic responses to temporal variation in resource supply (water and nutrients) and disturbances (browsing), three savanna tree species were used on this study. We choose species from two different savanna types: two species from a semi-arid savanna (*Acacia nigrescens* and *Colophospermum mopane*) and one species from a humid savanna (*Acacia karroo*) for our study. These species present great differences on they functional trait (see Table 5.1). All species selected are locally abundant, though associated with different soil types (Venter et al. 2003). Since these represent a substantial portion of the tree biomass in the local savanna, their responses to different environmental conditions will have substantial effects on the local vegetation structure.

Experimental set-up

To study the effect of changes in environmental conditions on the growth of savannah tree seedlings we followed the growth of three locally common and widespread species of savanna tree species (Table 5.1) during 15 months (Feb 2009-May 2010), divided in five 3-month periods (hereafter, Time 1 to Time 5). A total of 160 (30-day-old) seedlings per species were planted in long plastic bags (10 cm in diameter and 150 cm depth) filled with locally collected soil, which allowed the roots to grow naturally downwards. Bags were then randomly allocated to four different groups, each with 40 seedlings: one group being subjected to changes in water availability (variable water, W1), another group subjected to changes in nutrient level (added fertilizer N1), another to changes in browsing (with browsing, B1), and a control group subjected to constant conditions (continuous water, W0; no fertilizer, N0; no browsing B0). During the experiment's first three months (Time 1), all seedlings were subjected to the following conditions: no added nutrients (N0), no browsing (B0), and high water supply (W1) receiving 0.625 l per week per seedling (750 mm over three months) plus the natural rainfall in this three months (147.7 mm). For each group (Control, W, N and B) ten seedlings per species were harvested to obtain measurements of the root (root mass and root length, see methods description below) at the end of Time 1 and the remaining 30 grew under the treatment conditions until the end of the experiment. The seedling in the control group were maintained under constant conditions throughout the experiment (W1N0B0), while the remaining seedlings where subjected to changes: within the W group seedlings were subjected to changes in water (W1N0B0 vs. W0N0B0), within the N group seedlings were subjected to changes in nutrients (W1N0B0 vs. W1N1B0), and within the B group seedlings were subjected to changes in browsing (W1N0B0 vs. W1N0B1). For the seedlings in the C, B and N group, the high water supply (W1) was maintained throughout the experiment, while water provision for seedlings in the W treatment was reduced (W0) during Time 2 and Time 4. During these periods, seedlings in the W treatment received 0.2 l water per week per seedling for a total of 250 mm over three months (Table. S5.1). However, as seedlings were exposed to natural rainfall, all seedlings received more water than they would have naturally received in the study region (see Table S5.1). For seedlings in the N treatment, nutrients were added (5kg m^{-3}, i.e. 0.75 g per bag, of slow-release fertilizer Osmocote Exact 3-4 month N16-P9-K12) once at the start of Time 1, Time 3 and Time 5. Seedlings in the B treatment were subjected to simulated browsing (B1) by removing 80% of each shoot at the start of Time 2 and Time 4.

Measurements

Survival (alive or dead) and stem length (from the base to the tip - mm) of all seedlings were monitored monthly. Shoot growth rate (SGR) was calculated within each time period, using the measurements of the first (start) and third month (end) of that period, as

$$\boldsymbol{SGR} = \frac{Length_{end} - Length_{start}}{Length_{start}}.$$

At the end of the experiment, all seedlings were harvested, and the root lengths of the harvested seedlings (Time 1 and Time 5) were measured. Afterwards, roots and shoots (collected in Time 1 and in Time 5) were dried in an oven (80°C for 48 hours), and biomass (mg) was then measured. The root mass fraction (hereafter RMF), i.e.

$$\boldsymbol{RMF} = \frac{root\ \ biomass}{Total\ (root{+}shoot)\ biomass}$$

and root length fraction (hereafter RLF), i.e.

$$\boldsymbol{RLF} = \frac{root\ \ length}{Total\ (root{+}shoot)\ length}$$

were calculated for the seedlings harvested after Time 1 and after Time 5.

Statistical analyses

To test the effect of the changes in water supply, nutrient addition and browsing on the shoot parameters (stem growth rate in mm, SGR), general linear models (GLM) were applied using water (low *vs.* high water supply), nutrients (no addition *vs.* addition) and browsing (no browsing *vs.* browsing) and time (1 to 5) as explanatory variables, using individual seedling as random variable. All possible interactions between the variables were considered. We applied a Sidak *post hoc* comparison test for pairwise comparisons between the treatments during each time period.

To test the effect of the changing water supply, nutrient addition and browsing on RMF and RLF for Times 1 and 5, GLMs were applied using treatment and harvest time as explanatory variables. We applied a Sidak *post hoc* comparison test for pairwise comparisons between the treatments and harvest times. All analyses were performed using the software R (R Development Core Team 2011).

Table 5.2 – Effect of treatments, experimental time periods (ETP) and species on the stem growth rate. The variable treatment includes all experimental treatments (browsing, nutrients and watering) and control. The variable ETP includes all five time periods. Species variable includes all three study species (*A. karroo*, *A. nigrescens* and *C. mopane*).

Stem Growth Rate	F-value	P-value
Treatments	6.165	<0.0001
Experimental time period (ETP)	74.695	<0.0001
Species	17.616	<0.0001
Treatments * ETP	14.604	<0.0001
Treatments * Species	11.032	<0.0001
ETP * Species	26.100	<0.0001
Treatments * ETP * Species	8.073	<0.0001

Table 5.3 – Effect of treatments, experimental time periods (ETP) and species on the root length fraction (RLF) and root mass fraction (RMF). The variable treatment includes all experimental treatments (browsing, nutrients and watering) and control. The variable *ETP* includes the initial harvest (harvest time 1) and the final harvest (harvest time 5). Species variable included all three study species (*A. karroo*, *A. nigrescens* and *C. mopane*).

	RLF		RMF	
	F-value	P-value	F-value	P-value
Species	36.00	<0.0001	11.54	<0.0001
Treatment	51.22	<0.0001	8.94	<0.0001
ETP	77.89	<0.0001	0.49	0.485
Species * Treatment	2.45	0.025	2.71	0.014
Species * ETP	8.50	<0.0001	0.31	0.732
Treatment * ETP	48.52	<0.0001	7.69	<0.0001
Species * Treatment * ETP	0.63	0.704	1.35	0.236

Results

Effect of variation in soil resource availability on seedling growth

The statistical results of the effect of treatments, experimental time periods (ETP) and species on tree seedling aboveground (SMF) and belowground (RLF and RMF) growths are presented in Table 5.2 and 5.3, respectively. While the variation in water supply showed no clear effect on the stem growth of the tree seedlings (Fig. 5.1), it did change the RMF or RLF of the seedlings for all species compared to the control plants. The seedlings of all species showed lower RLF at the end of the experiment compared to the control plants (Fig. 5.2). Although this effect was only significant for the humid savanna species, it indicates that variation in water supply led to more investment in shoot growth than in root growth.

The greater availability of nutrients had a positive effect only on shoot growth rate of the non N-fixing species, *C. mopane*, after the second nutrient addition (Time 3) (Fig. 5.1). Although the RLF of this species was not affected, the RMF was initially (Time 1) enhanced, indicating an initial allocation of resources to roots, followed by allocation of resources to shoots (Fig. 5.2). At the final harvest, *C. mopane* had an equal RLF and RMF under the control or experimental conditions. Nutrient addition did not affect shoot or root growth of the N-fixing species from semi-arid savannas (*A. nigrescens*) and even negatively affected (Time 1) shoot growth of the N-fixing species from humid savannas (*A. karroo*). RMF of this species was, however, significantly enhanced after Time 1. This enhancement indicates that resources were diverted from shoot to root growth, but as with *C. mopane*, that initial positive effect disappeared after Time 5. The nutrient treatment significantly affected *A. karroo* and *C. mopane*, enhancing RMF after the first nutrient supply. However, this effect was not found at the end of the experiment (Fig. 5.2).

Effect of defoliation on seedling growth

After the first browsing event (Time 2), the seedlings of all species had a significantly higher growth rate than the control seedlings, independent of being spinescent or not (Fig. 5.1). For the species from semi-arid savannas, *A. nigrescens*, this increase was followed by a significant drop in growth rate during the following period (Time 3) compared to the control plants. Moreover, after the second browsing event (Time 4) when seedlings were 12 months old, no significant differences were found between the growth rate of the browsed plants and the control plants. At the start of the following period (Time 5), seedlings of the spinescent species (*A. karroo* and *A. nigrescens*) that had been browsed had a significantly enhanced stem growth, whereas the browsed seedlings of non-spinescent species (*C. mopane*) did not differ from the control plants. For all species, seedlings subjected to the browsing treatment had a significantly higher RLF at the end of the experiment (Time 5) than the control plants. Browsing treatment did not significantly affect RMF in any of the study species (Fig. 5.2).

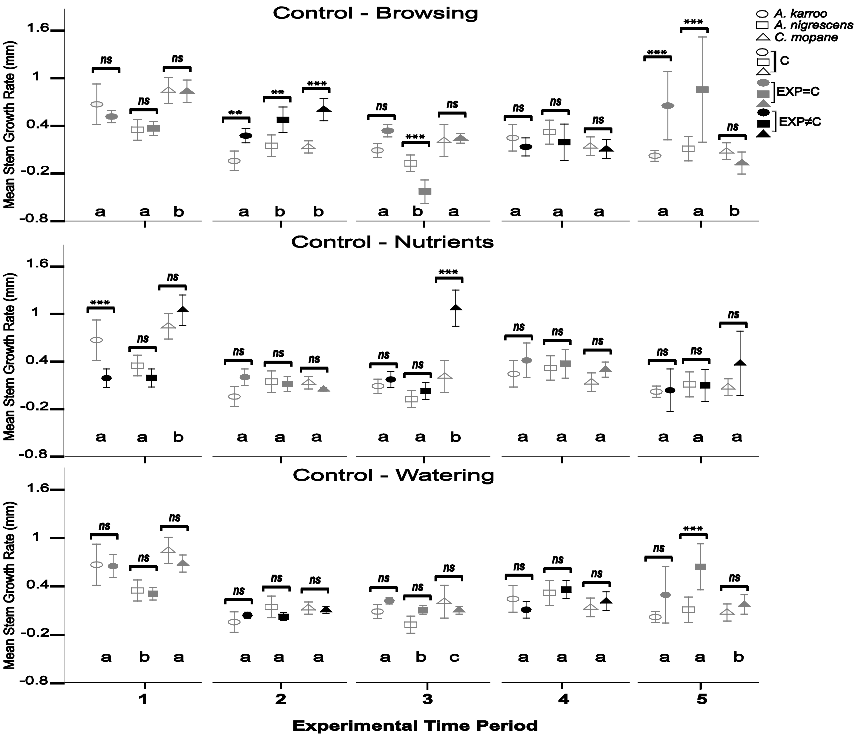

Figure 5.1 – Stem growth rate of the seedlings in the experimental and control treatments for the different experimental time periods. The open symbols represent the control treatment (C) and the full symbols the experimental treatments (EXP). The black bars show when the experimental treatments differ from the control treatments (browsing events, nutrients addition and drought periods). The letters illustrate when the species responses differ among each other during the experimental times. The stars represent the significant difference between the species groups (*, ** and *** = $p<0.05$, $p<0.01$ and $p<0.001$ respectively, and ns = not significant). The error bars represent 95% C.I. Statistical details are presented in Table 5.2.

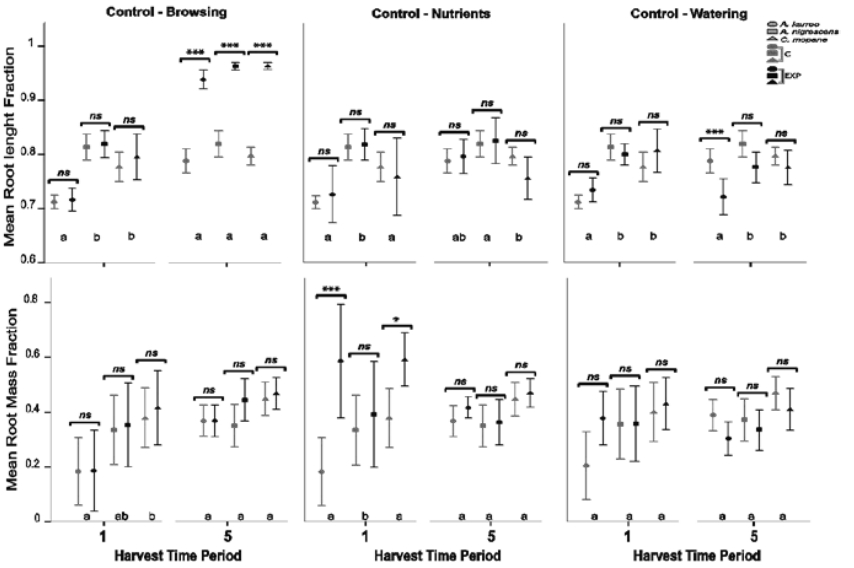

Figure 5.2 – The comparison of the root length fraction (RLF) and root mass fraction (RMF) in experimental and control treatments on the different harvest time periods. The grey bars represent the control treatment (C) and the black bars the experimental treatments (EXP). The letters illustrate when the species responses differ among each other during the experimental times. The stars represent the significant difference between the species groups (*, ** and *** = $p<0.05$, $p<0.01$ and $p<0.001$ respectively, and ns = not significant). The error bars represent 95% C.I. Statistical details are presented in Table 5.3.

Discussion

Explaining the composition and dynamics of plant communities is an important topic in plant ecology. However, there is little information on the effect of frequent environmental changes on tree seedling establishment, a critical stage in the plant population and community dynamics (Grubb 1977; Bond & Van Wilgen 1996). Our results suggest that, during early seedling development, the studied species are resilient to fluctuations in soil resource availability, independent of their traits (N-fixing and spinescence), and that aboveground growth is stimulated by periodic herbivory.

Effect of pulses in soil resource availability (water and nutrients)

Contrary to our expectations (Hypothesis 1), increase in nutrient supply did not lead to a higher investment in root growth, while variation in water availability led to a higher investment in shoot growth. Root lenth fraction (RLF) of the seedlings was lower for *A. karroo*, and shoot growth rate was higher for *A. nigrescens* (Figs. 5.2 and 5.3). These results contradict the optimal partitioning theory (OPT) that predicts allocation of biomass to the roots during periods of soil resource scarcity (Brouwer 1963; Reynolds & Thornley 1982; Bloom et al. 1985). Previous studies that support the OPT compare situations of high water availability with situations of low water availability. Although it has been stated that drought during the wet season is critical to the survival of savanna tree seedlings (Ward 2005; Kraaij & Ward 2006), we did not find a change in root traits for the seedlings that developed in conditions where droughts (treatment W0) occur during the wet season (treatment W1). The generality of OPT has been previously questioned as other studies on plant resource allocation also did not find support for the OPT (e.g., Ryser & Eek 2000; Tomlinson et al. 2012). It is possible that plants may take some time before they are physiologically able to immediately use pulses of soil moisture, which happens when a dry period is interrupted by a sudden rainfall event (Schwinning & Ehleringer 2001; Schwinning et al. 2003). Moreover, species that have evolved in a region where water availability is more erratic (i.e., species from semi-arid savannas: *A. nigrescens* and *C. mopane*) invested more in root growth (higher RLF) than humid savanna species (Fig. 5.3). This evidence supports the hypothesis that these species have evolved traits that give them more ability to cope with variation in water supply and that allow them to invest more in shoot growth (Fig. 5.2). RMF and RLF were lower in the species from humid savannas compared to the species from semi-arid savannas, but the single species from humid savannas did not significantly increase shoot growth. In semi-arid savannas there is a negative correlation between the coefficient of variation and median annual rainfall (see e.g., Ward 2009), which results in more frequent droughts in semi-arid savannas. Thus, the plants must adopt recruitment strategies that are designed to better cope with the unpredictability of temporal rainfall. It is possible that species from humid savannas are not adapted to respond quickly to a sudden decrease in soil water availability.

Although many plants are believed to rapidly increase nutrient uptake in order to capitalize on resource pulses (James & Richards 2006), and although water was not a limiting factor in our study, increased nutrient supply did not improve seedling growth rate in any of the study species, even in the N-fixing species (*Acacia*). Therefore, we have to reject Hypothesis 2 (see also Ludwig et al. 2001; Wakeling et al. 2010). This lack of effect could be explained by the inhibition of nodule development of N-fixing plants and nitrogenase activity due to high soil N availability (Armone el at. 1994; Dommergues 1995). Moreover, our results also agree with suggestion that N_2-fixation is induced by grass competition for nutrients, enabling tree seedlings to compensate for limited soil N and survive grass competition (Kambatuku et al. 2012).

Effect of defoliation on seedling growth

There is an increasing concern about the effects of woody cover expansion on conservation and local livelihoods in tropical grasslands and savannas (Ward 2005). Browsing is recommended as a useful way to control invasive woody species in these areas (O’Connor 1995). Despite the strong immediate effects of browsing on the aboveground parts of plants (Gill & Beardall 2001), our results show that tree seedlings are able to recover from foliar damage and compensate for the productivity of the lost leaf area particularly after the first browsing event (Fig. 5.2 and Hypothesis 3). This is in agreement with the suggestions of Belsky (1986). Such compensatory growth after herbivory was also reported in grasses (see also Prins et al. 1980; McNaughton 1983), where it is associated with higher resource allocation towards the stem with costs in terms of root growth. However, contrary to our expectations, such compensatory response did not occur at the expense of root growth because root mass increased with stem growth (Fig. 5.3) and root length (RLF) (Thomas et al. 2006; Snyder & Williams 2007).

All species, independently of the type of defense against herbivory (secondary compounds vs. spinescence), showed no difference in resource investment in root growth among the treatments (length and biomass). Moreover, compensatory growth occurred equally in all species (rejecting Hypothesis 4). As in less productive environments where plants cannot easily replace biomass lost to herbivory, investment in defenses can increase (Coley et al. 1985; Fine et al. 2004), even though chemical defenses are cheaper than physical defenses, we expected a lower regrowth response for the non-spinescent species which are from less productive enviroments (Hypothesis 3). However, spinescent species might have invested not only in stem length, but also in more and larger spines as a response to herbivory (Cooper et al. 2003), resulting in stem length growth similar to that of non-spinescent species. It is also possible that water availability influences compensatory growth (Whitham et al. 1991). A long-term fully crossed experiment where plants are subjected to a combination of grazing, water and nutrient regimes is needed to evaluate the influence of such interactive effects.

Concluding remarks

Our study shows that the savanna species used in this study are generally able to cope with changes in environmental conditions and that droughts and low nutrient supply have little influence on the aboveground and belowground growth patterns of tree seedlings. Moreover, the studied species responded similarly to the environmental changes independent of their traits (N-fixing and spinescence). Finally, our findings show that herbivory stimulated aboveground growth of savanna tree seedlings. Although previous theoretical studies predict that changes in environmental conditions lead to accentuated shifts in savanna community structure (reviewed by Sankaran et al. 2005), our findings show a high resistence of savanna tree seedlings. Although browsing has been recommended as a useful tool to remove the seedlings of invasive tree species in tropical grasslands and savannas (O'Connor 1995), our results suggest that the introduction of herbivores as bio-control agents to manage unwanted vegetation (e.g., Bolton & James 1985) is not appropriate in these habitats, because the wood plants increase their growth rate.

Acknowledgements

We are thankful to the staff of Southern African Wildlife College, SANParks, School of Biological and Conservation Sciences (University of KwaZulu-Natal) and members of the Resource Ecology Group of Wageningen University for technical support, especially Luisa Carvalheiro, Gerda Martin, Patricia Meijer, Herman van Oeveren, Anne-Marie van den Driessche, Catherine Yi van Ravenstein, Cradock Mthabine, Promise Mthabine, Lovemore Mthabine, Def Mlambo and Professor Ngomana. Funding and research assistance for this project were provided by The Stichting Shell Research.

Supplementary information

Table S 5.1 – Water availability in the different experimental time periods

	Rainfall (mm)	Water addition (mm)	Water addition (litres)
Time 1	147.7	750	7.5
Time 2	24.5	250	2.4
Time 3	4	750	7.5
Time 4	351.4	250	2.4
Time 5	249	750	7.5

Chapter 6

Synthesis: Surviving and growing amidst others - the effect of environmental factors on germination and establishment of savanna trees

Eduardo R.M. Barbosa

Understanding how plant communities change with ongoing environmental changes (e.g., climate, land-use) is a central issue in ecology (e.g., Sala et al. 2000; Lambin et al. 2001; Cramer et al. 2001; Walck et al. 2011; Smith 2012). Savannas are characterised by large regional and seasonal variability in water (Eamus et al. 1999; Kanegae et al. 2000; Kottek et al. 2006), nutrient resources (Venter et al. 2003; Schwinning & Sala 2004), and disturbances by fire (Barbosa et al. 1999; Russell-Smith et al. 2003) and herbivory (Van Langevelde et al. 2003; Staver et al. 2009). The variation of water and disturbances within a region usually follows a seasonal pattern and occurs within certain limits (Bourliere & Hadley 1983; Kottek et al. 2006), species within this ecosystem having evolved to tolerate such variations (Ward 2009). However, rainfall patterns and concentrations of atmospheric CO_2 are expected to change African savannas due to global climate change (Hulme et al. 2001; Staver et al. 2011). Moreover, accentuated land-use changes driven by the continuously increasing human population are also expected to affect this region (IPCC 2007), potentially affecting soil nutrient availability and herbivory intensity. African savannas are, therefore, considered to be one of the most susceptible to such changes in the climate (IPCC 2007). In this research I examined how disturbances and variation in environmental factors influence tree seed germination and seedling recruitment in African savannas.

Seed germination and juvenile growth are critical stages in the regeneration of trees and overall plant population dynamics (Higgins et al. 2000; Kitajima & Fenner 2000; Wiegand et al. 2006; Van Langevelde et al. 2011). During these early development stages, savanna trees are extremely susceptible to disturbances, such as herbivory and fire, as well as to competition for nutrients with neighbouring plants. Plant species within a community are expected to respond differently to environmental pressures, most of the differences in plant responses to environmental changes occurring during the regeneration phase of the life cycle (the regeneration niche theory, Grubb 1977). Large-scale patterns in tree communities are hence likely a result of divergent seedling strategies when selection pressures are most intense (Prior 2005).

To improve the ability to predict changes in plant species establishment, hence abundance, and the consequences for the plant communities of savannas, several ecological process models that take into account the effect of multiple environmental drivers (e.g., rainfall, soil properties, herbivory, fire) have been recently developed (e.g., Jeltsch et al. 1996; Higgins et al. 2000; Wiegand et al. 2003; Scheiter & Higgins 2009; Higgins et al. 2010; Wigley et al. 2010; Hirota et al. 2011; Staver et al. 2011; Higgins & Scheiter 2012). Apart from some notable exceptions (e.g., Coughenour & Ellis 1993; Metzger et al. 2005), due to the lack of empirical studies involving several plant species (Van Langevelde et al. 2011), most process models studies assume that generally species respond identically to environmental changes (e.g., Higgins et al. 2000; Wiegand et al. 2006).This lack of empirical knowledge critically limits our ability to understand the dynamics of plant communities, and consequently the effects of changes in environmental conditions (Ward 2005; Bond 2008; Lehmann et al. 2009). The aim of my research was, therefore, to evaluate how seedling of different African savanna trees species differs on their performance under different environmental conditions.

By running field multi-factorial experiments, where I manipulated the levels of soil moisture, soil nutrients, light irradiation, defoliation, as well grass occurrence (presence and absence) in the study plots, I obtained empirical evidence on how the different tree species respond to variations in the environmental conditions, during several stages of early tree establishment: from germination to seedling and juvenile tree (up to 18 months) (Fig. 6.1).

Based on the evidence collected, in this last chapter of the thesis, I synthetize my results and discuss how the differences in the responses to environmental factors could influence the tree species establishment (Fig. 6.2), and relate to possible changes on the community composition and dynamics and functional structure of savannas. I also relate my findings to the on-going debates on both tree-grass interaction and the vegetation shifts predictions due to climate changes. Lastly, I discuss the applications of my findings to savanna management and conservation. In each section I discuss the current state of knowledge and the contribution of the thesis to the current knowledge, and then conclude with suggestions for further research.

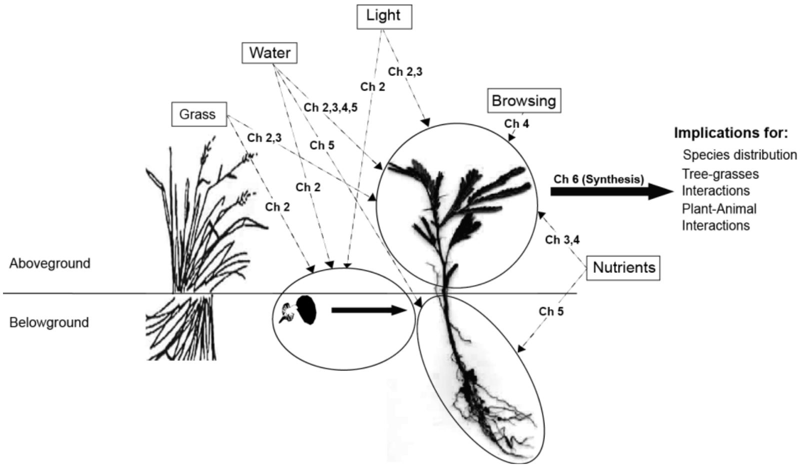

Figure 6.1 – Schematic outline illustrating the interrelationships between the different thesis chapters (Ch).

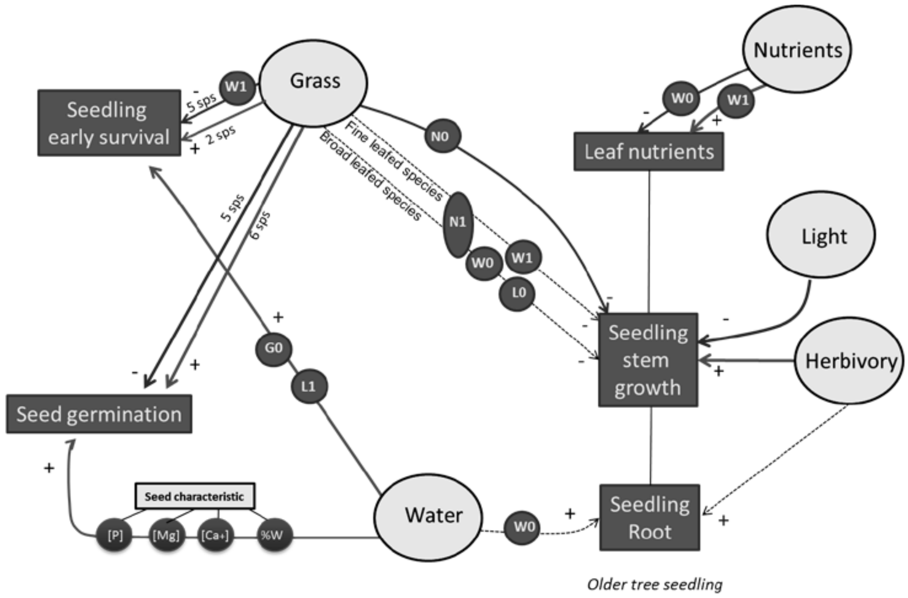

Figure 6.2 - Conceptual figure showing how the environmental factors in my experiments affect tree seed germination and seedling establishment and how these factors interact with each other. The positive signals represent positive effects and the negative signals represent negative effects. The dashed lines represent weak but significant effects. The blue circles represent the treatments used in the experiments, (G0) indicated grass presence, (G1) grass absence, (L0) shade, (L1) full sun, (W0) irregular water supply and (W1) evenly water supply. The orange circles represent seed characteristics that influence the water effect on germination, ([Ca+]) seed calcium concentration, ([Mg+]) seed magnesium concentration, ([P]) seed phosphorous concentration and (%W) seed water content. Fine leafed species represent species with fine leaves, N-fixing nodes and spinescent; broad leaved species represent species with broad leaves, without N-fixing nodes and not spinescent.

Consequences of changing environmental conditions for savanna tree establishment

Effects of changes in rainfall patterns

Plant primary production in savannas is especially dependent on an adequate supply of water, most of which stems from rainfall (Prins 1988). Rainfall is, therefore, thought to be the most important constraint to seedling survival in savanna systems (Kraaij & Ward 2006; Mosutakas et al. 2006; Meyer et al. 2007), although shade and hydraulic lift by established adult trees may also maintain soil moisture for a longer period (Belsky 1994; Holmgren et al. 1997; Phillips and Barnes 2002; Ludwig et al. 2004). Climate projections indicate a reduction in rainfall in southern African savanna (IPCC 2007), which would affect the current water supplies, potentially leading to a reduction in plant cover and productivity (Hulme et al. 2001; Sankaran et al. 2005; Staver et al. 2011). Such changes in the rainfall distribution can impact the balance between trees and grasses in savannas, critically affecting the vegetation dynamics in savannas.

In this thesis I investigated the role of soil moisture (evenly available vs. irregularly available Fig. 6.3) in germination (Chapter 2), early seedling survival (Chapters 2 and 3), seedling and juvenile establishment (Chapters 3 and 5) and leaf biomass and nutrient content (Chapter 4) of several abundant tree savanna species (see Table 1.1). Our initial expectation was that tree seedling establishment of most species would increase with increasing regularity of water availability (see Midgley & Bond 2001; Sankaran et al. 2005; Meyer et al. 2007; Ward & Esler 2011). Indeed, for some of the studied species germination and early survival rate were higher in soils where soil moisture was more regularly available, but for many others it was not (Chapter 2). Seed characteristics explained part of this variation. Under low soil moisture conditions (infrequent water supply and full sun), species with seeds with higher water content and magnesium concentration had higher germination rates, while species with higher calcium concentration had lower germination rate. Moreover, the beneficial effect of water was only detected during the early stages (up to 3 months old, Chapter 2). For later stages of tree seedling development, contrary to the expectations (see Higgins et al. 2000; Kraaij & Ward 2006), increases in water availability did not significantly increase aboveground (Chapters 3 and 5) and belowground (Chapter 5) growth, also not increasing biomass production (Chapter 4), or survival rates of juvenile (nine to 18 months old) (Chapters 3 and 5). However, water did have an indirect influence, as any beneficial effects of other environmental factors (such as light intensity or nutrient addition) were dependent on water availability and species functional traits. Broad-leafed species (which were also not N-fixing and not spinescent) were only benefited by added nutrients (which reduced the negative effect of grass presence) under the more irregular natural rainfall, while fine-leafed species only benefited from nutrients under even watering treatment (Chapter 3).

Although my study site is characterized by water scarcity (*ca.* 438 mm/season) where mostly all the rains fall at the beginning of the wet season (from November up to January), the rainfall distribution in the 2009-2010 wet season had two major rain peaks: November-December 2009 (320 mm) and April-May 2010 (202 mm), with an intermittent 3-month drought period (January to March) (Fig. 6.4). This particular rainfall distribution generated a more even water distribution during the wet season, and reduced the effect of water stress on seedling survival. Thus, although for most years a drought within the wet season (Fig. 6.4) may be a limiting factor for the establishment of seedling (Ward 2005; Kraaij & Ward 2006), the results of this thesis clearly show that, under more regular rainfall, savanna tree seedlings are not negatively affected by periods of lower soil water availability within the wet season (Fig. 6.3).

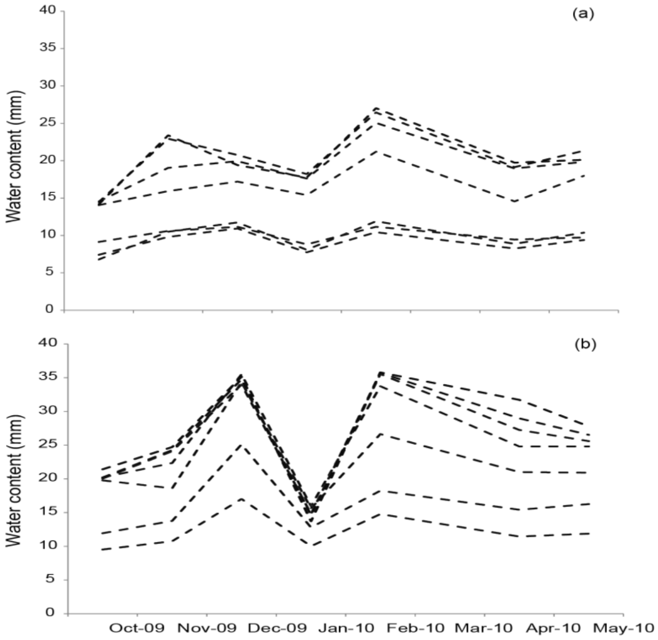

Figure 6.3 – Soil water soil content at different depths (10 to 70 cm) in plots where no nutrient were supplied and grass was absent, (a) under regular water supply and (b) under natural (irregular) rainfall, during the rainy season in the study year (2009/2010).

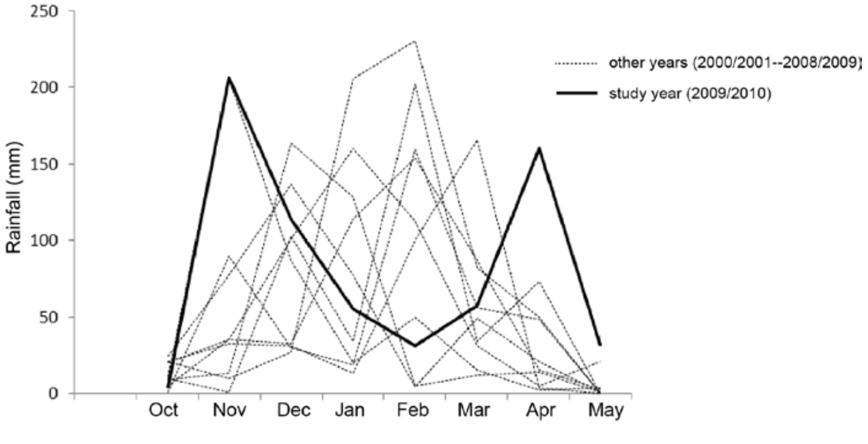

Figure 6.4 – Rainfall distribution during the rainy season in the study year (2009/2010) and in previous years (2000/2001 up to 2008/2009). The rainfall data were obtained from the Hans Hoheisen Research Facility weather station (10 km from the field site).

As climate projections indicate a reduction of rainfall of about 8 mm/yr in southern African savanna (IPCC 2007), the success of tree species to establish depend on their success in dealing with future changes in rainfall patterns. My results suggest that species vary in their responses to changes in rainfall patterns, and this variation may cause differences in species local abundance and diversity. In the future, with lower rainfall, broad-leafed tree species, such as *Colomospermum mopane* or *Peltrophorum africanum*, or tree species with high water content, such as *Dichrostachys cinerea* may have advantages, possibly increasing their distribution. Furthermore, such changes may also affect the tree-grass distribution in savannas. My results indicated that if indeed rainfall patterns become more erratic (with longer periods of drought) grass species will be favoured; and only under a scenario of increase of rain frequency trees species may be favoured, promoting transition from grassland to woodland savanna. This change is unlikely to occur under the current variation in rainfall patterns.

Effects of changes in the soil nutrient availability

Fire management (Kutiel & Shaviv 1992), air nitrogen deposition (Dentener et al. 2006), soil fertilization (Pritchard et al. 2007) or changes in herbivore densities (Augustine & McNaughton 2004) can change soil nutrient availability. However, it is unclear how such changes in soil nutrients affect savanna tree establishment (Huston 1980; Kutiel & Shaviv 1992). While some studies claim that tree recruitment is enhanced by soil nutrient enrichment (e.g., Schlesinger et al. 1996; Gillson & Ekblom 2009), others have not found any effect (e.g., Heisler et al. 2004; Sankaran et al. 2008). Some tree seedling experiments have even shown negative effects of fertilization, although these effects are thought to be mostly related to the increase of competition by grasses (e.g., Cohn et al. 1989; Kraaij & Ward 2006; Van der Waal et al. 2009). To better understand the effects of nutrient deposition effects on savanna vegetation, I evaluated how changes in soil nutrient availability affect tree seedlings using experiments where several factors (e.g., presence of grass and nutrient availability) are taken into account.

The lack of soil nutrients is expected to limit plant growth rate and to increase the risk that seedlings die during stress periods (Knoop & Walker 1985; Burke et al. 1990; Debain et al. 2005; Hermans et al. 2006). This study shows that for several abundant savanna tree species, nutrient addition neither substantially changes the survival rate of most of the study species (Chapters 2 and 4), nor does it always increase seedling stem growth (Chapter 3 and 5). While the increase in nutrient availability did not enhance seedling stem growth production per se, it reduced the negative effect of grasses on the seedling growth. However, this reduction differed between the species groups, affecting only broad-leafed species (which do not have N-fixing nodes) under natural rainfall, and only fine-leafed species (with N-fixing nodes) under even watering treatment (Chapter 3). This high tolerance to changes in nutrient availability suggests a high adaptability of the studied savanna tree species to the increases in soil nutrients, allowing them to cope with variability of environmental conditions.

Soil nutrient availability also determines the concentration of leaf nutrients in plants (Lambers et al. 2008). However, until now little was known about the effects of changes in soil nutrient availability on the nutrient concentrations in the leaves of savanna trees (but see Van der Waal et al. 2011). While it was expected that a higher nutrient availability would increase leaf concentrations (Lambers et al. 2008), I found that these effects were highly dependent on water availability as well as on species functional traits (Chapter 4). Soil nutrient input increased leaf nutrient content only under a regular watering regime, indicating strong dependence on water availability for plant nutrient uptake. The dependence on water may be explained by the fact that nutrient uptake to shoots is dependent on water movement within the plant (Hu & Schmidhalter 2005).These close relationships between water availability and nutrient soil availability have been previously suggested when it was found that fertilizers did not increase primary production in the absence of sufficient water, and increasing soil water availability did not result in an increased production without adequate nutrient supply (Breman et al. 1980; Alam 1999; Breman et al. 2001; Hu & Schmidhalter 2005). However, the most striking finding of this study was the fact that with an irregular water supply, the nutrient supply can lead to a strong drop in leaf nutrient quality. This decrease is possibly due to resources being allocated to organs that can support growth and survival after soil reserves are exhausted (Grime 1979; Nicotra et al. 2003), such as roots. For example, tree species from semi-arid savannas have been suggested to have higher resource allocation towards root elongation so that they can search more efficiently for deep water and avoid early death due to water stress (Bond 2008; Tomlinson et al. 2012). Further studies on root production in tree seedlings along soil nutrient and moisture gradients would help to confirm where extra nutrients are primarily allocated.

The increase of N deposition caused by industry, and agriculture (Smil 1990; Galloway et al. 1995; Vitousek et al.1997) is known to effect plant growth, affecting the ecosystem net primary production and plant diversity (Cleland et al. 2006; Clark & Tilman 2008; Lau et al. 2008). Differently from temperate ecosystems, where anthropogenic N stimulates plant growth (Vitousek & Howarth 1991; Aber et al. 1995; Schimel 1995), my results indicated N inputs into semi-arid savannas are unlikely to increase tree productivity and may even decrease in some cases (particularly under lower water availability). The direction and magnitude of the seedling responses to changes in nutrient availability depends on the nutrient requirements of different plant functional groups and species. Future experiments and long term monitoring schemes, with different savannas trees functional groups, are necessary to determine of the potential long-term responses of savannah tree communities to changes in N deposition.

Effects of changes in the vegetation cover (light availability)

Low light availability can limit carbohydrate accumulation in plants, which reduces their germination (Moris et al. 2000), limits their establishment (Kanz 2001; Walters et al. 2004), and slows their growth rates and prolongs their existence as juvenile plants

(Givnish 1988). In savannas, light limitation is not severe enough to limit plant production because tree density is low and most of the savanna trees only intercept ca. 50% of the sunlight (Belsky 1994; Ludwig et al. 2004). Conversely, excess of light might be a limiting factor, as direct sunlight rapidly dries out soils and demands a substantial transpiration cost of seedlings (Kitzberger et al. 2000). Hence, shade under established adult trees may, hence, favour tree seedlings by allowing them access to water for a longer period (Belsky 1994; Holmgren et al. 1997; Phillips & Barnes 2002; Ludwig et al. 2002), reducing seedling temperature and evapotranspiration (Bernhard-Reversat 1982), and therefore increasing their chances of survival. Adult trees can simultaneously have other positive (e.g., hydraulic lift) and also negative (e.g., competition for nutrients) influences on tree seedling recruitment (Belsky 1994; Holmgren et al. 1997; Ludwig et al. 2002).

The results of my study showed that while lower light availability (i.e. reduction of 80% of sunlight) did not affect early seedling survival of most species (first three months of growth) (Chapter 2), it did influence survival and growth of savanna of older seedlings (*ca.* nine months old seedlings) (Chapter 3). While shade per se had a negative effect on seedlings, for a particular group of species (broad leafed species) it had an indirect beneficial effect on seedling growth by reducing the negative effects of the presence of grass (Chapter 3). This beneficial effect is likely due to the direct negative effect of shade on grass biomass (personal observation; see also Uhl 1998; Holl 1999; Aide et al. 2000, Barnes & Archer 1999; Anderson et al. 2001), which may have reduced competition for resources between grasses and tree seedlings (Fig. 6.2)

The increase in atmospheric CO_2 might favor trees over grasses in African savannas (Scott 1999; Bond et al. 2003), shifting the savanna vegetation structure towards tree dominance, with diminishing grass suppression of faster-growing tree seedlings. Scheiter & Higgins (2009) presented a dynamic vegetation model that simulates the vegetation patterns of Africa in the year 2100 under projected increasing CO_2 levels. The authors predicted that around 30 % of today's grasslands might be transformed into savannas and around 45 % of today's savannas might be transformed into deciduous woodlands. If tree canopy cover does increase, species which are tolerant to shade (e.g., *Acacia gerrardii, A. nigrescens, Euclea divinorum and Schotia brachypetala*, Chapter 2) will have higher probabilities to establish, potentially changing tree community dynamics and composition.

Effect of herbivory levels (simulated by leaf defoliation) on seedling development

Tree seedlings are a common food source for herbivores (Belsky 1984; Fornara & Du Toit 2008). By decreasing the chances of successful tree establishment (Van Auken &Bush 1997; Ward & Esler 2011), herbivory regulates savanna structure (Prins & Van der Jeugd 1993). Seedlings employ strategies to cope with herbivory either through tolerance (e.g., see Palo et al. 1993) or through herbivory-avoidance strategies (see Fornara & Du Toit 2008).

The results of this study show that tree seedlings of three abundant African savanna species (*A. nigrescens, A. karroo and Colophospermum mopane*) are highly resistance to intensive browsing, increasing stem growth after browsing (Chapter 5). Compensatory growth after herbivory was previously reported in plants but mostly in grasses (McNaughton 1983) but also in dicotyledons (Prins et al. 1980), where it is associated with modifications in plant metabolism (e.g., higher resource allocation towards the stem at root growth costs). Moreover, contrary to our expectations, these compensatory responses did not occur at the expense of root growth. Like steam growth, root growth was also enhanced by herbivory (see also Thomas et al. 2006; Snyder & Williams 2007). These results suggest that although the use of browsing animals is recommended as a useful tool to control encroach woody species in semi-arid savannas (e.g., O'Connor 1995), this method might lead to an opposite effect on trees, namely strengthening of unwanted vegetation.

Changes in vegetation cover due to soil eutrophication can also strongly impact herbivore population dynamics (Bakker et al. 2004; Carvalheiro et al. 2010). Soil eutrophication may have an additional effect by affecting the leaf nutrient levels of tree seedlings (as shown in Chapter 4) and hence their palatability to herbivores (Olff et al. 2002; Grant & Scholes 2006; Prins & Van Langevelde 2008; Ahrestani et al. 2011). Our study shows that, under the irregular water supply that characterizes the savanna climate, increase of soil nutrient availability will decrease the quality of tree seedlings as resources for herbivores by changing nutrient content in leaves (see Chapter 4). Therefore, even when overall food quantity (plant biomass) does not change, increases of soil nutrients can impact herbivores, as food quality (leaf nutrient content) is lower. As some specialist herbivores species are less likely to adapt to changes in vegetation (e.g., Owen-Smith & Cooper 1988; Carvalheiro et al. 2010), these changes in food quality may alter the herbivore communities composition.

The role of tree seedlings for tree and grasses co-existence

Savannas exhibit enormous spatio-temporal variability in woody and herbaceous biomass and structure (Bond 2008; Lehmann et al. 2009). Different types of explanations (e.g., equilibrium, non-equilibrium and disequilibrium models) have been proposed to describe tree and grass co-occurrence in savannas (Fig. 6.5, adapted from figure 1 of Van Langevelde et al. 2011). Although these models agree on the crucial role of tree seedling recruitment in the long-term dynamics of savannas, they disagree on the long-term importance of competitive interactions between tree seedlings and grass for savanna dynamics. The equilibrium models assume that long-term coexistence of trees and grasses in savannas does not depend on rainfall variation, disturbances, or direct competition between grasses and trees. Coexistence is rather related to differences in the ecological niches (Walter 1971; Scholes & Archer 1997). However, the equilibrium models do not take into account differences between tree life stages. Similarly, non-equilibrium models (Higgins et al. 2000; van Wijk & Rodriguez-Iturbe 2002) assume that tree recruitment is not limited by competition between tree seedlings and grass but is limited only by disturbance and environmental factors (e.g., drought). In contrast to the first two explanation for the coexistence of trees and grass, disequilibrium models (Menaut et al. 1990; Jeltsch et al. 2000) assume that competitive interactions between trees and grasses occur at all life stages. In this model, the grasses have superior competitiveness over the tree seedlings and juveniles, however, such competiveness advantage changes toward the trees as they reach adulthood. Moreover, local conditions determine the competitive advantage of each life form and disturbance prevents long-term general dominance by one life form.

Supporting the assumptions of the disequilibrium savanna dynamics models, I found that grass was an important factor for savanna tree seed germination (Chapter 2), with many woody species being affected by the presence of grass. I also found here a considerable level of inter-specific variability between woody species. Although early seedling survival of several species was negatively affected by grass, other species were positively affected (Chapter 2). My results also indicate that grass mainly benefits germination and early survival of tree species in drier conditions (e.g., under full sun light and low water availability). This suggests that grass may help some species to retain moisture, providing seeds with a better opportunity for germination and early seedling survival (e.g., Anthelme & Michalet 2009). However, in later development stage (around 9-month-old seedlings) these positive effects were no longer detected (Chapter 3). In this later stage, grass strongly negatively affected seedling growth of all species and reduced the final shoot growth (primary and secondary) and biomass (stem and leaf), independently of water, nutrient or shade regime. These results suggest that belowground competition for resources plays an important role in seedling establishment and possibly reduces the period of suitable growing conditions for tree seedlings. Therefore, although grass presence may facilitate initial seedling establishment (Chapter 2), grass presence is the most limiting factor for savanna seedling growth, even under favourable recruitment conditions (e.g., constant water supply and high nutrient availability, see Chapter 3). Therefore, my results show that a transition from grassland to woodland savanna can only occur if grass abundance is severely affected (e.g., through high levels of herbivory).

Implications for conservation and management of savanna systems

Savannas are geographically extensive and occur in nearly all continents, supporting abundant and diverse wildlife assemblages (Scholes & Archer 1997). Savannas are also important for the socio-economic lives of the people living in tropical regions (Tothill & Mott 1985), especially in areas with large and fast-growing human and livestock populations (Solbrig & Young 1993). Such rising human population places added demands on the ecological services provided by savannas, such as food for livestock or the harvest of plants for food, fuel and construction. This overexploitation of the natural resources damage the ecosystem capacity to recover, compromising its productivity and threatens its biodiversity (e.g., du Toit & Cumming 1999; Carvalheiro et al. 2011). By providing substantial empirical information on how tree species' responses differ in germination, survival and establishment under varying environmental factors, my research contributes to the understanding of the consequences of climatic and land-use changes to plants and herbivore community dynamics.

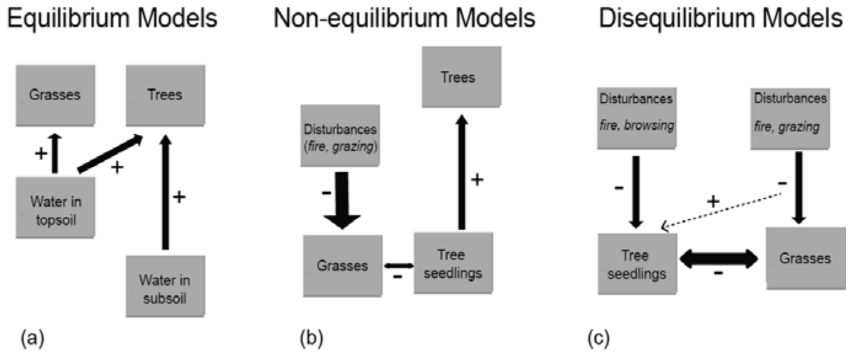

Figure 6.5 - Schematic representation of the three main groups of theoretical models on co-existence of savanna trees and grasses: (a) Equilibrium models: niche separation (e.g., the two-layer hypothesis by Walter 1971), (b) Non-equilibrium models (e.g., Higgins et al. 2000; van Wijk & Rodriguez-Iturbe 2002): tree recruitment is not limited by competition between tree seedlings and grass but by disturbance (fire and herbivory) and environmental factors (e.g., drought), (c) Disequilibrium models (e.g., Menaut et al. 1990; Jeltsch et al. 2000): competitive interactions between trees and grasses occur during all life stages, with local conditions determining the competitive advantage of each life form, and disturbances preventing dominance of one life form over another. Full arrow with '+' sign indicates positive direct interactions, while full arrow with '-' sign indicates direct negative interaction. The dashed arrow indicates an indirect effect. The sub-figures (a) and (b) were adapted from Van Langevelde et al. 2011.

One of the major concerns for the conservation of arid and semi-arid habitats like savannas is the invasion of woody plants in tropical grasslands and savannas (bush encroachment) (Ward 2005; Kraaij& Ward 2006) which is thought to reduce the carrying capacity for grazing animals (Dean & Macdonald 1994). The high level of herbivory by domestic grazers is suggested to be the most critical factor causing bush encroachment because it reduces aboveground grass biomass, the fuel needed for grassland fires that regulate savanna trees (Prins 1982; Prins & Van der Jeugd 1993; Bush & Van Auken 1995; Schuster 1964; Van Auken& Bush 1997; Asner et al. 20009). However, my results show that grass presence is the most important factor to control savanna tree seedling growth per se (Chapter 3), independently of fire. In semi-arid African savannas where grass biomass is less abundant than in other more humid regions (i.e. due to the lower water and nutrient availability), the intensity of fire will be lower (Van Wilgen & Scholes 1997; Enslin et al. 2000); and so the impact of fire on adult trees will be limited. Consequently, the use of controlled fires (a common management measure to control the expansion of woody species, see Tropolle 1980) in semi-arid savanna may be not an adequate measure to control expansion of woody cover, as it will mostly affect the grasses, decreasing their competitive ability, and increasing chances of tree establishment success. Another suggested measure to control invasive woody species in semi-arid savannas involves the increase of browsing animal density (e.g., O'Connor 1995). However, the results of my work show that high levels of browsing might lead to an opposite effect on trees: browsing might strengthen the presence of unwanted vegetation rather than limit it (Chapter 5).

Given the importance of tree seedlings for the dynamic of plant community (Higgins et al. 2000; Wiegand et al. 2006; Van Langevelde et al. 2011), management measures that aim to prevent bush encroachment should consider recruitment control of tree seedlings, a practice that is still not common (Angassa & Oba 2008). The findings of my research provide, detailed information on the requirements of different savanna tree species to germinate and establish. First of all, for a given species to gain advantage over another and be able to increase its abundance, the grass abundance needs to be substantially suppressed and specific climatic conditions need to occur (e.g., *C. mopane* would need lower rainfall and high light exposition; while *Acacia species* would need a higher and regular rain fall). This information is essential to better predict which tree species are more likely increase its local abundance and turn into an invasive woody species, and hence can help develop more efficient targeted management strategies.

Final remarks

Despite the strong limiting effect of grass on the establishment of savanna seedlings, my study shows the wide diversity of responses of the different savanna trees to variation in growth conditions. This broad range of species recruitment responses associated with the high spatial and temporal variation in climatic conditions within savannas is likely responsible for the high level of tree diversity in this ecosystem, with a given species only having advantage over others under specific micro-climatic conditions. These findings support the regeneration niche theory's claims that plant species within a community respond differently when exposed to variations in environmental conditions (Grubb 1977). These different responses allow plant species to coexist and explain the high diversity of plant species in savannas. The findings of this study provide insightful information to help better predict changes in savanna habitats and guide management strategies in the face of human pressures and the future prospects of environmental change.

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Summary

Savanna ecosystems are characterized by a continuous grass layer intermixed with a discontinuous layer of trees and shrubs. A complex set of environmental drivers, such as water, soil nutrients, solar radiance, fire and herbivory, determines vegetation structure and composition in savannas. Such environmental drivers are expected to be strongly affected by future global climatic and land-use changes, potentially modifying savanna vegetation, and consequently savanna fauna. The ability to predict changes in plant community composition is therefore important for management and conservation of savannas. However, the mechanisms controlling plant establishment and growth in savannas are still unclear. Germination and seedling establishment are critical recruitment stages in the life cycle of plants and can influence plant community composition. A better understand of the factors influencing plant species recruitment and their ecology is needed. This thesis focuses on seedling recruitment of several savanna tree species.

Water stress is probably the single greatest constraint to tree seedling survival in savanna systems: tree seedling recruitment and survival are hypothesized to be limited by soil moisture availability. Shade by established adult trees may facilitate tree seedling recruitment by maintaining high soil moisture availability. Chapter 2 deals with germination and early seedling establishment of several tree species. I expected that tree species would germinate and establish best under high moisture conditions (high water and shade), while under stress conditions (i.e. low soil moisture due to low water supply and full sun, and in the presence of grasses) plants would suffer. The observed variability of seedling performance among the tree species under stress conditions may be explained by differences in functional traits. Higher soil moisture mostly benefited germination of species with seeds with high calcium concentration and low water content. On the other hand, low soil moisture conditions benefited germination of tree species with seeds with higher magnesium and phosphorus concentration and water content. Furthermore, under low soil moisture availability, grass presence facilitated germination of most tree species but its effect on early survival (positive or negative) differed among species. The findings of this chapter confirm a large difference in the tree species responses to environmental variation during early recruitment, which potentially affect the plant community composition and dynamics under different environmental conditions in savannas. Seed trait differences among the species partly contribute to explain such variability. Therefore, considering inter-specific variation among tree species and information on seed traits can improve the ability to predict and manage the impacts of environment changes.

For later stages of seedling development (up to 9 months), the effect of environmental variation (water, nutrient and light supply, as well as grass presence or absence) on survival and establishment of semi-arid savanna tree seedlings differed between species (Chapter 3). All species were expected to respond positively to higher resource availability, and negatively to the presence of grass. Indeed, the results of this chapter clearly show that grass presence strongly suppressed seedling establishment. However, recruitment strategies varied among species, particularly under high stress conditions (water stress or low light). In some of the studied tree species, light shortage (i.e., shade) reduced the negative effects of the presence of grass on growth. Furthermore, nutrient availability also reduced the negative effect of grasses, although for certain species (broad-leaf species) this effect occurred only under natural rainfall, while for others (fine-leaf species) it occurred only under regular water provision.

Increased atmospheric nitrogen deposition, intensification of agricultural fertilizer use and large herbivore management interventions are on-going processes that increase soil nutrient levels in many savannas. As nutrient concentration in the leaves (i.e., plant quality for herbivores) also depends on soil nutrient availability, I expected in Chapter 4 that both biomass production and leaf nutrient concentration would increase with increasing soil nutrient availability. Contrary to my expectations, differences in soil nutrient levels (low vs. high) did not affect biomass production of any of the tree species, independently of water availability (uneven vs. even water supply). However, leaf nutrient content of the seedlings did differ significantly with different water and nutrient levels. Soil nutrient input increased leaf nutrient content, but only when water was applied regularly, indicating that plant nutrient uptake strongly depends on water availability. Under irregular rainfall patterns, nutrient input significantly reduced leaf quality. Given that large herbivore populations depend on plant nutrient content for their nutritional requirements, increases in nutrient deposition and rainfall levels will likely impact herbivore populations and their browsing patterns, altering the functional structure of ecosystems even if overall plant biomass remains unaffected.

In Chapter 5, the effect of fluctuations of environmental conditions on above and belowground growth of juveniles of three savannas tree species (*Acacia karroo*, *A. nigrescens* and *Colophospermum mopane*) during the first 18 months was tested. While it was expected that low soil resource availability would result in high biomass allocation towards roots, experimental simulation of dry events within the wet season or pulses of nutrient availability did not have a clear effect on the seedlings' aboveground and belowground growth. Furthermore, the results of this chapter demonstrated that browsing stimulated stem regrowth and root elongation of savanna tree seedlings, suggesting that the three studied species have compensatory growth in response to frequent herbivory, quickly recovering the loss of biomass. This result puts in question the usefulness of herbivory or human land-cleaning in controlling invasive woody species in tropical grasslands and savannas.

In conclusion, the results of this thesis demonstrated that savanna tree species are generally able to cope with differences in resource availability during seedling establishment, being mostly limited by grass competition for resource. Furthermore, this study shows that during early stages of the life-cycle, when exposed to the same environmental conditions, tree species within a plant community differ in their responses, only having advantages over other species under specific conditions. This inter-specific variation may allow tree species coexistence, explaining the diversity of plant species in savannas.

Samenvatting

Savannes worden veelal gekenmerkt door een aaneengesloten grasvlakte met daarin groepjes van bomen en struiken. Een complex geheel van milieufactoren, zoals water, bodemnutriënten, zonnestraling, vuur, en herbivorie, bepalen de vegetatiestructuur en samenstelling van savannes. Dergelijke milieufactoren zullen naar verwachting sterk worden beïnvloed door de wereldwijde veranderingen in klimaat en landgebruik, wat zou kunnen leiden tot veranderingen in de in savannes aanwezige flora en fauna. Het vermogen om veranderingen in de samenstelling van plantengemeenschappen te voorspellen is dan ook belangrijk voor het beheer en behoud van savannes. Echter, er is nog weinig bekend over de factoren die sturing geven aan vestiging en groei van planten in savannes. Kieming en vestiging van een zaailing zijn cruciale stadia in de levenscyclus van een plant en deze stadia kunnen dan ook van invloed zijn op de samenstelling van plantengemeenschappen. Het is noodzakelijk om een beter inzicht te verkrijgen in de factoren die van invloed zijn op de ecologie en vestigingskansen van verschillende plantensoorten in savannes. Dit proefschrift richt zich op de kiemings- en vestigingskansen voor zaailingen van een aantal in savannes voorkomende boomsoorten.

De vestigings- en overlevingskansen voor zaailingen van bomen worden naar verwachting voornamelijk bepaald door de beschikbaarheid van water in de bodem. Het beschaduwen van de bodem door volwassen bomen zou vestigings- en overlevingskansen voor zaailingen kunnen faciliteren, doordat het kan zorgen voor een verhoogde beschikbaarheid van vocht in de bodem. Hoofdstuk 2 behandelt de kieming en het vroege vestigingsstadium van zaailingen voor verscheidene boomsoorten. Ik verwachtte dat kieming en vestiging van zaailingen het best zou zijn onder vochtige omstandigheden (hoog bodemvochtgehalte en veel schaduw), terwijl onder stressvolle omstandigheden (laag bodemvochtgehalte door weinig aanvoer van water en door een positie in de volle zon, en de aanwezigheid van grassen) de bomen zouden lijden. De waargenomen variatie in prestaties van zaailingen van verschillende boomsoorten onder deze stressvolle omstandigheden zou wellicht kunnen worden verklaard door verschillen in functionele eigenschappen. Hoge bodemvochtgehaltes waren vooral voordelig voor kiemingskansen van boomsoorten waarvan de zaden een hoog calciumgehalte en een laag vochtgehalte hebben. Anderzijds waren lage bodemvochtcondities vooral voordelig voor boomsoorten waarvan de zaden hoge concentraties aan magnesium, fosfor en water hebben. Verder bleek dat onder lage bodemvochtcondities, de kansen op kieming voor de meeste boomsoorten vergroot werden door de aanwezigheid van gras, maar diens effect op de vroege overleving van zaailingen (zowel positief als negatief) was verschillend voor de verschillende boomsoorten. De bevindingen van dit hoofdstuk bevestigen de grote verschillen in reacties van boomsoorten op variatie in omgevingsfactoren gedurende de periode van vroege ontwikkeling van de zaailing. Dit zou de samenstelling en dynamiek van plantengemeenschappen in savannes onder verschillende milieuomstandigheden kunnen beïnvloeden. Verschillen in de eigenschappen tussen zaden van verschillende boomsoorten dragen bij aan het verklaren van deze variabiliteit. Door de variatie tussen boomsoorten en informatie over zaadeigenschappen in beschouwing te nemen is men beter in staat om de impact van milieuveranderingen te voorspellen en te beheren.

Tijdens latere stadia in de ontwikkeling van de zaailing (tot 9 maanden) bleek het effect van variatie in milieuomstandigheden (aanbod van water, nutriënten en licht, alsook aan- of afwezigheid van gras) op overleving en vestiging van boomzaailingen uit half-droge savannes te verschillen tussen de soorten (hoofdstuk 3). De verwachting was dat alle soorten positief zouden reageren op een toename in de beschikbaarheid van voedingstoffen, maar negatief op de aanwezigheid van gras. De resultaten van dit hoofdstuk laten inderdaad duidelijk zien dat de aanwezigheid van gras het vestigen van zaailingen sterk onderdrukt. Echter, de gevolgde ontwikkelingsstrategieën verschilden per soort, vooral onder hoge stresscondities (waterstress of weinig licht). In sommige van de onderzochte boomsoorten zorgde een tekort aan licht (d.w.z. veel schaduw) voor een vermindering in het negatieve effect van de aanwezigheid van grassen op de groei. Daarnaast verminderde de beschikbaarheid van voedingstoffen ook het negatieve effect van grassen, hoewel dit effect er voor bepaalde soorten (breedbladige soorten) alleen was onder natuurlijke neerslagomstandigheden, terwijl het voor andere soorten (smalbladige soorten) alleen gold onder een regime van regelmatige bewatering.

De concentraties aan bodemnutriënten in veel savannes nemen continu toe door huidige processen als verhoogde atmosferische stikstofdepositie, intensivering in het kunstmestgebruik en veranderingen in het beheer van grote herbivoren. Omdat de concentratie van nutriënten in de bladeren (d.w.z. de kwaliteit van planten voor herbivoren) afhankelijk is van de aanwezigheid van nutriënten in de bodem, verwachtte ik in hoofdstuk 4 dat zowel de biomassaproductie als de concentratie van nutriënten in bladeren zouden toenemen met een toename in beschikbaarheid van nutriënten in de bodem. In tegenstelling tot mijn verwachtingen, vond ik geen effect van verschillende bodemnutriëntengehaltes (laag versus hoog) op de biomassaproductie van de verschillende boomsoorten, onafhankelijk van de beschikbaarheid van water (regelmatige versus onregelmatige watergift). Echter, de concentratie van nutriënten in de bladeren van zaailingen was wel significant verschillend onder de verschillende water- en bodemnutriëntengehaltes. Toevoeging van bodemnutriënten zorgde voor een toegenomen concentratie van nutriënten in de bladeren, maar alleen onder regelmatige watervoorziening. Dit geeft aan dat de opname van nutriënten door planten sterk afhankelijk is van de beschikbaarheid van water. Onder omstandigheden van onregelmatige watervoorziening, zorgde de verminderde opname van nutriënten voor een significante afname in bladkwaliteit. Gezien het feit dat grote herbivoren afhankelijk zijn van nutriënten in planten om aan hun nutritionele behoeftes te voldoen, zal een toename in regenval en in depositie van nutriënten waarschijnlijk invloed hebben op herbivoren en hun begrazingspatronen. Dit zou de functionele structuur van ecosystemen kunnen veranderen, zelfs als de totale plantenbiomassa onveranderd blijft.

In hoofdstuk 5 werden de effecten van fluctuaties in de milieuomstandigheden op ondergrondse en bovengrondse groei van drie boomsoorten (*Acacia karroo, A. nigrescens* en *Colophospermum mopane*) gedurende hun eerste 18 maanden getest. Hoewel de verwachting was dat een lage beschikbaarheid van voedingstoffen in de bodem zou resulteren in een hoge biomassa-allocatie naar de wortels, heeft experimentele simulatie van zowel droge periodes binnen het regenseizoen als tijdelijke verhogingen in de beschikbaarheid van nutriënten geen duidelijk effect laten zien op de ondergrondse en bovengrondse groei van de zaailingen. Daarentegen lieten de resultaten van dit hoofdstuk zien dat vraat door herbivoren zowel de hergroei van de stam als het verlengen van de wortels van de boomzaailingen stimuleerde, wat suggereert dat de drie bestudeerde boomsoorten "compenserende groei" laten zien in reactie op frequente begrazing. Hierdoor wordt het verlies aan biomassa snel hersteld. Dit resultaat doet de vraag rijzen over het nut van begrazing of houtkap bij het controleren en beheren van invasieve houtige soorten in tropische graslanden en savannes.

Kortom, de resultaten van dit proefschrift laten zien dat boomsoorten van savannes over het algemeen goed in staat zijn om te gaan met verschillen in de beschikbaarheid van water, bodemnutriënten en zonnestraling tijdens het vestigingsstadium van zaailingen, waarbij competitie voor deze voedingstoffen met grassen de ontwikkeling van zaailingen voornamelijk beperkt. Bovendien toont deze studie aan dat boomsoorten binnen een plantengemeenschap tijdens de vroege stadia van de levenscyclus, wanneer ze blootgesteld staan aan dezelfde milieuomstandigheden, verschillen in hun reacties op deze milieuomstandigheden waardoor ze alleen voordeel hebben over andere soorten onder specifieke omstandigheden. Deze interspecifieke variatie zou ruimte kunnen bieden aan co-existentie van boomsoorten, wat de diversiteit aan plantensoorten in savannes zou kunnen verklaren.

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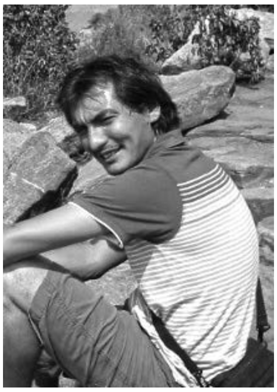
Curriculum vitae



Eduardo R.M. Barbosa was born on 18 July 1976 in Brasil, and grew up in the state of Paraná. After high school he enrolled for a 5-year course on biological science degree at the Universidade Estadual do Paraná (UEL), which was completed in 2000. He completed his degree with an internship at "Mata dos Godoy" Natural Park, from which he wrote his degree dissertation on the "Effect of natural corridor to fragmented forest birds communities". During his degree he participated in several research projects of the Universidade Estadual do Paraná, and gave Biology classes in local secondary schools in 1999 and 2000. After completing the honours programme he worked for a year as an environmental consultant for the Municipality Department of Environmental Affairs of the Londrina, where his work included production of environmental impact reports. In 2002 he worked in Caiman Ecological Refuge, Pantanal, Brasil where he worked with bird communities and eco-tourism.

In 2003 he moved to Lisbon to start with the MSc Environmental and Life Sciences at Faculdade de Ciências da Universidade de Lisboa (FCUL). For his MSc he studied reptile habitat preferences on several natural areas in Southern Portugal with the aim of identifying endangered hotspot areas for the development of mitigating measures. During this time he also worked as research assistance for FCUL collecting data for the Atlas of Reptiles and Amphibians of Portugal and for the reptile species monitoring project in Alqueva dam region. In 2005-2006, Eduardo worked as research assistant at University of Bristol (UK), where his work included research on propagation of impacts of invasive species on native plant and insect communities.

In 2007 he moved to Wageningen University to start a PhD at the Resource Ecology Group under the supervision of Herbert Prins and Steven de Bie. His PhD research focused on the role of seedling establishment for the community dynamics of savanna trees. Part of the research was integrated on the Global GEST experiment (global experiment in seedling trees). GEST is worldwide experiment running in field sites located in savannas from Africa, Asia, Australia and the Americas. His field work run in the savannas of South Africa. The results of his research culminated in this thesis. During the time in South Africa, Eduardo gave several theoretical and practical classes on plant physiology and ecology for the graduation course in Natural Resource Management of the Southern African Wildlife College. He also co-supervised an MSc student from Wageningen University.

Presently, he received a post-doctoral grant from the Brazilian Science Foundation (CAPES) to work for 1 year at University of Brasilia (Brazil) with Prof. Fabian Borghetti. The grant is linked with the Nuffic/Capes program. The research will focus on the influence of climate changes to the distribution of neotropical plant species, and how this changes effect the balance between different ecosystems found in semi-arid areas in Brazil (Cerrado, Caatinga and Forest).

List of Publications

Tomlinson, K., Sterck, F.J., Bongers, F., Silva, D. D. A., Barbosa, E. R. M., Ward, D., Bakker, F.T., Van Kaauwen, M., Prins, H. H. T., De Bie, S., Van Langevelde (2012) Biomass partitioning and root morphology of savanna trees across a water gradient. *Journal of Ecology* **100**: 1113 – 1121.

Van Langevelde, F., Tomlinson, K.W., Barbosa, E.R.M., Bie, S. de, Prins, H.H.T. Higgins, S.I. (2011) Understanding tree-grass coexistence and impacts of disturbances and resource variability in savannas. In: Hill, M. & Hanan, N. (eds.) *Ecosystem function in savannas.* CRC Press, Boca Raton, USA.

Carvalheiro, L.G., Barbosa, E.R.M. & Memmott, J. (2008) Pollinator networks, alien species and the conservation of rare plants: Trinia glauca as a case study. *Journal of Applied Ecology* **45**: 1419 -1426.

PE&RC PhD Education Certificate

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)

- Competitive interactions between savanna trees and grasses: experiments on the multiple factor that influence the early seedling recruitment (2006/2007)

Writing of project proposal (4.5 ECTS)

- Competitive interactions between savanna trees and grasses: experiments on the multiple factor that influence the early seedling recruitment

Post-graduate courses (4.2 ECTS)

- Consumer resource interaction; PE&RC/SENSE/FE (2006)
- Survival analysis; PE&RC (2007)
- Basic GIS course; SAWC/Peace Parks Foundation (2009)
- Generalized linear models; PE&RC (2010)

Laboratory training and working visits (1.5 ECTS)

- Methods of pre-treatment and breaking dormancy of tree seeds; Nursery, SANPARKS, KNP (2007)

Deficiency, refresh, brush-up courses (8.8 ECTS)

- Ecological methods (2006)
- English IV (2007
- Basic statistics; PE&RC (2007))

Competence strengthening / skills courses (1.8 ECTS)

- Scientific writing; Centa (2010)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.2 ECTS)

- PE&RC Weekend (2007)
- PE&RC Day (2007)

Discussion groups / local seminars / other scientific meetings (3.3 ECTS)

- LOCORES (lowveld co-ordinated research forum) (2008)
- Day symposium: elephant management strategies (2009)
- Mini symposium: how to write a world class paper (2010/2011)
- Wageningen Evolutionary and Ecology Seminar (WEES) (2010/2011)
- Oral presentation at climate related ecological research within centre of ecosystem mini-ecosystem; Wageningen University (2011)

International symposia, workshops and conferences (9.7 ECTS)

- Poster presentation at 6th annual Kruger National Park science network meeting; Sukukuza, South Africa (2008)
- Oral presentation at 7th annual savannah science network meeting; Sukukuza, South Africa (2009)
- Poster presentation at DIVERSITAS Open Science Conference 2; Cape Town, South Africa (2009)
- Poster presentation at 12th European Ecological Federation (EEF) Congress; Avilla, Spain (2011)

Lecturing / supervision of practical's / tutorials (0.9 ECTS)

- Vegetation dynamics in savannas-lecture in the higher certificate in nature conservation; Southern African Wildlife college; Hoedspruit, South Africa (2008)
- Vegetation dynamics in savannas-lecture in the higher certificate in nature conservation; Southern African Wildlife college, Hoedspruit, South Africa (2009)
- Plants propagation- lecture in the higher certificate in nature conservation; Southern African Wildlife college, Hoedspruit, South Africa (2009)

Supervision of MSc student; 180 days

- C.Y. van Ravenstein: effects of resource availability and interaction with grasses on allocation patterns and morphology of different savanna tree seedling species.

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