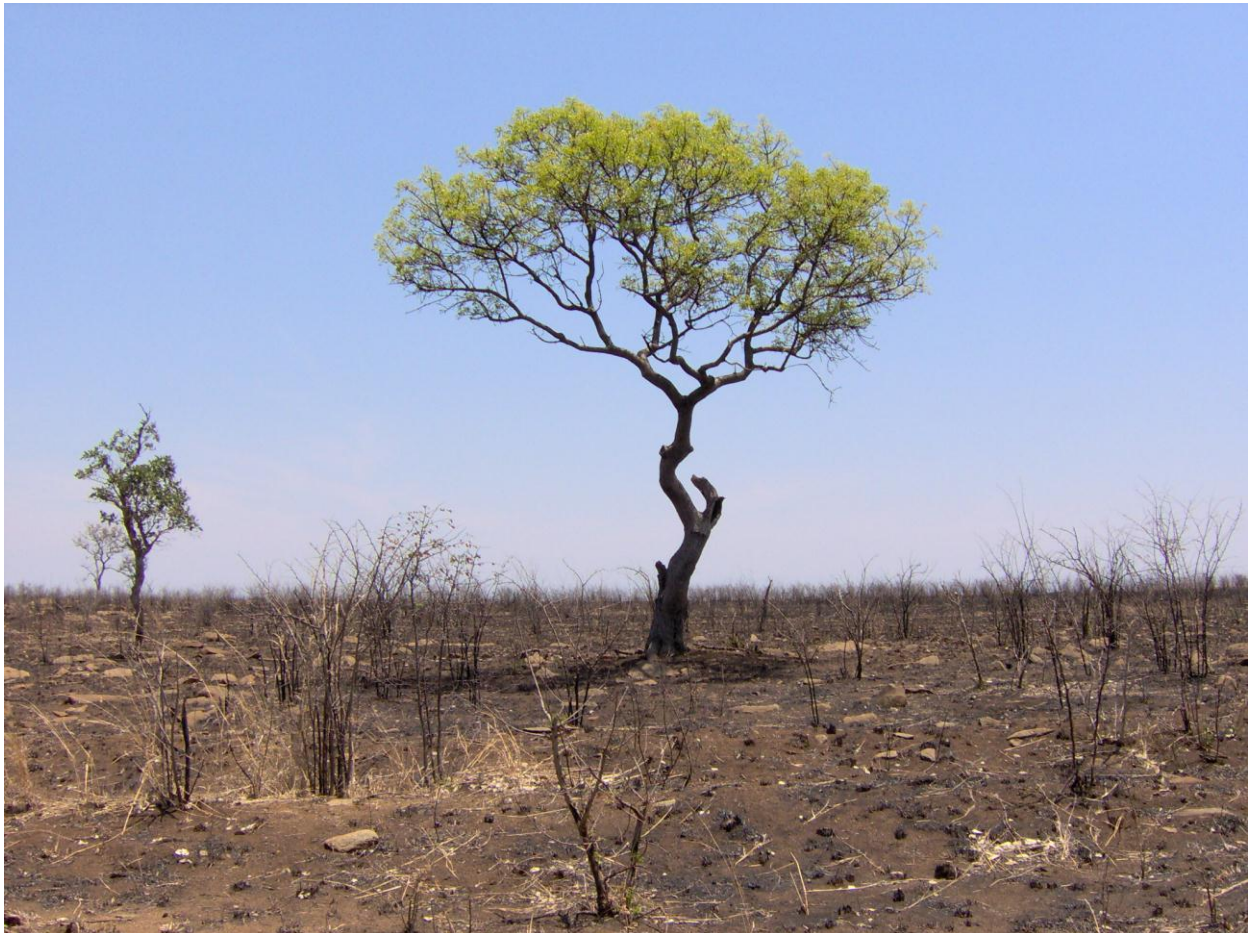


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Niche differentiation in savannas: exploring competition-based hypotheses for inter-life form coexistence



Richard J.T. Verweij



UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

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University of Cape Town

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Supervisor: Dr. E.C. February
Senior Lecturer
Department of Botany, University of Cape Town

Declaration

This thesis is my own unaided work, both in concept and execution, and apart from the normal guidance from my supervisor, I have received no assistance except as stated below:

The root severing experiment as described in Chapter 3 was proposed -in rudimentary form- by Dr. Edmund February and Prof. William Bond in the project proposal 'Mechanisms of tree/grass interactions in savannas', which formed the basis for the Tree-Grass Programme. I took the lead in the design and execution of the experiment, as well as analyzing the data and the writing of the paper that resulted from the work. Prof. Steven Higgins offered statistical advice and suggestions for the text.

The tracer addition experiment described in Chapter 4 has been designed by Dr. Andrew Kulmatiski and Prof. Karen Beard. The fieldwork was a team effort to which I contributed significantly, as well as to data analysis, interpretation of the results, and the writing of the manuscript. All the water samples were analyzed by me using the facilities in the isotope lab at the University of Cape Town. The parts of the resulting joint paper that I cannot reasonably claim to be my work are not included in this thesis.

Niche differentiation in savannas: exploring competition-based hypotheses for inter-life form coexistence

Richard J.T. Verweij



Thesis Presented for the Degree of

DOCTOR OF PHILOSOPHY

In the Department of Botany

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Abstract

Competition-based models predict that coexistence of trees and grasses in savannas may be possible if trees and grasses occupy different niches when exploiting limiting resources such as water and nutrients. I investigated two competition-based models, each of them considering a different axis for niche separation: the two-layer hypothesis, which is based on differences in rooting depth, and the phenological niche separation hypothesis, which is based on differences in timing of resource acquisition.

A field experiment in which I monitored leaf-level responses following tree root severing showed that severing deep roots of *Terminalia sericea* showed no substantial response, where severing shallow roots resulted in high levels of water stress. The results suggest that trees are dependent on resources in the surface layers (upper 50 cm), and that access to deep water (>50cm) does not seem to promote tree-grass coexistence. Results of an isotope tracer study, in which deuterated water ($^2\text{H}_2\text{O}$) was injected to different soil depths (5, 20, 50, and 120 cm) and during four sampling periods (Oct, Nov, Feb, and Apr), revealed that plants can partition soil resources over small spatial scales, relatively close to the soil surface.

The phenology of trees and grasses being out of phase may create a niche for trees to monopolize resources, such as nitrogen, early and late in the growing season. The phenological strategy followed by *Sclerocarya birrea* and the biomechanical properties of this species allow it to store water that can be used to leaf out well before the rains of the following growing season start. *T. sericea* is more drought tolerant, and consequently it is able to retain its leaves for longer, extending its growing season substantially over that of *S. birrea*. The consequence is that it does not have any stored water to leaf out early and is therefore reliant on the first rains of the following growing season. It therefore cannot avoid competition for nitrogen in the early growing season, as opposed to *S. birrea*. My results show that niche separation along environmental axes on spatial and/or temporal scales, although unlikely to be the only mechanism of coexistence in savannas, may play a more significant role than has been appreciated recently.

Acknowledgements

This PhD study was undertaken within the framework of the Tree-Grass Programme, which was initiated in 2002 by Dr. Edmund February and Prof. William Bond, both from the University of Cape Town. They gave me the opportunity to come to South Africa to do my research in one of the most beautiful places on Earth, for which I am very grateful. I am also indebted to South African National Parks for granting me permission to carry out my fieldwork and experiments in the Kruger National Park, and the NRF for funding the research.

The following people were key to the success of my PhD research and I hereby would like to express my gratitude to them. My supervisor Edmund February provided me with invaluable scientific advice and assistance in every phase of my project, in any way he could. Steven Higgins, William Bond, Andrew Kulmatiski and Karen Beard have supported me in many aspects of the work, through ideas, discussions, technical and statistical help. I very much enjoyed our collaboration as well as my work visits to Frankfurt (Germany) and Logan (USA). Many thanks to the H.O.D. of the Department of Botany, Prof. Jeremy Midgley, for welcoming me to take up my PhD study at UCT. I thank my (former) lab colleagues Klaudia Schachtschneider, Adam West, Tim Aston and Matthew Britton for their advice, help with lab/fieldwork and their friendship in the past years.

Without the support and encouragement of the Tree-Grass Programme's successive field managers Louise Swemmer, Corli Coetsee, Benjamin Wigley and Henri Combrink, and the field assistants Annoit Mashele, Patrick Ndlovu, Mightyman Mashele and Adolf Manganyi, this research would not have been possible. The people at the UCT isotope facility John Lanham, Ian Newton and Shaamielah Davids were of great assistance to me during the many hours I spent with the isotopic analyses of my samples. The secretarial/administrative staff of the Department of Botany, Sandy Smuts, Ncumisa Thungela and Natalie Allies, as

well as Patricia Khoza of KNP's Scientific Services, provided me with much appreciated logistical/administrative help. Scientific Services of the Kruger National Park are acknowledged for arranging research permits and for providing general support, and the section rangers and game guards for late permits and protection.

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Richard Verweij, December 2011

Table of Contents

<i>Abstract</i>	<i>vii</i>
<i>Acknowledgements</i>	<i>viii</i>
<i>Chapter 1:</i> General introduction.....	
.....	1
<i>Chapter 2:</i> Study sites and species	
.....	11
<i>Chapter 3:</i> Water sourcing by trees in a mesic savanna: responses to severing deep and shallow roots	19
<i>Chapter 4:</i> Vertical, horizontal and temporal patterns of water use by trees and grasses suggest resource use differentiation in the upper soil layers.....	41
<i>Chapter 5:</i> Stress or strategy? Plant water status and vulnerability in two tree species with asynchronous phenology	59
<i>Chapter 6:</i> Relating plant traits to plant functional type: trade-offs in water use and growth	75
<i>Chapter 7:</i> Synthesis.....	
.....	89
<i>Chapter 8:</i> References	
.....	97

CHAPTER 1

GENERAL INTRODUCTION

University of Cape Town

CHAPTER 1:

General Introduction

INTRODUCTION

One of the key unresolved questions in plant community ecology is how multiple species coexist alongside each other in a given ecosystem, while competing for the same resources. To explain this coexistence, there are a number of different hypotheses, including niche-mediated coexistence (Silvertown et al. 1999, Silvertown 2004; Araya et al. 2010) and neutral theories (Hubbell 2001; Leigh Jr 2007). Niche-mediated coexistence occurs when species specialize in a particular region of niche space, where it is superior to its competitors (Silvertown 2004). Coexistence requires that species respond to ecological heterogeneity in different ways (Leibold & McPeck 2006). Therefore, species must show trade-offs, i.e. develop traits that confer an advantage over competitors for performing one function, but simultaneously are a disadvantage in performing another (Chesson 2000; Chase & Leibold 2003). The niche theory implies that species must be different enough to coexist. An additional criterion for coexistence is that intra-specific competition is stronger than inter-specific competition (Chesson et al. 2004). Another much-debated theory, the unified neutral theory of biodiversity and biogeography, criticizes the niche theory by arguing that very similar species co-occur as well, and that the differences between species are irrelevant to their success (Hubbell 2001). The neutral theory proposes therefore that the ecological niche is not necessary to explain species coexistence at all, but that stochastic demographic processes (birth, death, dispersal, speciation) are enough to predict species assemblies (Hubbell 2001). The neutral theory explains coexistence as the result of chance, along with historically-contingent initial population size (for reviews of Hubbell's and other neutral theories, see Leigh Jr 2007; Chase & Leibold 2003). There is, however, a general consensus among community ecologists that natural communities are influenced by both niche and

stochastic factors (Chave et al. 2002; Adler et al. 2007; Mutshinda & O'Hara 2010), albeit at different spatio-temporal scales.

TREE-GRASS COEXISTENCE IN SAVANNAS

The savanna is commonly defined as a vegetation type with a well-developed, continuous grassy layer, and with a prominent, discontinuous woody layer of trees and/or shrubs. The woody plants may be scattered to form an open, park-like landscape, or be more dense to form typical bushveld (Frost et al. 1986; Bredenkamp 2002). Savannas occur on all continents of the world, occupying one fifth of the global land area, predominantly in the seasonal tropics (Hutley & Setterfield 2008). Average annual rainfall in regions where savannas occur ranges between 200mm and 2000mm, with a distinct dry season, varying in length from 2-9 months (Hutley & Setterfield 2008). Savannas occur on a range of different soil types, which may or may not be rich in nutrients, and support high animal biomass, with animal densities generally increasing with increasing soil fertility (East 1984, Fritz et al. 2002).

The coexistence of trees and grasses is unique for savannas. Trees and grasses are two very different life-forms; they differ in size, height, life span, root diameter, root/shoot ratio, leaf/stem ratio, etc., yet they depend on the same limited set of resources. Tree-grass interactions have been extensively described (e.g. Belsky 1994; Scholes & Archer 1997; Jeltsch et al. 2000; Ludwig et al. 2004; Hempson et al. 2007; Scheiter & Higgins 2007; Simmons et al. 2007, 2008). It is unmistakable that -compared to open grasslands- habitat heterogeneity in the system is dramatically increased in the presence of a (discontinuous) tree component. For example, grass production under tree canopies may be negatively affected as a result of decreased light availability and below-ground competition with the tree for resources. On the other hand, nutrient availability under tree crowns is often shown to be higher under trees than in open grassland (Belsky 1994, Simmons et al. 2008), hydraulically

redistributed water may partly compensate competition for water and/or water lost through transpiration may be reduced as a result of shade (Ludwig et al. 2004). The manner in which trees and grasses divide up these limited resources, without the one excluding the other from the community, has intrigued researchers for some time (Walter 1971; Walker & Noy-Meir 1982; Sarmiento 1984). Many mechanisms have been suggested, but evidence from field and model studies have provided mixed results. Two main groups of hypotheses were distinguished by Sankaran et al. (2004): competition-based models (e.g. Walter 1971; Sala et al. 1997; Fernandez-Illescas & Rodriguez-Iturbe 2003) and disturbance-based, demographic bottleneck models (e.g. Jeltsch et al. 1996, 2000; Higgins et al. 2000, 2007; Beckage et al. 2009). Competition-based models explain tree-grass coexistence from the niche perspective, on either spatial or temporal scales. These models are based on differences in rooting depth (e.g. Walter 1971) or timing of resource acquisition (e.g. Ovalle & Avendano 1987; Scholes & Archer 1997). Demographic bottleneck models take life stages explicitly into account and focus on disturbance and climatic variability limiting tree recruitment and growth (Higgins et al. 2000; Sankaran et al. 2004; Higgins et al. 2007; Beckage et al. 2009; Baudena et al. 2010), with resource availability determining growth rate while time between fires determines the time available to reach fire escape height into taller size classes (Higgins et al. 2000). Researchers proposing a third theory, patch dynamics, claim that this approach integrates many of the mechanisms suggested earlier (Wiegand et al. 2006, Moustakas et al. 2009, Meyer et al. 2009). The patch dynamics hypothesis proposes savanna landscapes to be composed of numerous patches, a few hectares in size, which are in different states of transition between grassy and woody biomass. This cyclical succession between open savanna and woody dominance may be driven by variability in rainfall and inter-tree competition (Wiegand et al. 2006).

The very different explanations for tree-grass coexistence in savannas are believed to be caused by the differences in the scale at which research is carried out. Whether empirical evidence is supportive or not for a coexistence hypothesis also depends on the system

investigated (Meyer et al. 2009). At present, most researchers acknowledge that multiple mechanisms are simultaneously in play, some more prominently than others depending on local conditions (Frost et al. 1986; Jeltsch et al. 2000). There may not be one unifying mechanism overriding all others; all described mechanisms are in all probability contributing to the maintenance of coexistence of trees and grasses at the landscape level. This legitimizes and requires further research on several scales, to show the relative importance of each contributing mechanism. The aspiration to understand these mechanisms is not merely academic, but has a practical side too. A thorough understanding of the processes responsible for the long-term coexistence of trees and grasses in savannas is important if the system –be it a conservation area or communal farming land- is to be kept in a desired state, through proper management. Woody plant increases in tropical grassy biomes have been widely reported over the last century, especially in southern African savannas (Archer et al. 1995; Wiegand et al. 2005; Kraaij & Ward 2006; Wigley et al. 2009). This so-called “bush encroachment” is defined as “the slow proliferation of woody plants [often thorny thicket-forming species] at the expense of grasses in savannas and grasslands” (Wigley et al. 2009). In conservation areas, bush encroachment negatively impacts biodiversity. In communal and commercial farming areas the undesired woody species cause the loss of grazing potential, resulting in severe economic losses throughout southern Africa (Ward 2005; Wigley et al. 2009). Changes in land use are thought to be major causes of woody invasion of grassy biomes, but how these changes undermine the unstable balance between trees and grasses is unclear. The replacement of indigenous herbivores by livestock, the exclusion of fire, and the introduction of artificial water points, may induce a disturbance of the balance between resource driven recruitment of woody species, and modifying agents preventing woody species domination (fire, herbivory). However, there is still a lack of consensus about the causes of bush encroachment (Ward 2005), highlighting the poor understanding we still have of the mechanisms driving savannas.

OUTLINE OF THESIS

The focus of this thesis is on two competition-based mechanisms explaining tree-grass coexistence: the two-layer hypothesis and the phenological niche separation hypothesis. Of the currently existing competition-based models, the most prominent and persistent is the two-layer hypothesis, first proposed by Walter (1971). In this competition-based model, grasses are assumed to be superior competitors for water in the surface soil layers, while trees are only able to persist because of access to deeper soil moisture. This equilibrium model does have some support (Walker & Noy-Meir 1982; Weltzin & McPherson 1997). However, the broad applicability of this model has been questioned, as savannas occur also in shallow soils where a vertical separation may not be possible (e.g. Sarmiento 1984). Several studies have shown a substantial overlap in the rooting niches of trees and grasses, suggesting that there may be competition for resources throughout the soil profile (Le Roux et al. 1995; Seghieri et al. 1995; Mordelet et al. 1997; Ludwig et al. 2004; February & Higgins 2010). I aim to test the hypothesis that tree and grass coexistence in savannas can, in part, be explained by the vertical partitioning of soil water. The questions I seek to answer are 1) to what extent do trees and grasses partition rooting niches? 2) To what extent do trees rely on soil water that is beyond the reach of grasses? 3) Does access to deep soil water enhance the performance of trees?

Chapter 2 is a description of the study site and the species used in the study. In Chapter 3, I describe a manipulative experiment in which shallow and deep roots of *T. sericea* were severed to test the dependence of this locally dominant tree on water in shallow and deep soil layers. “Deep” is in this experiment defined as “beyond the vertical reach of grasses”, and “Shallow” as “within the grass-roots layer”. If this species avoids competition by sourcing water from different (deeper) soil layers than grasses (as predicted by the two-layer hypothesis), then I expected to find severe water stress developing in trees with severed deep-growing roots.

As a result of difficulties with below-ground research, plant access to soil resources is a fundamental aspect of terrestrial life that remains poorly understood. Stable isotope techniques have the potential to provide better insights in the way plants access resources than can be achieved by e.g. root excavation or trenching techniques. In Chapter 4, the results of a depth-controlled isotope tracer experiment are reported. This experiment provides detailed measurements of the location, timing, and amount of water used by grasses and trees in low-nutrient, moderately deep, sandy soils.

A hypothesis that has received less attention is the phenological niche separation hypothesis (Scholes & Walker 1993, Sala et al. 1997, Scholes & Archer 1997, House et al. 2003). This hypothesis suggests that tree-grass coexistence is possible because of a temporal separation of leaf phenology. Phenological studies in savannas and tropical dry forests have shown that timing of phenophases may differ considerably between trees (Monasterio & Sarmiento 1976; Borchert et al. 1994; Shackleton 1999; Archibald & Scholes 2007). These and other studies have also noted that leaf display by trees is often not strictly bound to the start and end of the rainy season (Monasterio & Sarmiento 1976; Scholes & Walker 1993; Seghieri et al. 1995; Chapotin et al. 2006; Archibald & Scholes 2007) as is the case with savanna grasses (Prins 1988, Seghieri et al. 1995; Scanlon et al. 2005). By leafing out immediately after or even before the first rains, trees are able to monopolize resources and reach maximum production rates early in the growing season, thereby avoiding competition with grasses for resources. Trees may also retain leaves well into the dry season, when grasses have already senesced, thereby maximizing photosynthetic potential (Jolly & Running 2004). I will describe how different tree species adopt different (phenological) strategies to overcome the competition for water in a seasonally dry environment. I will subsequently investigate seasonal water use by trees, and suggest possible explanations for their strategies. In light of this hypothesis, the questions I ask are: 4) To what extent is plant available water a driver for leaf abscission or leaf flush? 5) What resource use strategies are used by the dominant tree species in the study

area? 6) How do these strategies contribute to avoiding competition for resources with grasses?

Variations in the timing of leaf out and leaf fall have often been observed in semi-arid and mesic savannas, not only between trees and grasses, but also between deciduous trees (e.g. Monasterio & Sarmiento 1976; Shackleton 1999). The ability to extend the growing season depends on the environmental drivers influencing leaf phenology. In Chapter 5 I demonstrate that two co-occurring tree species with very different leaf phenology patterns, cope with seasonal drought, intra- and inter-life form competition for water in different ways. I determine the extent to which plant available water is a driver for leaf abscission and leaf flush. I do this by monitoring the seasonal changes in plant water status for both tree species and determined their drought tolerance. Additionally, I relate leaf abscission to plant water status along a rainfall gradient.

The questions I ask in Chapter 6 are: 1) what do the observed water use patterns in the two study species tell about hydraulic constraints, 2) can the differences in phenology be explained in terms of differences in biomechanics, and 3) what are the implications for the strategies displayed by the study species?

Chapter 7 provides a general discussion and synthesis. In this synthesis chapter I review the different approaches used to answer the overall question of how trees and grasses manage to divide resources without outcompeting each other. I conclude this thesis by suggesting further work on specific questions relating to niche mechanisms in savannas.

CHAPTER 2

STUDY SITES AND SPECIES

University of Cape Town

CHAPTER 2:

Study area and species

STUDY AREA

I conducted the research I present in this thesis in the Kruger National Park (KNP), South Africa. The KNP is located within the savanna ecoregion (Rutherford 1997; Fig 2.1). Savannas in southern Africa occur within a wide biogeographic range. Annual rainfall varies from 235mm in the Kalahari, to over 1000mm in some moist savanna types in the east. Furthermore, the altitude varies from sea level to more than 1700m above sea level (Bredenkamp 2002). Covering an area of 412,545 km², the savanna occupies 32.54% of South Africa (Driver 2004). Savannas are well represented in the north-eastern provinces of South Africa, extending southwards in the lowland areas of Kwazulu-Natal and the Eastern Cape. The savannas of South Africa are very species rich, supporting more than 5700 plant species, 167 mammal species, 532 bird species, 61 reptile species, and 22 amphibian species (Bredenkamp 2002).

The savanna ecoregion is the core of the wildlife, ecotourism and meat-production industries. Savannas are vulnerable to human-induced disturbances. Outside protected areas, threats to the savanna ecosystem include rapidly expanding development of settlements and the associated need for firewood and building materials, diminishing water supply, agriculture and overgrazing (Bredenkamp 2002). Clearing land for alternative land uses may result in invasions of alien species, a problem affecting much of the world's savannas (Hutley & Setterfield 2008). Of the area covered by savannas in South Africa, only a few percent fall within official conservation areas, with most vegetation types within this biome not achieving the IUCN 10% target (Reyers et al. 2001). However, vast areas of savanna land are privately owned and used for game farming and big game hunting. Provided that these private land

owners employ judicial stocking rates, these areas can be considered as protected (Bredenkamp 2002).

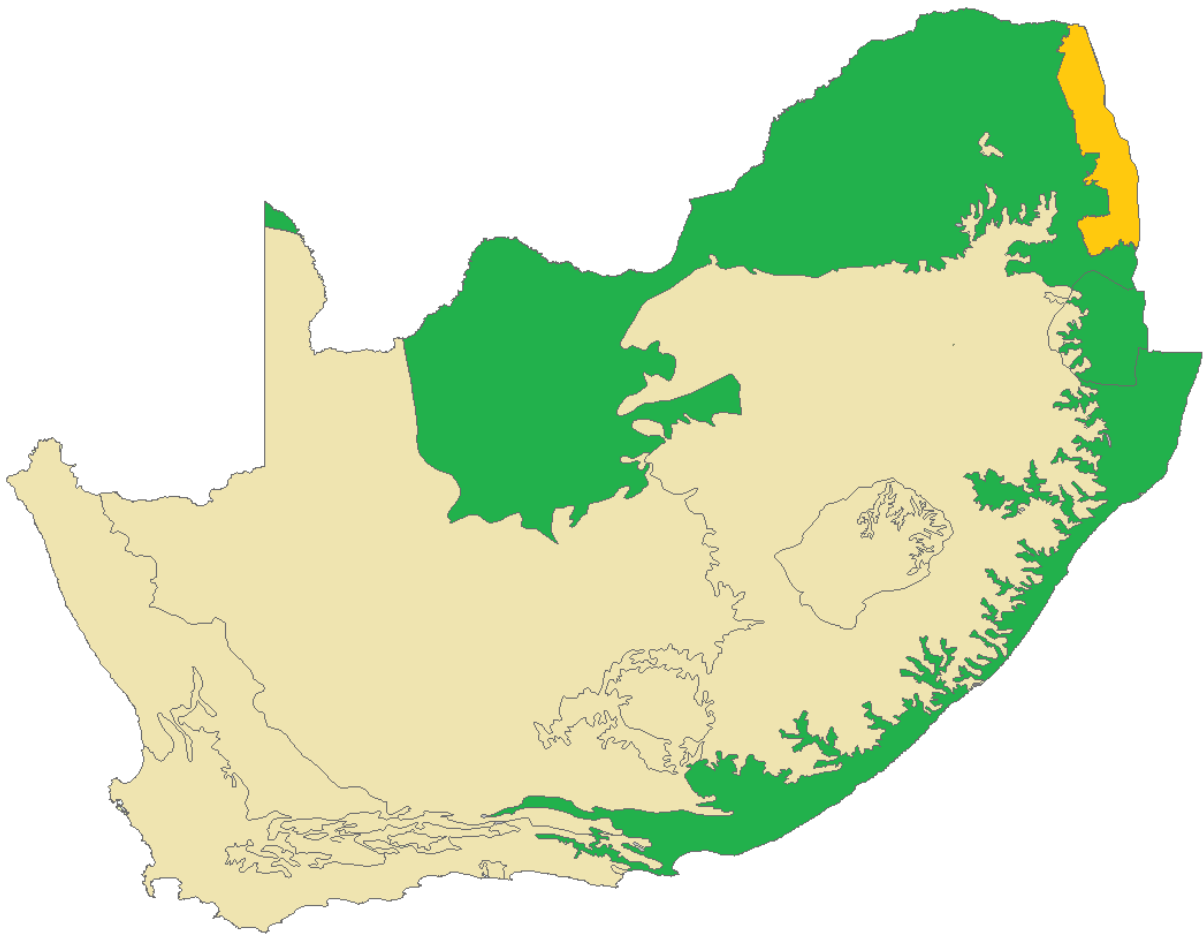


Fig 2.1: Biome map of South Africa, indicating the extent of the savanna ecoregion (green). The location of the Kruger National Park, which falls entirely within the savanna biome, is highlighted in orange. Adapted from Rutherford (1997).

The Kruger National Park is situated partly in Mpumalanga province and partly in Limpopo province; it borders Mozambique in the east and Zimbabwe in the north, while in the west it is bordered by high density communal areas and by private and provincial game reserves (Mabunda et al. 2003). The park encompasses a total area of approximately 20,000 km², and measures from north to south about 350km, with an average width of 60 km. The park forms part of the lowveld savanna, lying at an average altitude of 300 m above sea, varying from 900 m in the southwest to 200 m in the east (Codron et al. 2007). The savannas of the Kruger National Park can generally be divided into two types: those growing on nutrient-poor

substrates underlain by granites, and those growing on nutrient-rich substrates underlain by basalts (Fig 2.2a). Many ecological features of savannas can be predicted on the basis of this classification (Scholes & Walker 1993). For example, broad-leafed savannas occur on the highly weathered, nutrient-poor sandy soil substrates, whereas the fine-leafed, thorny savannas are restricted to recently-formed, nutrient-rich clay soils (Scholes & Walker 1993; Hutley & Settersfield 2008). Gertenbach (1983) identified 35 “landscapes” within the KNP, describing the heterogeneity in terms of geomorphology, climate, soil, vegetation and associated fauna. Most of the fieldwork for my research was carried out in the Gertenbach landscape no.1, called “Lowveld Sour Bushveld of Pretoriuskop”, which is situated in the south-western corner of the KNP in the vicinity of the Pretoriuskop tourist rest camp, along the Shabeni experimental burn plots. The undulating landscape has distinct uplands and bottomlands and is characterized by granite rocky outcrops, locally known as “koppies”. The altitude varies between 550-650 meters above sea level.

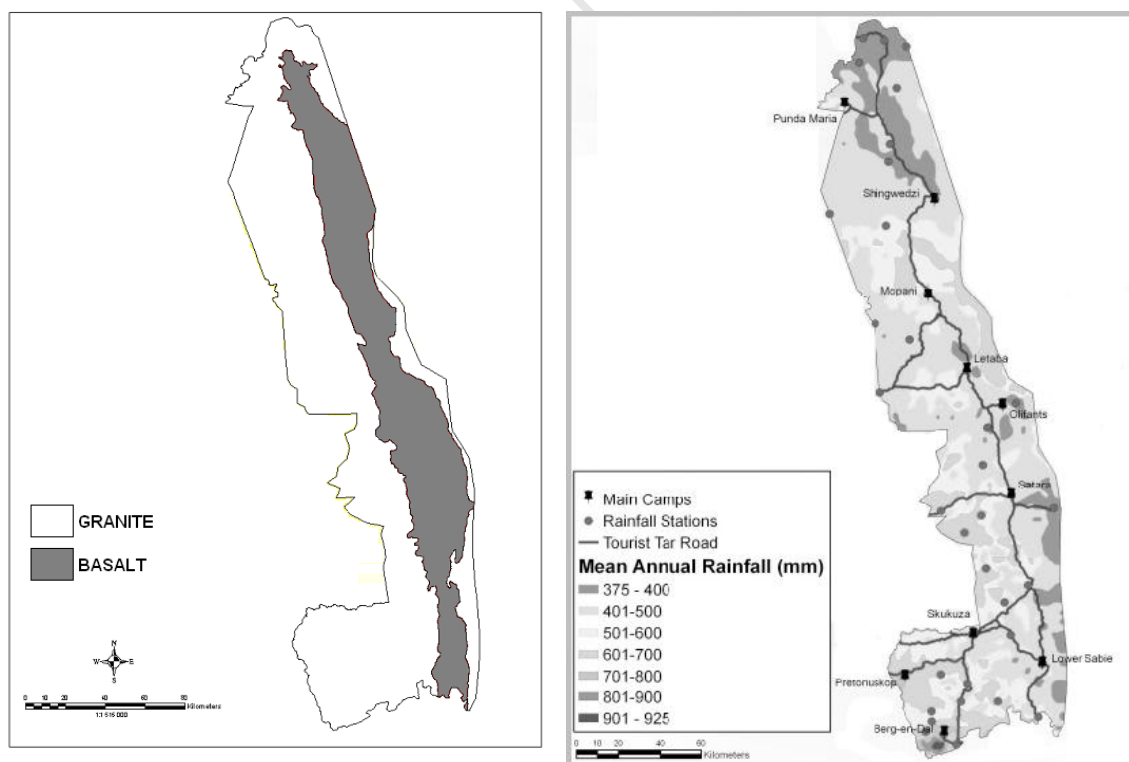


Fig 2.1: a) Simplified geological map of Kruger National Park and b) distance-interpolated mean annual rainfall for the KNP. Adapted from Scientific Services, KNP.

The vegetation structure around Pretoriuskop can be described as open tree savanna, with a woody component dominated by *Terminalia sericea* Burch. ex DC, *Dichrostachys cinerea* (L.) Wight et Arn and *Sclerocarya birrea* subsp. *caffra* (Sond.) J.O. Kokwaro. Dominant grasses in the area include the tall-growing species *Hyperthelia dissoluta* (Nees ex Steud.) Clayton, *Hyparrhenia hirta* (L.) Stapf and *Heteropogon contortus* (L.) P. Beauv. ex Roem. & Schult.

The climate of the KNP is subtropical with warm, wet summers and mild winters. Rain falls mainly during the austral summer, from October-April, the remainder of the year is dry. Precipitation decreases from south to north, with at Pretoriuskop an average of 730mm, and up north at Pafuri 400mm rain per annum (Fig 2.2b). The long-term average for the study area near Pretoriuskop and the measured rainfall of the study period are shown in Fig 2.3.

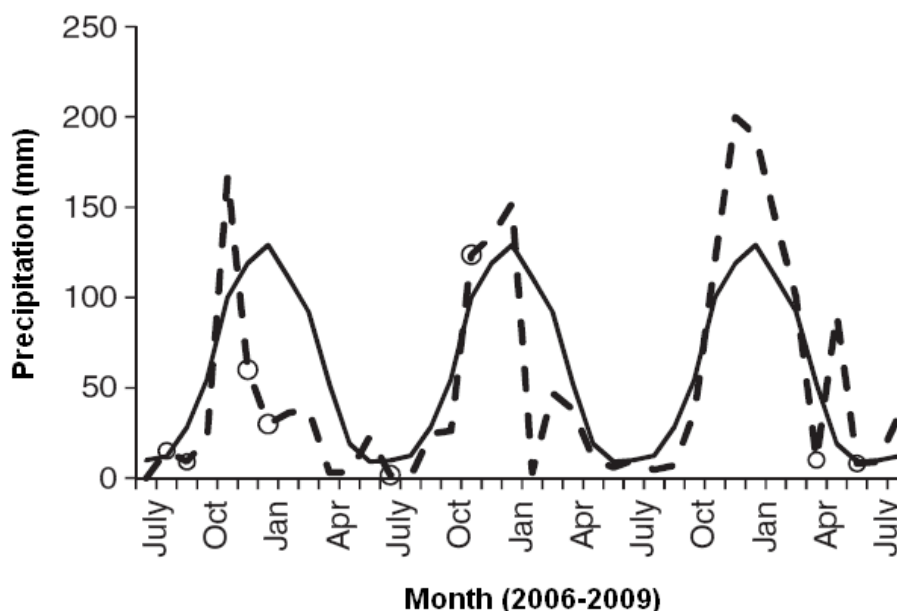


Fig 2.3: Mean (solid line, data from 1961-1990, Zambatis 2006) and observed (broken line) precipitation from July 2006 to July 2009, at the Pretoriuskop enclosure in the southern section of the KNP, where some of the fieldwork was done. Open circles indicate dates for which missing data were inferred from nearby meteorological stations.

STUDY SPECIES

I selected two dominant tree species in the study area as my main study species. Both tree species are widely distributed throughout the savannas of southern Africa (Fig 2.4). The study

species were chosen because of their dominance in the area and because they represent different ends of the 'deciduousness' spectrum, with very different leaf phenological patterns (see Chapter 5). *Terminalia sericea* Burch. ex DC (family: Combretaceae), is a medium-sized deciduous tree, common as a shrub or bush 6-9m tall, but individual trees may reach 23m in height (Coates Palgrave 2002). The rough, deeply fissured bark is dark grey or brownish often peeling, exposing a brownish under-bark. The blue-green leaves are clustered towards the tips of the branchlets and covered with silvery silky hairs; *T. sericea* is a good timber species and is used for charcoal and fuel wood. Some parts of the tree have medicinal properties (Orwa et al. 2009). It flowers in September and January and fruits in January to May, fruit remaining on the tree almost until the next flowering season. Pollination is usually by flies (Orwa et al. 2009). The leaves of *T. sericea* exhibit high concentrations of condensed tannins (Cooper & Owen Smith 1985), making it unpalatable for browsing ruminants. The species is associated with soils of low clay content and low calcium and nitrogen contents (Ben-Shahar 1991), and has a wide distribution throughout southern Africa (Fig 2.4a).

Sclerocarya birrea ssp. *caffra* (Sond.) J.O. Kokwaro, a member of the Anacardiaceae family, is a medium to large dioecious tree. Hereafter I will refer to this species as *Sclerocarya birrea*, or *S. birrea*, by which I solely mean the sub-species *caffra*. *S. birrea* is distributed throughout the eastern, low-altitude regions of southern Africa (Fig 2.4b). It reaches heights of usually 9-12 m, but trees up to 18 m have been recorded. The tree is single stemmed with a grey bark (which is used for medicinal purposes), a dense, spreading crown and deciduous foliage. Young twigs are thick with spirally arranged composite leaves containing 2-23 leaflets at their ends (Hall et al. 2002). In South Africa flowering occurs from September to November, and fruiting from January to March. The fruit is abscised when ripening commences so that final ripening takes place on the ground. It is rich in vitamin C, and eaten by animals and humans (Wynberg et al. 2002).

The population structure of *S. birrea* in protected areas such as the Kruger National Park has been shown to lack immature trees and is characterized by unsuccessful regeneration and recruitment (Hall et al. 2002). In protected areas, the most serious threat to the establishment

and survival of *Sclerocarya birrea* regeneration are intense fires (Jacobs & Biggs 2002), making the timely implementation of appropriate fire management practices an essential conservation tool (Hall et al. 2002).

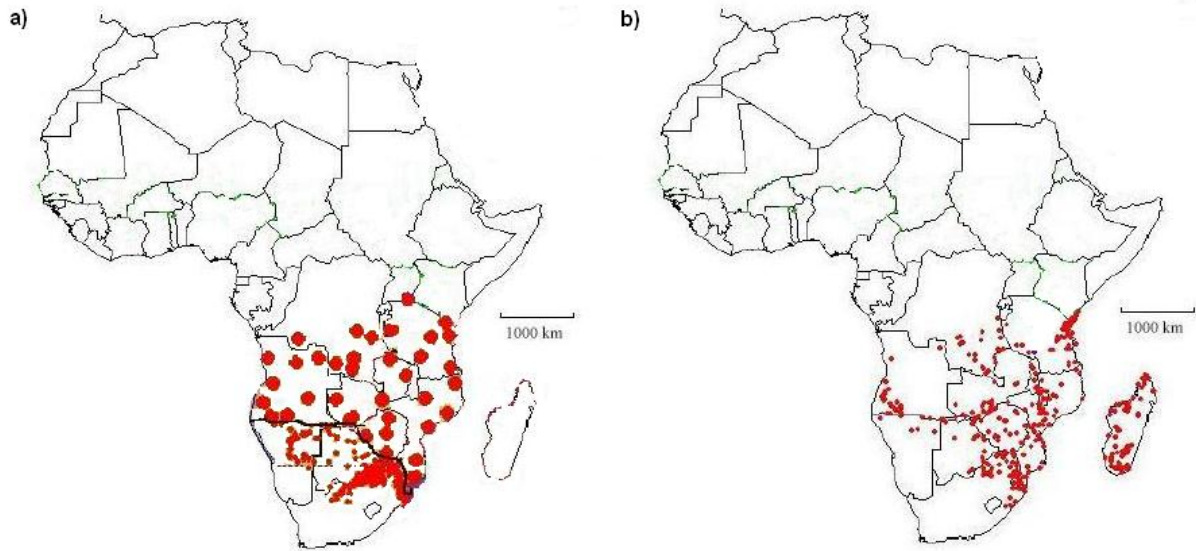


Fig 2.4: Distribution maps for *T. sericea* (a) and *S. birrea* (b). The distribution map for *T. sericea* is composed of field records (small dots) and locality information from herbarium specimens. The distribution map for *S. birrea* is adapted from Hall et al. (2002).



Fig. 2.5: Physiognomy of the broad-leafed savanna at the study site at Pretoriuskop.

CHAPTER 3

WATER SOURCING BY TREES IN A MESIC SAVANNA: RESPONSES TO SEVERING DEEP AND SHALLOW ROOTS

University of Cape Town

CHAPTER 3:

Water sourcing by trees in a mesic savanna: responses to severing deep and shallow roots

Introduction

One of the world's most extensive biomes, the savanna, is generally defined as a discontinuous layer of trees overlying a continuous layer of grasses, and is characterized by a distinctly seasonal rainfall pattern (Frost et al. 1986). The mechanisms enabling the coexistence of trees and grasses in tropical and subtropical savannas have been comprehensively researched (for reviews see: Scholes and Archer, 1997; Sankaran et al. 2004; Midgley et al. 2010). The results of this research has led to a general agreement among savanna ecologists that resource availability, fire frequency and intensity, and herbivory influence savanna structure in complex ways. However, the factors preventing competitive exclusion of any of these two life-forms are not well understood and the relative importance of the different processes shaping the structure of savannas is under continuing debate (Higgins et al. 2000; Van Langevelde et al. 2003; Sankaran et al. 2008; Coetsee et al. 2010; Higgins et al. 2010; Midgley et al. 2010).

Rainfall is regarded as a key determinant of potential woody cover in savanna systems. In a continental scale analysis, Sankaran et al. (2005) showed that rainfall and maximum woody biomass are positively correlated for systems receiving between 100-650 mm of rainfall per year (Sankaran et al. 2005; Sankaran et al. 2008). Above this threshold, rainfall is presumed to be sufficient for woody biomass to increase to a state of complete canopy closure, but disturbances such as fire and herbivory prevent this from happening (Bond et al. 2003; Sankaran et al. 2005).

A number of studies have suggested that trees in savannas with their deeper roots have access to deep soil water that is unavailable to grasses (Walter 1971). In this competition-

based model, Walter (1971) assumed grasses to be superior competitors for water in the surface soil layers, with trees predicted to persist only where enough water percolates beyond the reach of grasses. This model, referred to as the two-layer hypothesis, has empirical support (Walker and Noy-Meir, 1982; Weltzin & McPherson, 1997), but several studies have shown a substantial overlap in root niches between trees and grasses (Le Roux et al. 1995; Seghieri et al. 1995; Mordelet et al. 1997; Ludwig et al. 2004; February & Higgins 2010; Kulmatiski et al. 2010). Despite all this research, the extent to which trees and grasses partition resources is still unclear. Studies of soil resource competition in savannas have relied on inference from rooting patterns and/or from stable isotopes. Root distribution may not indicate actual water uptake, whereas stable isotope tracer studies merely provide a snapshot of water uptake patterns.

In this study, I further explore below ground resource partitioning between trees and grasses in mesic savannas. To examine the extent to which trees rely on water uptake from soil layers that are either within or beyond the vertical reach of grasses I conducted a manipulative experiment in which I simply restricted tree roots to the grass-roots layer. Root trenching is commonly used to study the effect of water competition between trees and understory crops on crop yield in agroforestry systems (Powell & Bork, 2006; Burner et al. 2009). In natural systems, Ludwig et al. (2004) found increased grass production after tree root trenching in a savanna, while Barberis & Tanner (2005) showed increased survival and growth in tree seedlings in response to trenching in a tropical forest. Although the effects of root severing on the water relations of mesquite trees have been examined (Ansley et al. 1990; Ansley et al. 1991) there has been no research testing the two-layer hypothesis using manipulation of tree water access in shallow and deep soil compartments through the severing of tree roots. In this study I manipulate the vertical and horizontal access of trees to the rooting zone of grasses, by severing the taproot and the lateral roots using a novel trenching technique which minimizes soil disturbance. I then monitored a range of water stress indicators, including water status and leaf-level responses, throughout the 2006-2007 growing season. If, as is predicted by the two layer hypothesis, trees are able to avoid

competition with grasses for resources through deep rooting, then trees should respond more strongly to taproot severing than to lateral root severing.

Materials and Methods

STUDY SITE

The study was conducted at a site along the Shabeni experimental burn plots near Pretoriuskop (25°07'S; 31°13'E) in the southern section of the Kruger National Park, South Africa. The vegetation at the study site is a broad leaved woodland classified as Pretoriuskop Sour Bushveld dominated by *Terminalia sericea*, *Sclerocarya birrea* and *Dichrostachys cinerea* with a dense grass layer dominated by tall grass species such as *Hyperthelia dissoluta* and *Setaria sphacelata*. The climate of the region is characterized by hot, wet summers and mild, dry winters; the growing season lasts from October to April. Average daily maximum and minimum temperatures are 30.6°C (January) and 9.3°C (June), respectively, with an average annual rainfall of 736mm (Zambatis, 2006). Elevation is ca. 600m. The granite derived nutrient poor soils are moderately deep (between 1m and 1.5 m), well drained and coarse grained (Barton et al. 1986). On average, the soils contain 0.87% carbon and 0.05 % nitrogen with an average of 200 mg kg⁻¹ total phosphorus (Feig 2004).

The experiment was initiated in September 2006, 6 weeks before the first rains of the new growing season. From mid-January until the end of March 2007, normally the peak of the growing season, there was very little precipitation (Fig. 3.1). The rains returned in April, but no rain fell in May. A weather station (MC Systems (Pty) Ltd, Cape Town, South Africa) at the study site recorded temperature and precipitation. Volumetric water content of the soil was recorded through a nest of three soil moisture sensors (ECH₂O-20, Decagon Devices, Pullman, WA, USA), which were placed at 10cm, 30cm and 50cm depth below the soil surface. All experimental trees were less than 50m from the soil moisture probes.

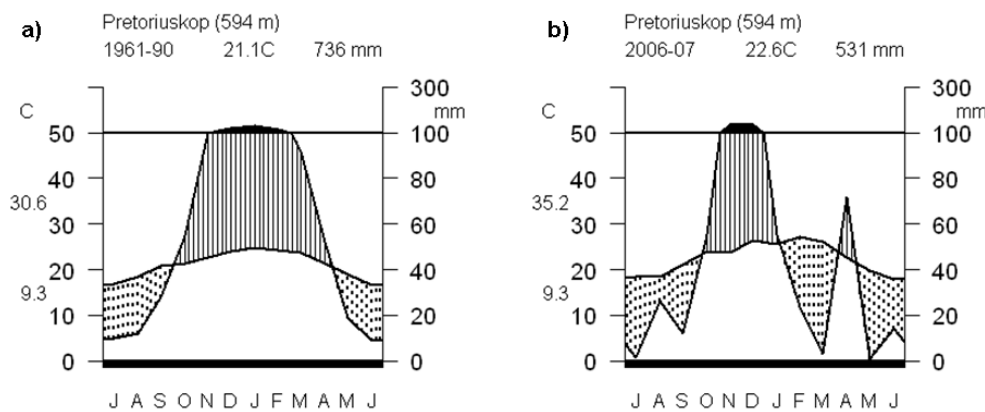


Fig. 3.1: Walter & Lieth climate diagrams for Pretoriuskop, for long-term averages (a) and for the year of the study (b). Long-term data was adapted from Zambatis (2006), data from the year of study was collected with a weather station at the study site.

ROOT SEVERING

Terminalia sericea Burch. ex DC., a locally dominant savanna tree species of medium stature (4-10m) with a wide distribution range (Coates Palgrave 2002), was selected as the study species. I randomly assigned three treatments to 18 single-stemmed saplings (average height: 3.4 m (± 0.46), average DBH: 6.4 cm (± 0.93)). These treatments were named “Control”, “Deep” and “Shallow”. The trees in the “Control” treatment had unrestricted access to all soil layers, no severing was performed. The root bole of the trees in the “Deep” treatment was partially excavated prior to severing taproots growing beyond a depth of 50cm using a hammer and chisel. This treatment restricted tree roots to soil horizons with the highest percentages ($\pm 90\%$) of grass roots (February & Higgins 2010). For the “Shallow” treatment, all lateral roots were severed to a depth of 50cm, 1m away from the bole of the tree using a custom designed root-cutting tool (Fritz-snipper). This device is a sharpened mild-steel blade (300 mm wide, 600 mm long and 6 mm thick) attached to a solid metal rod. The blade is driven into the soil by pounding on the metal rod. This method is novel in the sense that it has the advantage of minimizing soil disturbance, as opposed to traditional

trenching techniques which are much more intrusive. The distance of 1 meter was chosen as I aimed at reducing a significant amount of lateral root volume without undermining the trees' anchorage by severing too close to the bole. Based on findings by Hipondoka & Versfeld (2006) in a dry savanna, as well as excavations at Pretoriuskop, I concluded that the lateral roots of *T. sericea* have a potential extent of several meters (average length of main lateral root reported in Hipondoka & Versfeld (2006) was 4.31m). An assumption I made is that roots growing laterally away from the tree remain in the top 50cm of the soil along their entire length. Roots were severed on the 7th and 8th of September 2006, 6 weeks before the first rains of the new growing season.

CANOPY FULLNESS

I predicted that root severing would induce water stress which would, in turn, lead to a reduction in leaf area. Canopy fullness (% of crown area occupied by leaves) was used as a proxy for leaf area, and was visually estimated by two independent observers. The average of the two estimates was used in the analysis. Estimates ranged from 0% (completely leafless crown) to 100% (a full crown with fully matured, green leaves). Canopy fullness was estimated every two weeks from the first week of September to the end of November 2006, after which estimates were done monthly until the end of May 2007.

LEAF SIZE AND SPECIFIC LEAF AREA

To examine whether changes in resource availability induced changes at the leaf level I determined leaf size and specific leaf area (SLA). Leaf size and SLA influence the energy budget and the gas exchange of a leaf, and are therefore expected to be related to water and nutrient availability (Jones 1983; Cornelissen et al. 2003). I define leaf size as the one-sided projected surface area of a fresh leaf, including the petiole. SLA is defined as the one-sided area of a fresh leaf, divided by its oven-dry mass (Cornelissen et al. 2003). Three months

after root severing, leaf size and SLA were determined for 60 fully matured, outer canopy leaves of each of the 18 individual trees. Fresh leaves were sampled in plastic zip-lock bags and stored in a cool box. Leaf size was measured on the day of sampling using a Li-3100 leaf area meter (Li-cor, Lincoln, Nebraska, USA). After leaf size was measured, samples were dried in an oven for 72 hours at 70°C before weighing to determine SLA.

PERCENTAGE NITROGEN AND STABLE CARBON ISOTOPE RATIOS

I anticipated that root severing would increase resource stress. The level of water stress under which plants fix carbon is reflected in the stable carbon isotope ratio of the products of carbon fixation (Farquhar & Richards 1984; Martin & Thorstenson 1988). A broad relationship exists between carbon isotope discrimination of plant dry matter and the efficiency with which this dry matter is formed relative to amounts of water which the plant transpires (Dawson & Ehleringer 1993). For carbon, the $^{13}\text{C}:^{12}\text{C}$ ratio is approximately 1.1 percent. Natural carbon isotope composition is usually expressed as deviation in per mil (‰) from the isotope composition of a particular standard, as natural variations in this ratio span only a few percent of this 1.1 percent. These deviations are symbolized by $\delta^{13}\text{C}$, and defined as:

$$\delta^{13}\text{C} = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] * 1000, \text{‰}$$

where $R = ^{13}\text{C}:^{12}\text{C}$ (Pate & Dawson 1999). When plant available moisture is high, the stomatal aperture is large. Under these conditions, the leaf internal (C_i) CO_2 concentration is high relative to concentrations of CO_2 outside the leaf (C_a) and the heavier carbon isotope (^{13}C) is strongly discriminated against by Rubisco (the primary carbon fixing enzyme in C_3 plants). When plant available moisture is low, the stomatal aperture is smaller, which leads to reductions in C_i/C_a ratio, which in turn reduces the level of discrimination against ^{13}C . As stomatal aperture regulates the plant's C_i/C_a ratio, it follows that the ratio of $^{13}\text{C}/^{12}\text{C}$ of plant material, typically expressed as $\delta^{13}\text{C}$, reflects the plant water use efficiency (WUE) averaged

over the growth period (Farquhar & Richards, 1984; Martin & Thorstenson, 1988). For C_3 plants, $\delta^{13}C$ values range from -20 to -34‰ (Pate & Dawson 1999).

I determined plant $\delta^{13}C$ values and percentage total N for the same leaf material used to determine SLA. This was done using a mass spectrometer: an instrument which separates charged atoms or molecules based on their mass-to-charge ratios and motions in the presence of a strong magnetic field (Dawson & Brooks 2001). A stable Isotope Ratio Mass Spectrometer (IRMS) uses a magnet to bend a beam of ionized particles towards a series of Faraday cups which convert particle impacts to electric current. Samples were combusted in a Flash EA 1112 series elemental analyzer (Thermo Electron Corporation, Milan, Italy). The gases were passed to a Delta Plus XP IRMS (Thermo Electron, Germany), via a Conflo III gas control unit (Thermo Finnigan, Germany). In-house standards were calibrated against an IAEA (International Atomic Energy Agency) standard. For carbon this was Pee-Dee Belemnite, which is CO_2 obtained from the carbonate shell of a Cretaceous mollusc, *Belemnitella americana*, from the Pee Dee Formation in South Carolina, USA (Pate & Dawson 1999). Nitrogen is expressed in terms of its value relative to atmospheric nitrogen. Precision of duplicate analysis is 0.1‰ for carbon and 0.2‰ for nitrogen.

XYLEM PRESSURE POTENTIAL

Where $\delta^{13}C$ values provide an integrated index of plant water stress, xylem pressure potentials (XPP) provide an instantaneous estimate (Scholander et al. 1965). Plant moisture stress was estimated using a Scholander-type pressure chamber (PMS Instrument Company, Albany, OR, USA). The pressure chamber is an extensively used technique to determine plant water status (Tyree 1997, Cochard et al. 2001, Hempson et al. 2007). A leaf is placed inside a pressure chamber with the petiole protruding through a rubber seal to the outside air (Fig 3.2). When compressed gas is forced into the pressure chamber, the gas

pressure is presumed to be transmitted directly to the xylem fluid raising the pressure in the xylem (i.e. it becomes less negative). When XPP is increased to a value slightly above atmospheric pressure, i.e. $XPP > 0$, then water begins to flow out of the vessels at the cut end of the petiole. The gas pressure needed to force out the liquid is equivalent to the negative pressure the water column was under, prior to admitting compressed gas to the chamber. Summarizing, the less water is available to the plant, the higher the plant moisture stress, the more negative the pressure potential of the water column. Typically, researchers determine XPP before dawn, when no transpiration takes place and water potential (Ψ) of the plant is in equilibrium with the Ψ of the soil, and/or at noon, when vapour pressure deficit (VPD) is at its presumed maximum and therefore the XPP is most negative. Here, both pre-dawn and mid-day XPP's were used as instantaneous indicators of plant moisture stress. XPP's were estimated once before and on seven occasions after root severing. For the XPP measurements, one twig containing at least one green leaf was sampled for all six trees in each of the three treatments. XPP of each tree was determined within one minute after sampling.

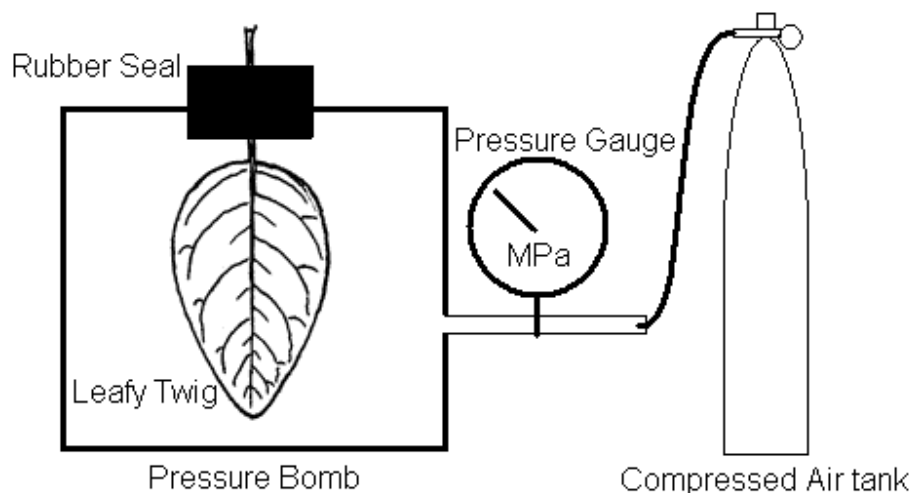


Fig 3.2: Schematic representation of the Scholander Pressure Chamber.

XYLEM VULNERABILITY TO CAVITATION

A vulnerability curve is a plot of the percentage loss of conductivity and the pressure that induced that loss of conductivity (Tyree et al. 1994). To translate the XPP's of the plant after root severing into loss of hydraulic conductivity I constructed xylem vulnerability curves. Vulnerability curves were constructed for each of five randomly chosen *T. sericea* saplings using an adjusted version of the air injection method of Sperry & Saliendra (1994; Fig 3.3). These saplings were not the same ones used for the root severing experiment, but they were sampled at the same site. Stem segments with an inside bark diameter of approximately 5mm were excised from these saplings in the field on the 6th of June, 2008, then wrapped in plastic and stored in a cool box until analysis. Starting the vulnerability measurements in the laboratory that same day, it took a total of three days to complete the vulnerability curves for all sampled saplings. The segments were recut under water to a length of 18-21cm. The segments were then de-barked and notched with a razor blade by making two small overlapping incisions about 50mm apart and 0.5mm deep around the centre of the segment. This was done to allow air to enter the vascular system, which minimizes the time required to embolize the xylem at a given air pressure (Sperry & Saliendra 1994). The segments were then flushed under a pressure of 0.15 MPa for an hour using degassed, de-ionized water, to remove any pre-existing embolisms. After flushing, the segments were placed in a double-ended pressure chamber (PMS Instrument Company Albany, OR, U.S.A.), with both ends of the segment protruding. The upstream end of the segment was connected to a slightly elevated water reservoir, adjusted in height to apply pressure high enough to allow flow through the stem segment, but low enough to prevent re-filling of cavitared vessels (typically 1-5 kPa). After first equilibrating the system for at least 3 minutes, conductivity was measured by collecting the flow over 1-minute intervals at the open, downstream end of the segment in pre-weighed vials filled with absorbent paper. The average flow over 8 intervals was taken as a relative measure of conductance. While measuring conductivity, the pressure in the chamber was kept at 0.1MPa to avoid xylem leakage inside the chamber. By forcing air into

the vascular system with increasing pressures in steps of 0.5-1.0 MPa, the conductivity through a stem segment was progressively reduced. After every sequence of conductivity measurements, the pressure was increased by 0.5-1.0 MPa steps. The pressure was maintained for 15 minutes before conductivity was measured as described above. I expressed the reduction in conductivity as a percentage of the initial (before pressurizing), maximum conductivity value. This procedure was repeated until the cumulative loss of conductivity was more than 95%. The vulnerability curve is the average cumulative percent loss of conductivity plotted against applied pressure.

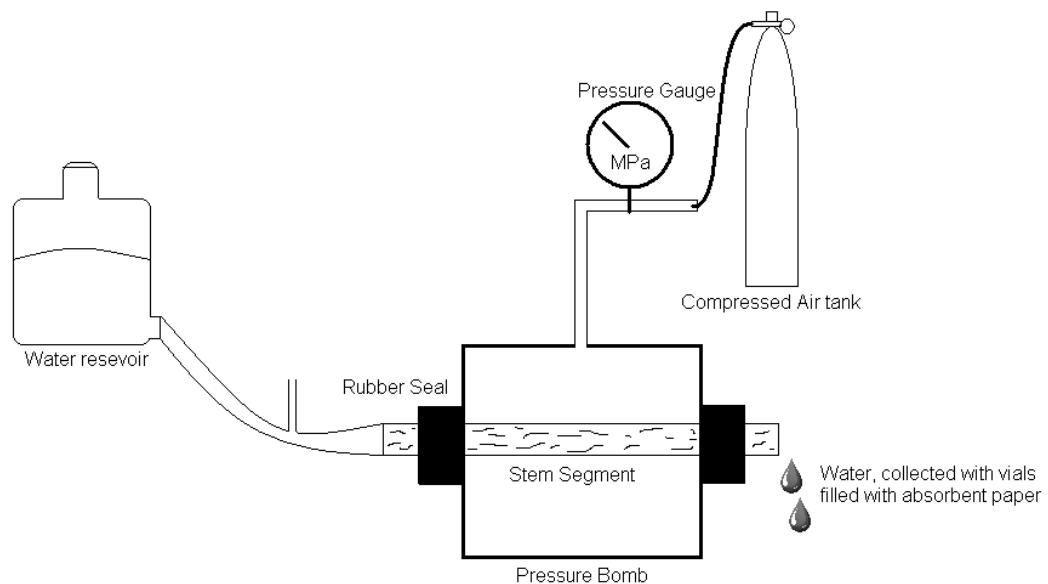


Fig 3.3: Schematic depiction of the adjusted Sperry apparatus in the conductivity measuring arrangement. When in the pressurization arrangement, the stem segment is detached from the water reservoir and the pressure chamber is submerged under water. The flushing section for removing pre-existing embolisms is not shown.

Statistical analyses

Leaf size, specific leaf area, %N and $\delta^{13}\text{C}$ values were analyzed using a One-Way ANOVA with a Posthoc Tukey-HSD test. The canopy fullness data and the xylem pressure potential data were analyzed using linear mixed-effect models (LME). Even though the observers did not estimate canopy fullness to the nearest 1% these estimates were treated as continuous. The analysis is a repeated measures analysis. Following guidelines provided by Crawley (2002, p. 685 onwards) I define the repeated nature of the analysis by including "tree" (the individual tree) as a random variable. Time is included as an ordered fixed effect. That is, it is not included as a continuous factor but as an ordered factor (an ordered factor preserves the fact that time 2 > time 1, etc, in the analysis). Model selection was based on AIC (Akaike, 1974). The vulnerability curve for *T. sericea* was fitted with a Weibull function as advocated by Neufeld et al. (1992). R version 2.7.0 (R Development Core Team, 2008) was used for the statistical analyses.

Results

Mean canopy fullness was 43.3% (± 12.4) before the start of the experiment; an analysis of variance identified no discernible differences between the individuals assigned to the different treatments (ANOVA, $F_{2,15}=0.0974$, $p=0.9078$). Three days after root severing, the leaves of all trees in the "Shallow" group started to abscise, resulting in bare trees one week after severing. The trees in the "Control" group and in the "Deep" group gradually discarded their leaves in the period leading up to the first week of October. Leaf-up started in all trees of all treatment groups immediately before and up to 1 week after the first considerable rainfall event (26 mm on 20 October 2006). By the end of November, canopy cover was less than 70% in 3 out of 6 individuals from the "Shallow" group, and in 1 out of 6 from the "Deep" group, whereas all trees in the "Control" group had full canopy cover (80-90%). During the drought from mid-January until late March 2007, 4 of the 6 trees in the "Shallow" treatment

group gradually discarded their leaves and replaced them with new leaves only after the rains returned again on the last day of March.

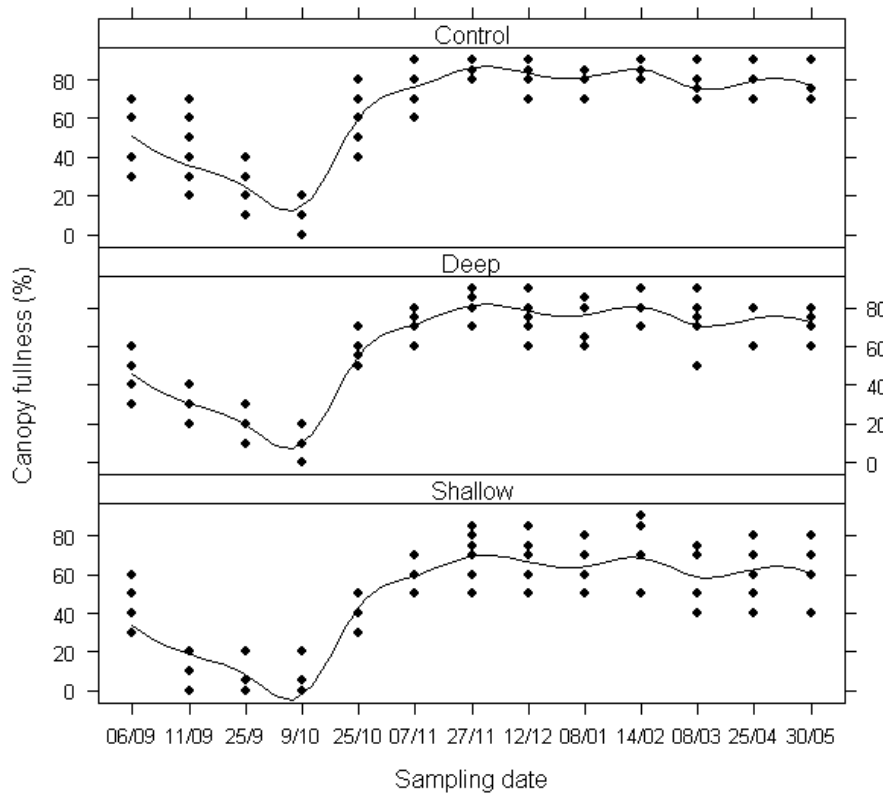


Fig. 3.4: Canopy fullness (% green leaves) during the experiment for each of the three treatments. Polynomials represent fitted linear mixed-effect models. Treatments were applied on 7 and 8 September, i.e. between the first two sampling dates.

Leaf size was significantly different between all groups (ANOVA, $F_{2,33}=13.040$, $p<0.001$), with leaves in the “Shallow” group being smallest and leaves in the “Control” groups largest (Fig. 3.5a). Specific leaf area (SLA) was significantly lower in the “Shallow” group (Fig. 3.5b; ANOVA, $F_{2,33}=5.5426$, $p=0.0084$). The percentage leaf nitrogen (%N) was higher in the shallow group, but not significantly (Fig 3.5c; ANOVA, $F_{2,15}=1.8874$, $p=0.1857$). No significant differences in $\delta^{13}\text{C}$ were detected between treatments (Fig. 3.5d; ANOVA, $F_{2,15}=1.5068$, $p=0.2533$). Pearson correlation coefficients for the variables predawn XPP, mid-day XPP, leaf size, SLA, $\delta^{13}\text{C}$ and %N are shown in Table 3.1. The XPP values from the 8th of March 2007 were included in the analyses; at this sampling date the trees experienced the most severe water stress as a result of a mid-season drought. All correlations with XPP were

significant, indicating that root severing did indeed have physiological effects at the leaf-level.

None of the other relationships were significant, except the one between $\delta^{13}\text{C}$ and %N.

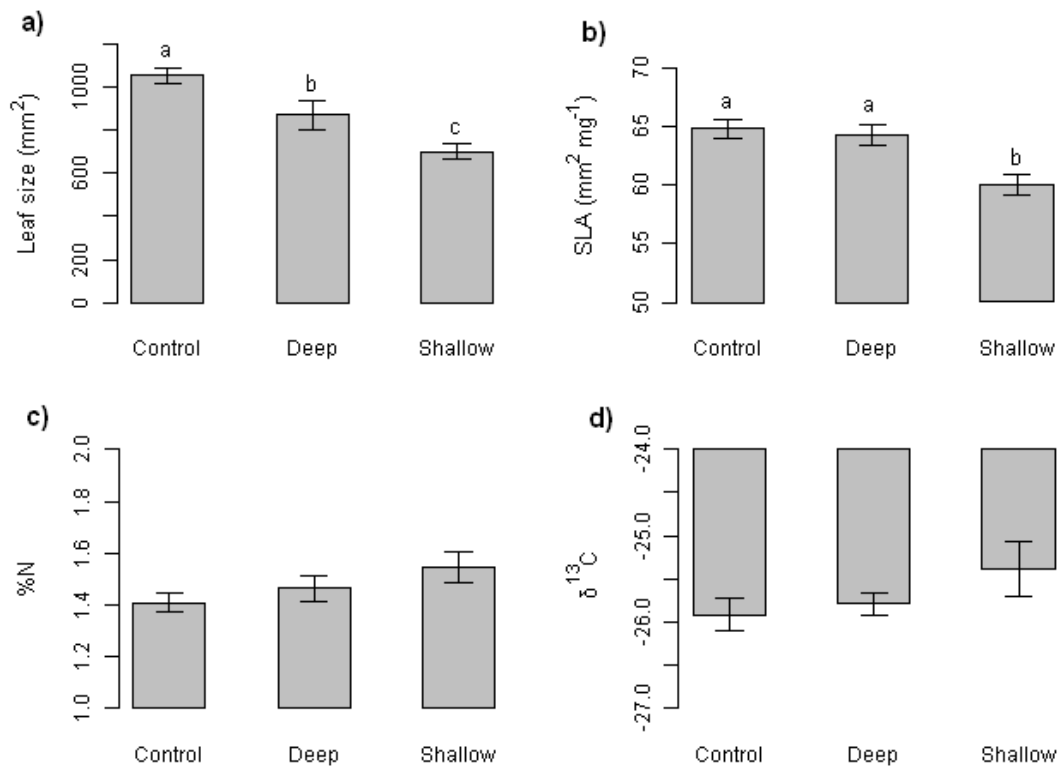


Fig. 3.5: Leaf traits for each treatment, three months after root severing, two months after leaf flush. a) Leaf size, b) Specific leaf area (SLA), c) %Nitrogen, d) $\delta^{13}\text{C}$. Different letters denote significant differences, error bars represent standard errors.

Table 3.1: Pearson correlation coefficients for predawn (XPPp) and midday (XPPm) xylem pressure potentials on 8 March 2007, and the leaf-level traits leaf size, SLA, $\delta^{13}\text{C}$ and %N. ***, **, and * denote statistical significance at the 0.001, 0.01 and 0.05 levels, respectively.

XPPp					
0.98	***	XPPm			
0.63	**	0.64	**	LeafSize	
0.58	*	0.60	**	0.29	SLA
-0.70	**	-0.69	**	-0.27	-0.27 $\delta^{13}\text{C}$
-0.74	***	-0.73	***	-0.44	-0.28 0.77 *** %N

Soil moisture content for the sampling period is shown in Fig. 3.6a. Predawn xylem pressure potentials are plotted against time in Fig. 3.6b, with sampling date defined as an ordered factor. No significant differences in XPP were found at the first time step (ANOVA, $F_{2,15} = 0.4935$, $p=0.62$), indicating that before the root severing, trees in all groups experienced similar levels of water stress. To examine the effects of soil moisture on XPP, I fitted a linear mixed-effect model (LME, see Table 3.2) with “treatment” and “time” as fixed effects and “tree” as a random effect. Soil moisture data was collected at three depths (10cm, 30cm and 50cm). Model selection was based on AIC (Akaike, 1974). AIC estimates relative goodness of fit for a given set of statistical models; the preferred model is the one with the lowest AIC value. This revealed that the most parsimonious model considers only soil moisture at 10cm. Although soil moisture at 10 cm was selected and used in the model, this is to be interpreted as a proxy of water available to the plant and does not imply that water content at other depths is not important. This LME-model showed that soil moisture content ($F_{1,87} = 303.170$, $p<0.001$) and treatment ($F_{2,15} = 4.959$, $p=0.022$) had significant effects on predawn XPP. Similar effects (soil moisture: $F_{1,87} = 260.349$, $p<0.001$, treatment: $F_{2,15} = 4.075$, $p=0.039$) were shown for midday XPP. The fitted models of the “Shallow” and “Control” treatments deviate increasingly as soil moisture content decreases, whereas the difference between the “Deep” and “Control” treatments is marginal (Figs 3.6c and 3.6d).

Table 3.2: Analysis of Variance of the Linear Mixed-Effect Models (LME).

<i>Variable</i>	<i>Effect</i>	<i>numDF</i>	<i>denDF</i>	<i>F-value</i>	<i>Sig. level</i>
Canopy Fullness	Treatment	2	15	10.156	**
	Time	12	204	127.246	***
XPPp (time)	Treatment	2	15	7.767	**
	Time	6	102	264.819	***
XPPm (time)	Treatment	2	15	6.696	**
	Time	6	102	127.412	***
XPPp (soil moisture)	Treatment	2	15	4.959	*
	Soil moisture	1	87	303.170	***
XPPm (soil moisture)	Treatment	2	15	4.752	*
	Soil moisture	1	87	260.349	***

Significance levels: *** $P<0.001$, ** $P<0.01$, * $P<0.05$.

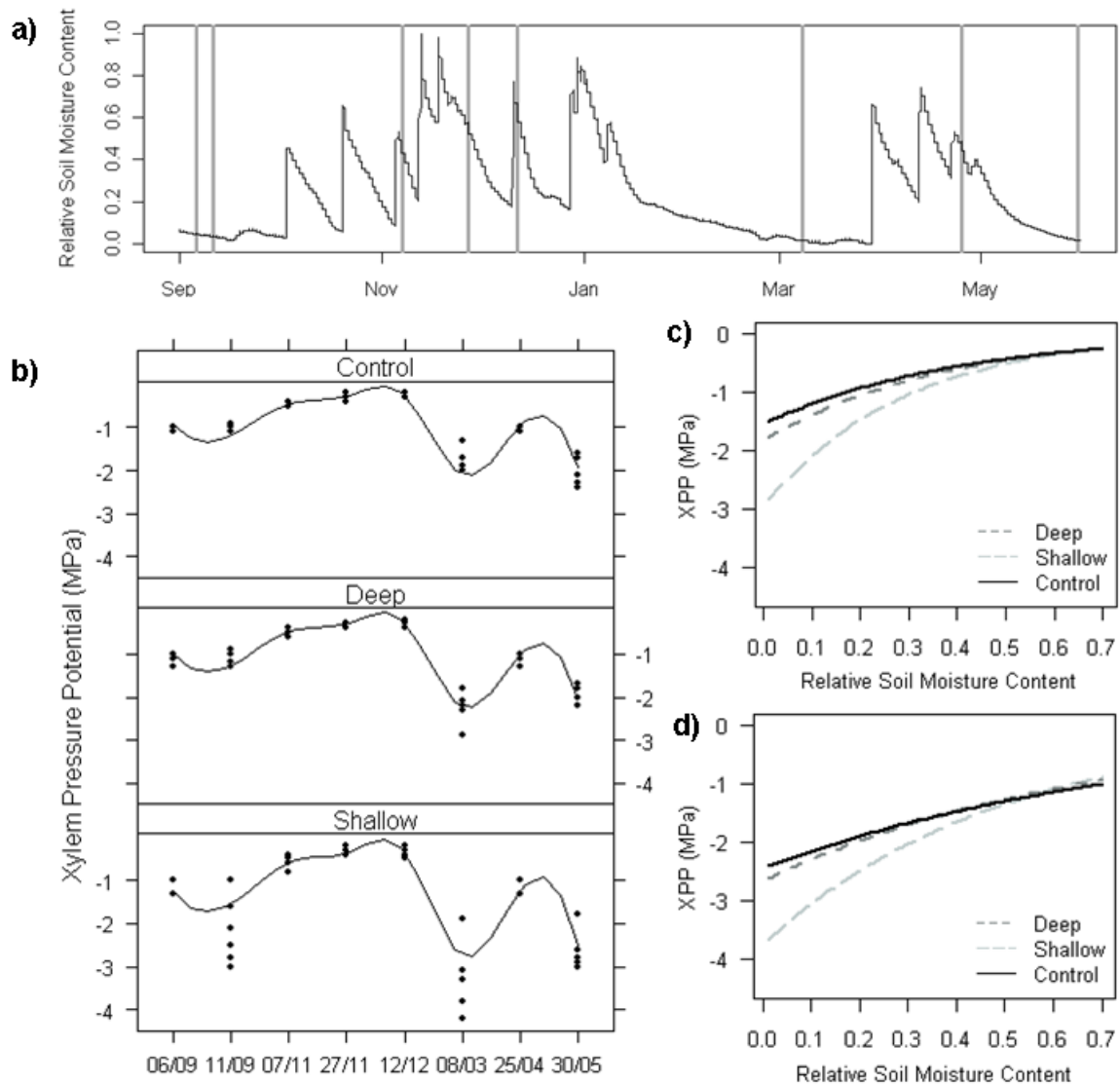


Fig. 3.6: a) Soil moisture content at 10cm depth over the 2006-2007 growing season. Volumetric water content was rescaled from 0 (driest value in the dataset) to 1 (wettest value in the dataset). Vertical lines are placed at sampling dates of the XPP measurements. b) Predawn xylem pressure potentials of trees in each of the treatments. Polynomials represent fitted linear mixed-effect models. c),d) Linear mixed-effect models of xylem pressure potentials as a function of soil moisture at 10cm depth for pre-dawn (c) and mid-day (d).

Fitting the Weibull function (following Neufeld et al. 1992) to the vulnerability data estimated using the air injection method reveals that a 50% loss of conductivity (P_{50}) occurs at -2.0MPa (Fig. 3.7a). Using the vulnerability curve to translate the measured XPP's into loss of conductivity reveals that trees in the "Shallow" group lost up to 70% conductivity (predawn) at the driest times (Figs 3.6b), whereas the control trees lost only 40%. For midday, the values are 80% (Shallow) and 60% (Control), respectively (Fig 3.7c).

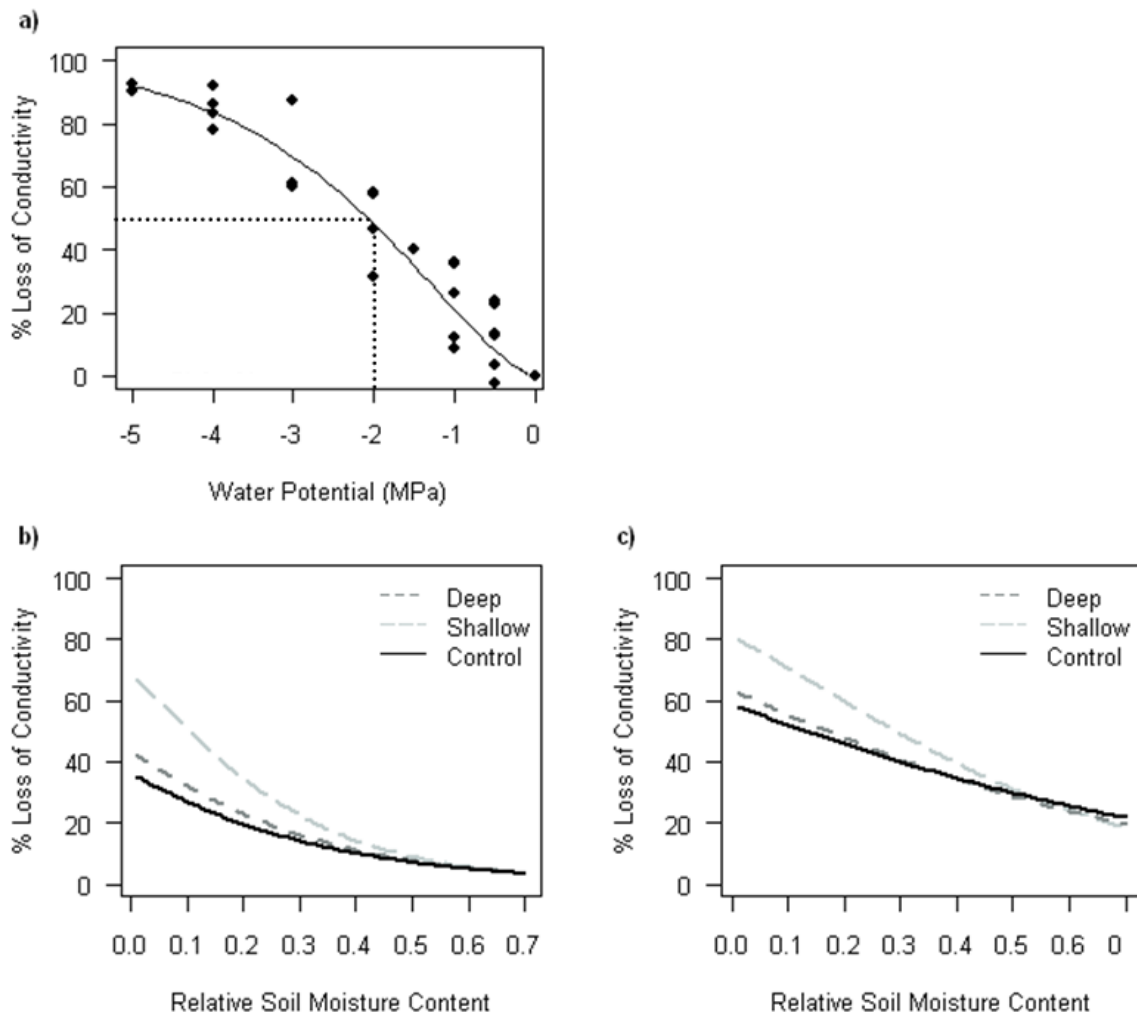


Fig. 3.7: a) Vulnerability curve for *T. sericea* as constructed with the air injection method. Conductivity is plotted as percent loss from initial value (PLC). The P₅₀ value, the pressure at which conductivity dropped to 50% from the initial value, lies at -2.0 MPa (dotted line). b), c) using the vulnerability curve for *T. sericea*, XPP's were translated into Percent Loss of Conductivity (PLC). PLC was then plotted against soil moisture content at 10cm depth, both for pre-dawn (b) and mid-day (c).

Discussion

The experiment did not lead to stem mortality regardless of treatment nor did my results show substantial response to the severing of deep roots. The results did however show that the severing of lateral roots to a depth of 50 cm had a negative effect on plant performance. These findings suggest that trees obtain the majority of resources from the surface layers of the soil and very little from the deeper layers. My results show that (1) trees are dependent

on resources in the surface layers (upper 50 cm), (2) they can take up resources from this layer, even in the presence of grasses, and (3) restricting access to deeper soil resources does not influence plant performance. When lateral roots were severed during the dry season, the taproot clearly failed to sustain the leaf biomass as the trees in this treatment immediately lost all leaves. My results concur with Ansley et al. (1990) and Ansley et al. (1991), who –in similar experiments- found responses to lateral root severing in honey mesquite varying from a slight reduction in stomatal conductance to leaf abscission, depending on subsoil characteristics. In my study, the effect of severing the taproots was negligible. What little effect was observed may be related to the disturbance of the fine roots when excavating part of the root system to sever the taproot. Trees in the “Shallow” group produced significantly smaller leaves, with significantly lower specific leaf area (SLA). Such responses in leaf size and SLA have often been observed in response to resource stress (Cornelissen et al. 2003). These findings are also consistent with studies that show that plants in dry environments have smaller leaves and lower SLA than plants in wetter environments (Wright et al. 2001; Lambers 2008).

Plants can regulate their gas exchange in several ways: through stomatal control, through changing the size of individual leaves or simply by changing the number of leaves they deploy and when they deploy them. All treatments initiated leaf deployment at the same time. Hence, the timing of leaf deployment was not influenced by root severing, although the “control” treatment had the most rapid rate of green-up. Trees subject to lateral root severing suffered the largest reduction in canopy cover during the mid-summer drought. These findings again suggest that lateral root severing had the most severe effect on carbon gain and that deep root severing had no discernible effect. *T. sericea* saplings thus appear to depend largely on their lateral roots for maintaining a water balance, even in dry periods during the growing season.

Factors determining the competitive outcomes of savanna trees and grasses may change with the ontogenetic stage of the trees (Higgins et al. 2000; Van der Waal et al. 2009). It has been suggested that savanna trees in different life-history stages need to overcome several

challenges before reaching the reproductive stage (demographic bottlenecks). First, seedlings need to overcome variation in seasonal rainfall and resource competition with grasses near the soil surface, where environmental conditions such as soil moisture and temperature fluctuate considerably. A seedlings' chance of survival and promotion to the next life-history stage would seem to improve in the absence of wet-season droughts and when/where grass production is suppressed (Davis et al. 1999; Kraaij and Ward 2006). My root-severing experiment was performed with established, 3-4 m tall saplings. My results imply that trees in this life-history stage do take up significant amounts of resources from the surface soil layers, despite the presence of grasses, and that access to deeper soil layers does not appear to enhance their performance. The factors preventing woody saplings in savannas (termed "Gullivers", *sensu* Bond and van Wilgen, 1996) from escaping to greater size-classes seem to be fire and herbivory rather than resource competition with herbaceous neighbours (Higgins et al. 2000; Midgley et al. 2010).

Research on the root systems of *T. sericea* conducted in Etosha National Park, Namibia (Hipondoka and Versfeld 2006), revealed that the vast majority of the excavated root systems at the dry end of a rainfall gradient (250mm rain/year) showed no taproot, whereas half of the trees in the wetter parts (500 mm) had taproots. The authors suggested that *T. sericea* adapts its rooting strategy to prevailing climatic conditions and hypothesized that the prevalence of taproots increases with precipitation (Hipondoka and Versfeld 2006). The occurrence of taproots in most excavated root systems of *T. sericea* at my study site confirms their hypothesis. Despite this, the ecological function of taproots in *T. sericea* at my study site is not immediately apparent, as my study suggests that they do not contribute much to the maintenance of the trees' water balance. This in contrast to studies done in other seasonally dry environments, which show that in periods of high water demand, deep roots play a very important role (e.g. Nepstad et al. 1994; Oliveira et al. 2005). Trees with deep roots have been shown to be capable of redistributing water from deeper, wetter soil layers to drier, shallow layers in a process called hydraulic lift (Richards and Caldwell 1987; Caldwell et al. 1998; Ludwig et al. 2004). My experiment does not provide direct evidence of hydraulic lift,

but the results encourage me to rethink the role of deep roots in this savanna system. It may be that the taproots in this species can only play a significant role in conjunction with the lateral roots. If that is the case, severing lateral roots would have simultaneously reduced the benefit of the taproot. More research is needed as my experiment was not designed to address the occurrence of hydraulic lift in *T. sericea*.

As I ran the experiment for one growing season only, one should be careful with the interpretation of the results. However, given the wide range of soil moisture contents during the year of study, I am confident that I sufficiently captured the relative importance of the lateral and taproots for maintaining the water balance under different conditions. In conclusion, my study shows that the common and locally dominant savanna tree *T. sericea* in the “Gulliver” life-history phase coexists with grasses and that a vertical rooting niche separation does not seem to promote this coexistence. In agreement with previous research in savanna systems (Le Roux et al. 1995; Mordelet et al. 1997; Kulmatiski et al. 2010), the results of my study therefore do not support Walter’s (1971) classic two-layer hypothesis of tree-grass coexistence.

CHAPTER 4

Vertical, horizontal and temporal patterns of water use by trees and grasses suggest resource use differentiation in the upper soil layers

University of Cape Town

Chapter 4:

Vertical, horizontal and temporal patterns of water use by trees and grasses suggest resource use differentiation in the upper soil layers

Introduction

The persistence of tree and grass coexistence in savanna ecosystems has intrigued ecologists for many years (e.g. Walter 1971, Scholes & Walker 1993, Sankaran et al. 2004, 2005), but this coexistence has not been satisfactorily explained to date. Resolving this fundamental problem will provide a basic understanding of the functioning of an ecosystem that covers over one-fifth of earth's land surface and supports a growing proportion of human, livestock, and mega-herbivore populations (Sankaran et al. 2004). Walter (1971) proposed that in savanna ecosystems there is no competition between trees and grasses for resources because woody plants are deeper rooted than grasses. This hypothesis, termed the two-layer hypothesis, has been integrated into models of species coexistence, hydrological cycles, biogeochemical cycles, and climate change (Walker & Noy-Meir 1982; Foley et al. 1996; Daly et al. 2000; Seyfried et al. 2005; Weng & Luo 2008). The two-layer hypothesis has provided an important explanation of savanna structure and function for the past four decades. Several studies, however, have suggested that niche partitioning in savannas is more complex than previously thought, as overlap between tree and grass rooting zones has often been shown, suggesting intense resource competition (Le Roux 1995, Seghieri et al. 1995, Mordelet et al. 1997, Ludwig et al. 2004, February & Higgins 2010).

Rooting strategies may differ between savannas. Studies in temperate savannas have demonstrated deep rooting by trees (Weltzin & McPherson 1997; Baldocchi et al. 2004; Asbjornsen et al. 2008), while studies in tropical and subtropical savannas have shown both

deep (Jackson et al. 1999; Schenk & Jackson 2002; Quesada et al. 2004; Goldstein et al. 2008) and shallow rooting by trees (Belsky 1994; Le Roux et al. 1995; Mordelet et al. 1997; Hipondoka et al. 2003; Rodriguez et al. 2007). Difficulties of belowground research have limited understanding of root uptake of soil resources. Measures of root mass are common, but are likely to be influenced by the presence of large and inactive roots (Chen 2004; Peek et al. 2005). Measuring root activity remains one of the most challenging problems in plant ecology (Casper et al. 2003; Schenk 2008b).

Isotope tracer techniques provide a way for addressing this challenge, as it allows the determination of the proportional contributions of several sources to a mixture. Natural variation in isotopic signatures ($\delta^2\text{H}$ and $\delta^{18}\text{O}$) have been used to determine the source of water taken up by plants and to study the functioning of root systems (Dawson & Ehleringer 1991; Ehleringer & Dawson 1992, West et al. 2007). If there is sufficient difference in isotopic composition between water in the soil profile and groundwater, the stable isotopic composition of water in the xylem sap of the plant can be used to define the mixing ratio of these sources of water for the plant (Dawson & Pate 1996). The method is based on the finding that isotopic composition of both hydrogen and oxygen in water is not altered when taken up by roots and transported from roots to the leaves (Zimmermann et al. 1967), i.e. no fractionation takes place. Techniques that rely on natural variation in stable isotope concentrations usually only allow broad distinctions in water use (e.g. 0-50 vs 50-100cm; Le Roux et al. 1995; Jackson et al. 1999; Ludwig et al 2004; Kulmatiski et al. 2006). Techniques that introduce tracers into the soil are more promising because they can constrain descriptions of water use to specific locations (i.e. vertical, horizontal, and temporal; Gottlein 2005). In tracer experiments, a known quantity of isotope is added to a pool and then recovered in a recipient pool after a known amount of time (Dawson et al. 2002).

Elements such as lithium (Li), strontium (Sr), and cesium (Cs) have been used as tracers (Mamolos et al. 1995; Bonanomi et al. 2005; Rodriguez et al. 2007). However, studies using

deuterated water ($^2\text{H}_2\text{O}$) show the most promise because plant roots typically do not distinguish $^2\text{H}_2\text{O}$ from $^1\text{H}_2\text{O}$ and, unlike some tracer elements, $^2\text{H}_2\text{O}$ is unlikely to accumulate variably in different plant parts (Dawson & Ehleringer 1993; Takahashi 1998; Webb & Longstaffe 2003; but see Ellsworth & Williams 2007). $^2\text{H}_2\text{O}$ has been used in irrigation treatments, but the wide wetting front associated with irrigation only allows broad distinctions in water use (Schulze et al. 1998; Moreira et al. 2000; Schwinning et al. 2002; Sternberg et al. 2005). $^2\text{H}_2\text{O}$ injections offer a better approach for measuring root activity, but few studies have tested this technique. Those that have used the technique injected into only one or two shallow soil depths (Bishop & Dambrine 1995; Plamboeck et al. 1999; Penuelas & Filella 2003; Sternberg et al. 2005; Hawkins et al. 2009).

The aim of this chapter is to test the hypothesis that tree and grass coexistence in savannas can, in part, be explained by vertical partitioning of the soil water resource. To do this, the timing and location and horizontal extent of water use by trees and grasses were measured in a mesic savanna using an isotope tracer. Deuterated water ($^2\text{H}_2\text{O}$) was injected into the soil at four depths (5, 20, 50, and 120 cm) and during four sampling periods (Oct, Nov, Feb, and Apr 2007–2008). Plant tissues were sampled following the tracer addition to determine the extent to which plants absorbed water from each soil depth. Plants were also sampled at several distances (0, 0–2, 2–3, and 3–5 m) from the tracer addition area to determine the horizontal foraging area of trees and grasses. Soil samples were taken to determine how the tracer moved in the soil.

Materials and Methods

STUDY SITE

The study site is situated 3 km south of Pretoriuskop in the Kruger National Park, ($25^{\circ}20'76$ S, $31^{\circ}28'32$ E; elevation 655 m). The site can be described as a broadleaf or woodland,

mesic savanna dominated by deciduous trees and shrubs (Sankaran et al. 2005; Archibald & Scholes 2007). Mean annual precipitation is approximately 746 mm, occurring primarily during the summer. A dry period occurred during the wet season of 2007-2008, with monthly precipitation of 11 and 51% of mean values in February and March, respectively. The grass layer was dominated by C_4 grasses such as *Hyparrhenia filipendula* (Hochst.) Stapf, *Hyparrhenia dissoluta* (Steud.) Hutch., *Cenchrus ciliaris* (L.) Link, *Panicum maximum* (Jacq.), and *Setaria sphacelata* (Schumach.) Stapf & C.E. Hubb); Dominant woody species at the site were the trees *Terminalia sericea* (Burch. ex DC.), *Sclerocarya birrea* (A.Rich.) Hochst., and the erect shrub *Dichrostachys cineria subsp. africana* (Brenan & Brummitt). Mid-season ground cover was $57 \pm 12\%$ grasses, $19 \pm 10\%$ trees, $8 \pm 5\%$ shrubs, and $6 \pm 2\%$ forbs (mean \pm standard deviation (SD)). Tree diameter at breast height (DBH) was 4 ± 4 cm (range 1–31 cm). Tree canopy radii were 3.9 ± 1.3 m (range 1.5–6.9 m). Soils are sandy loams with little coarse fragment and a dense layer from 80 to 150+ cm.

TRACER INJECTION

Along each of four 240-m-long transects, located 60 m apart, eight circular plots (5 m radius) separated by 30 m were placed (4x8=32 plots in total). Even-numbered plots were randomly assigned to a depth (5, 20, 50, or 120 cm) and sampling period (Oct, Nov, Feb, and Apr 2007–08) treatment (e.g. 20 cm in Nov). Each treated plot, therefore, was 60 m from any other treated plot. Odd-numbered plots were used as controls. In a 1-m² circle at the centre of each treated plot, 100 pilot holes were drilled by hand using a 10-mm-diameter steel rod to the assigned depth. These holes were drilled to make entering the soil easier and to avoid clogging of the needle as much as possible. Into each hole, two ml of 70% ²H₂O followed by 2 ml of tap water was injected directly to the bottom of the pilot hole, using standard 20 ml syringes fitted with aluminium tubing to length (5, 20, 50, 120 cm), connected to 16 gauge hypodermic needles (Vita Needle, Needham, MA, USA). The tap water injected after the tracer was intended to force out all tracer from the syringe and needle, and prevent tracer

contamination as the needle was removed from the hole. Holes were filled with soil to the approximate density before excavation, to prevent evaporation of the tracer. While the injection did create a pulse of water around the injection point, it was not expected to increase plant growth, as the 400 ml of added water represented only 0.05% of annual precipitation and increased volumetric soil water content by 7.9% in the 5024 cm³ volume.

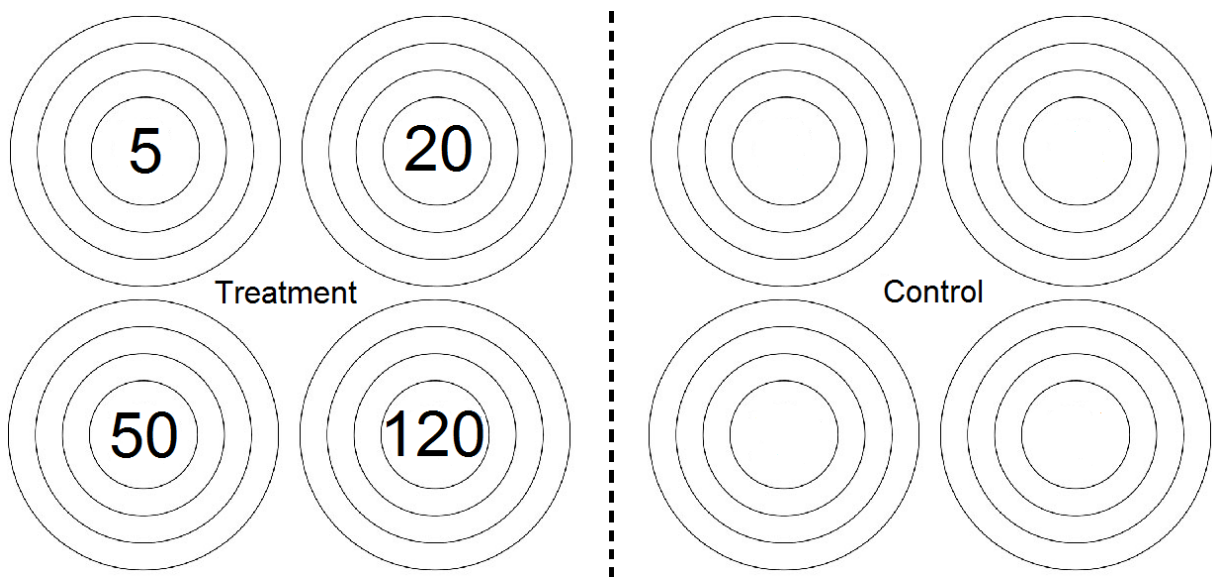


Fig 4.1: Experimental design. Each sampling period (Oct, Nov, Feb, Apr), eight (not previously used) plots (4 treatment, 4 control) were sampled (4x8=32 plots). Concentric circles have radii of 1m, 3m, 4m, and 5m, respectively. Values in the centre of each plot depict depth of tracer addition. Relative position of each plot in this figure does not match the situation in the field (see description in the text).

SAMPLING OF PLANT MATERIAL

One day following tracer injection, the first plant samples were removed from the 1-m² tracer addition area and the 0-2m area outside the tracer addition area. This was repeated two days later for the 2-3m area and three days later for the 3-5m area. Samples removed from the 120-cm plots were removed 1 day later than samples from the 5-, 20- and 50-cm plots to allow time for xylem flow to move the tracer to the sampled plant materials. Similarly, tree samples removed from > 1 m above the ground were sampled 1 day later than other samples from the same distance from the tracer addition area. Xylem flow rates of 1–5 m per day were used to determine when samples should be taken (Fravolini et al. 2005; Meinzer et al. 2006), and this assumption was tested by repeat sampling of trees (0–2 m from centre) and grasses (tracer addition area) in one plot (first 5 cm tracer injection) over 5 days. One week following tracer injection in November 2007, soil cores (5 cm diameter) were taken from the centre of both treated and control plots to confirm the location of the added tracer. These soil cores were split into eight depths (0-15, 15-30, 30-45, 45-60, 60-75, 75-90, 90-105 and 105-120 cm) to identify the location of the pulse.

For all plant samples, non-transpiring tissues were used so that samples represented the mean water uptake by plant roots (Dawson & Ehleringer, 1993). Grasses were sampled from the root crown. Tree twigs or stems were sampled from below the height of the first leaves. Control samples were collected throughout the sampling period following the methods used for treated plots. At least three replicate samples of each species sampled in treated plots were also taken from control plots. It was attempted to sample all individual trees and all grass species in plots. Forbs were not sampled. Samples of common species were composited to produce three to five replicate samples for each species in each distance from the pulse. Less common species were composited by functional type (i.e. grass or tree). As much plant material as could fit in the bottom 10 cm of the prepared sample vial was taken.

All sampling tools (hands, trowels, clippers, and steel rod for filling samples into vials) were moved 20 m outside the plot and triple rinsed with tap water before taking the next sample.

This sampling design resulted in 430 plant and 67 soil samples. Plant and soil samples were immediately placed into borosilicate (19 mm outer diameter) tubes, sealed with parafilm, placed on ice, transported to a freezer within 6 hours, and extracted within 2 weeks. Water samples were extracted from plant tissues and soils using a batch cryogenic distillation procedure as described by Vendramini & Sternberg (2007) in a laboratory in Skukuza, Kruger National Park (see Fig. 4.2). Tubes with samples were placed in liquid nitrogen, secured to a vacuum extraction line with an Ultrator fitting (Swagelok, Solon, OH, USA), evacuated to 10 milliTorrs pressure, flame sealed, and inserted sample-up in a custom-milled 12cm-deep aluminium block. A matching aluminium block was placed above the samples and heated to 90°C. After 1 hour of heating, liquid nitrogen was added to a foam tub that surrounded the bottom aluminium block. Samples were heated (top) and frozen (bottom) until ice placed against the sample tube created no condensation in the sample vial (typically 6 hours). Sample vials were cracked open, sap extracts were placed in labelled 2ml vials. Samples typically produced 1-4ml of sample water, limiting concern regarding fractionation as a result of evaporation during sample transfer from sample vials to 2ml vials.

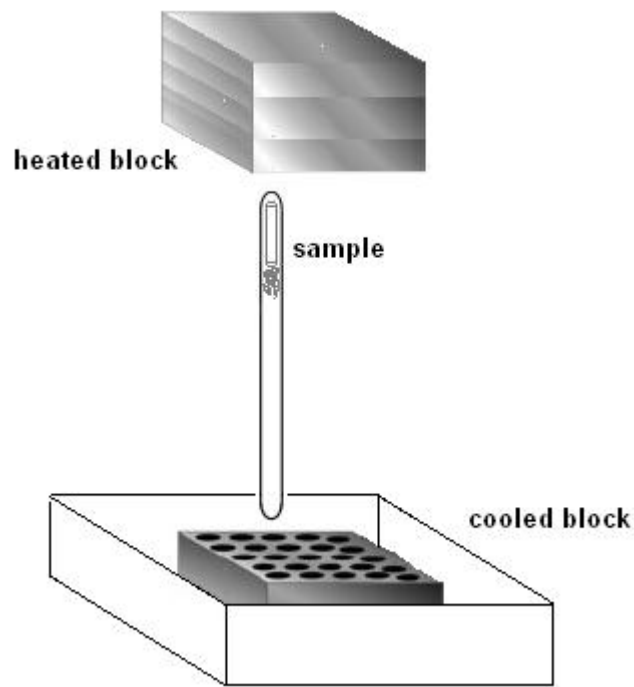


Fig 4.2: Batch cryogenic distillation set-up after Vendramini et al. (2007).

The samples were analyzed for $^2\text{H}/\text{H}$ ratios in the Stable Light Isotope Laboratory of UCT, with an AS-3000 autosampler (Thermo Scientific, Germany) connected to a Delta Plus XP IRMS (Thermo Electron, Germany). All isotope values are expressed in delta (δ) notation as the $^2\text{H}/\text{H}$ ratio relative to an international standard (Vienna Standard Mean Ocean Water). Analytical precision (2σ) was 0.9‰ for δD . Tracer was assumed to be present in samples with $\delta^2\text{H}$ values that were at least two SDs above mean concentrations in paired control samples. Because raw tracer concentrations are biased by differences in rooting area among species (i.e. tracer was added to a larger proportion of the rooting zone of a plant with a small rooting area relative to a plant with a large rooting area), tracer uptake was normalized by depth or distance to allow a direct comparison of water uptake between trees and grasses (Bishop & Dambrine 1995; McKane et al. 2002; Schwinning et al. 2002). The proportional uptake for a plant functional type by depth or distance is:

$$\frac{S_n - C}{\sum_{n=i}^j (S_n - C)}$$

, where S_n is the mean $\delta^2\text{H}$ value of samples from a treatment level n (e.g. 5 cm depth), and C the mean $\delta^2\text{H}$ value of control samples for that functional type (e.g. trees).

Statistical Analysis

Differences in tracer uptake were tested using analysis of variance (ANOVA) computed using the MIXED procedure accounting for autocorrelation of repeated measures in SAS/stat for Windows, Release 9.1.3 (SAS Institute Inc., Cary, NC, USA). To compare the proportion of tracer uptake between trees and grasses for each depth or distance from the tracer addition area, the fixed effect was plant type (trees and grasses). Month was used as a random effect. To compare tracer uptake for trees and for grasses among depths for each time period (month), the fixed effect was depth and replicate samples from each plot were used as random effects. Tukey post hoc pairwise comparisons were used to determine mean differences.

Results

TRACER DETECTION

A total of 300 grass samples and 130 tree samples were extracted from experimental plots. Of these, 119 (40%) grass and 23 (18%) tree samples demonstrated isotope ratios that were 2 SDs or more above controls (i.e. received tracer; Table 4.1). Of the 75 control samples, only one plant sample demonstrated an isotope ratio that was 2 SDs above the mean,

indicating there was little to no contamination. Soil and plant samples were taken to confirm the location of the tracer and to determine how quickly tracer moved through soil and plants, respectively. Soil samples taken 1 week following tracer injection showed clear differences in tracer concentration with depth among the treatments, although redistribution was also apparent (Fig. 4.3).

Table 4.1: Tracer uptake by depth, distance, and plant type

Depth	Distance from tracer addition (m)							
	0		0 - 1.5		1.5-2.5		2.5-4.5	
	Grass	Tree	Grass	Tree	Grass	Tree	Grass	Tree
5 cm	22/22	0/0	15/22	2/9	5/18	1/7	1/19	1/13
20 cm	23/23	1/3	11/22	2/7	5/17	2/9	1/17	6/14
50 cm	17/18	0/0	6/20	1/9	4/18	1/10	2/17	3/15
120 cm	3/17	0/0	3/19	2/12	0/16	1/12	1/15	0/10

Each pair of values shows the number of samples analyzed and the number of samples with $\delta^2\text{H}$ values that were 2 SD or more greater than mean $\delta^2\text{H}$ values for control samples

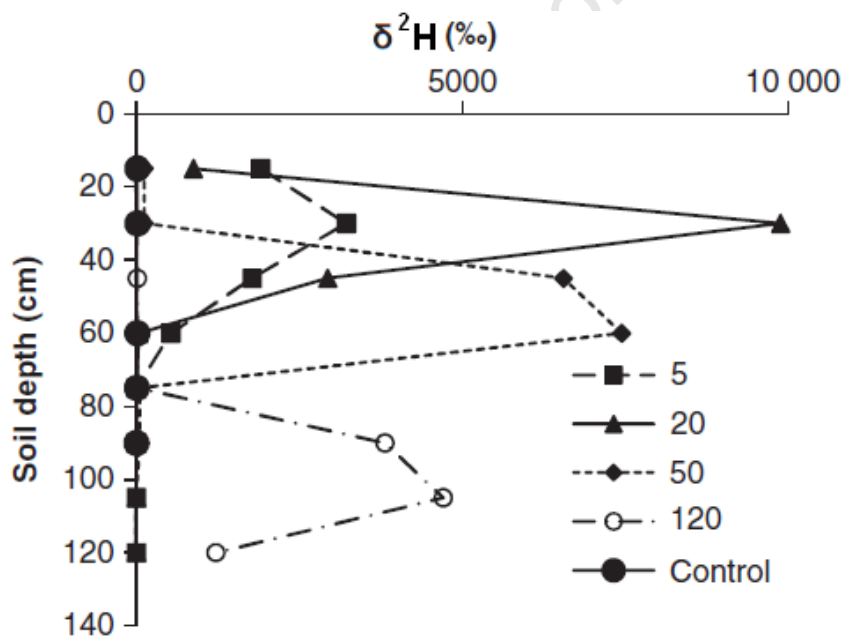


Fig 4.3: Tracer concentration by depth (control, 5, 20, 50, or 120 cm) at 1 wk following tracer injection.

Repeated vegetation sampling indicated that grasses realized a peak in tracer concentrations 1–2 days after tracer injection at 5 cm depth and trees realized a peak in

tracer concentrations 1–3 days after tracer injection at that depth (Fig. 4.4). Trees absorbed 24, 59, 14 and 4% of tracer from the 5, 20, 50 and 120 cm depths, respectively, while grasses absorbed 61, 29, 9 and 0.3% of tracer from the same depths (Fig. 4.5a). Neither trees nor grasses, therefore, absorbed > 5% of their tracer from 120 cm depths.

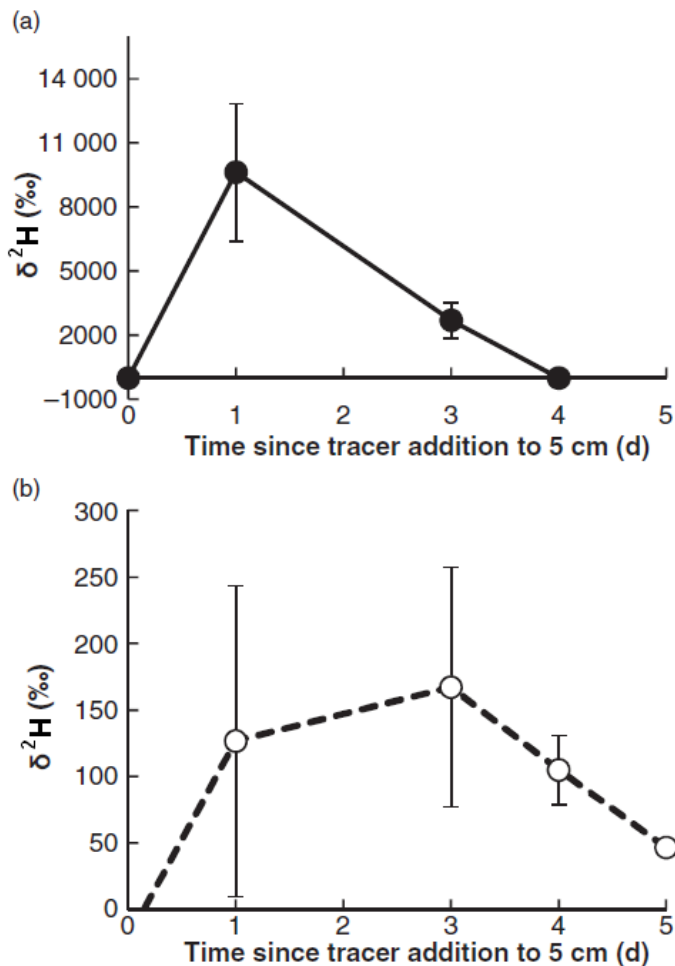


Fig. 4.4: Tracer concentration in grass (a) and tree (b) samples taken by day following tracer injection. Grasses were sampled in the tracer addition area and trees were sampled in the tracer addition area and within 2 m of the tracer addition area. Error bars signify \pm SE.

Grasses absorbed a greater proportion of water from the 5 cm depth than trees but there were no significant differences in the proportion of tracer uptake between trees and grasses at the other depths. In the horizontal plane, there was no difference in tree uptake among

distances from the pulse (Fig. 4.5b). By contrast, grasses foraged close to the stem. Almost all (98%) grass uptake occurred in the tracer addition area (Fig. 4.5b). As a result, trees absorbed a smaller proportion of tracer close to the stem than grasses, but absorbed a larger proportion of tracer 2, 3 and 5 m from the stem than grasses (Fig. 4.6b). Trees absorbed more tracer in November and April than in October or February ($F_{3,12} = 6.26$, $P < 0.01$; Fig. 4.6). Trees absorbed more tracer from 20 cm than from 5, 50 or 120 cm in November (Fig. 4.6b; $F_{3,16} = 11.07$, $P < 0.01$), and more tracer from 20 cm than from 50 or 120 cm in April (Fig. 4.6d; $F_{3,33} = 2.90$, $P = 0.05$).

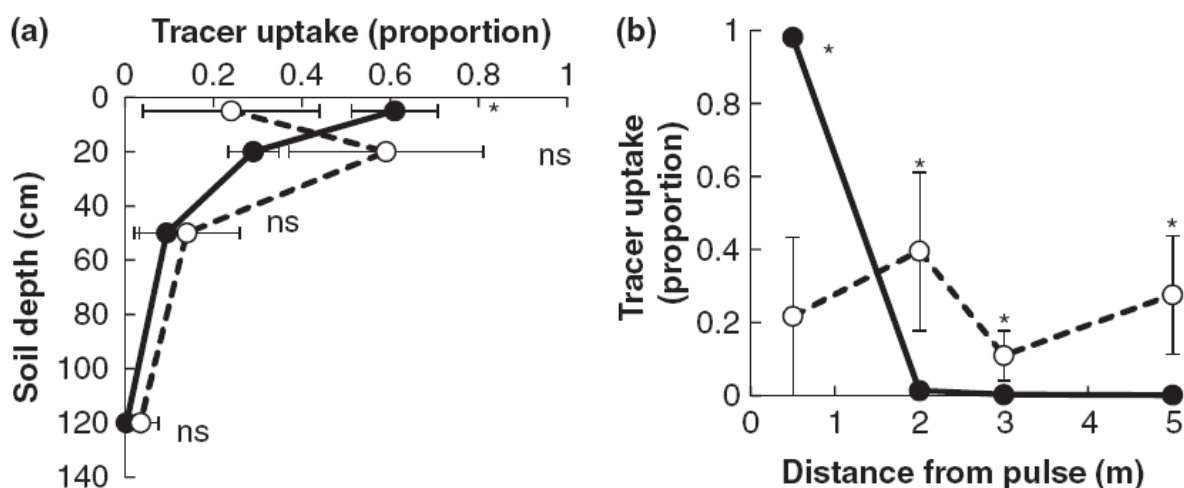


Fig. 4.5 Proportional tracer uptake (\pm SE) by trees (open circles) and grasses (closed circles) by depth (a) and distance (b). Sample sizes (n) at 5, 20, 50 and 120 cm depth are 81, 79, 73, 67 (grass) and 29, 33, 34, 34 (trees), respectively. Sample sizes (n) at 0.5, 2, 3 and 5 m distance are 80, 83, 69, 68 (grass) and 3, 37, 38, 52 (trees), respectively. Standard errors reflect variation between sampling dates. *, significant differences between trees and grasses within a depth or distance using $P < 0.05$; ns, no significant difference.

Grasses absorbed more tracer in November than April ($F_{3,11} = 3.55$, $P = 0.05$; Fig. 4.6). In October, grasses absorbed more tracer from 20 cm than from 50 cm (Fig. 4.6a; $F_{3,26} = 4.45$, $P = 0.01$). In November, grasses absorbed more tracer from 5 and 20 cm than from 50 and 120 cm, and more tracer from 50 cm than from 120 cm (Fig. 4.6b; $F_{3,75} = 4.89$, $P < 0.01$). In February, grasses absorbed more tracer from 5, 20 and 50 cm than from 120 cm (Fig. 4.6c; $F_{3,72} = 2.32$, $P < 0.01$). In April, grasses absorbed more tracer from 5 cm than from 50 or 120 cm (Fig. 4.6d; $F_{3,67} = 4.48$, $P < 0.01$).

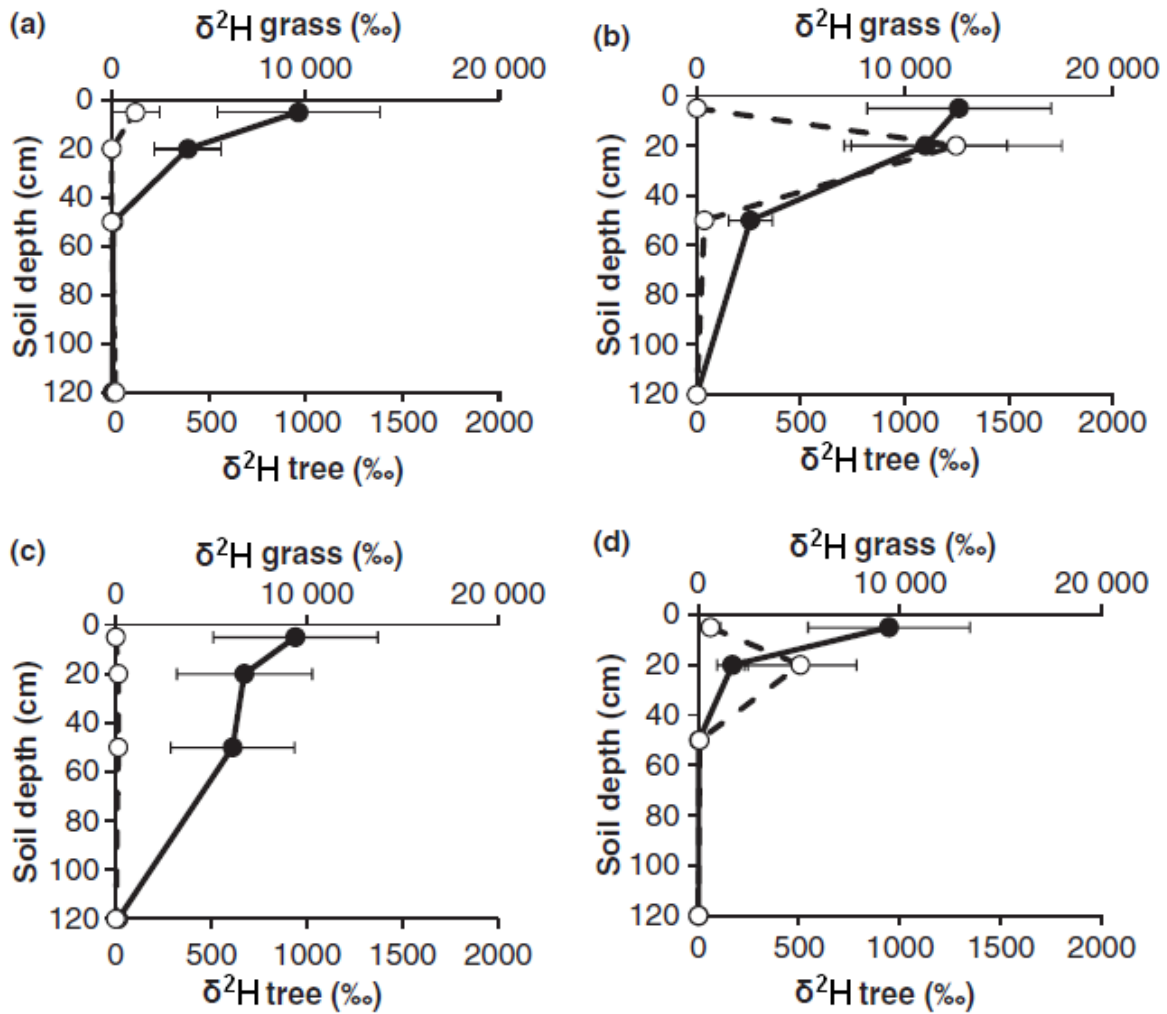


Fig. 4.6: Tracer concentrations in trees (open circles) and grasses (closed circles) in Oct (a), Nov (b), Feb (c), and Apr (d). Note that the top and bottom horizontal axes indicate grass and tree deuterium concentrations in, respectively delta notation. Standard errors reflect variation from replicate samples within a plot.

Discussion

Ecologists have long wondered how savanna trees and grasses access soil resources, but the difficulties associated with belowground research often prevent direct measurements of root activity. By injecting tracers at four depths up to 120 cm in a sandy, mesic savanna, I found that trees absorbed 83% and grasses absorbed 90% of their water from the top 20 cm of soil, indicating the importance of shallow soil layers, also for trees. This notion concurs with the findings reported in Chapter 3. Results of the tracer study provided mixed support for

the two-layer hypothesis. Trees and grasses did partition soil resources, but this partitioning did not reflect, as suggested by the two-layer hypothesis, the ability of trees to access deep soil water that was unavailable to grasses. More specifically, trees absorbed most of their soil water from 20cm depths and grasses absorbed most of their soil water from 5cm depths. Trees, however, did not absorb a larger proportion of water from 50 or 120cm depths than grasses: trees did not have exclusive access to deep soil water. Because trees and grasses partitioned soil resources over a narrow vertical range, techniques that measure soil resource use must distinguish differences of a few centimeters (McKane et al. 2002; Ogle et al. 2004). Previous studies have not made such precise measurements and concluded that trees and grasses did not partition shallow soil resources (Le Roux et al. 1995; Mordelet et al. 1997; Hipondoka et al. 2003; Chapter 3 in this thesis). Previous studies based on measurements of root mass suggest that the two-layer hypothesis will be most important in sandy, mesic savannas where deep infiltration provides a resource to deeply rooted trees (Schenk & Jackson, 2002). That trees and grasses in our sandy, mesic study site could partition resources within the top 20 cm of soil suggests that access to deep (e.g. > 1 m) soil water is not a prerequisite for resource partitioning among trees and grasses. Our results are consistent with the hypothesis that most root activity occurs at the shallowest possible depths (Schenk 2008a). This hypothesis and our results suggest that vertical niche partitioning may be important in savannas with or without deep soil water infiltration (i.e. any savanna type) and not just in sandy, mesic savannas. The ability of trees to maintain leaf area longer than grasses has been used to support the two-layer hypothesis, but our measurements suggest two alternative explanations. Leaf production before seasonal rains (Archibald & Scholes 2007) has been used to suggest that trees use deep soil water (Walter 1971; Scholes & Archer 1997). I found that, when there were no early-season rains, *S. birrea* used stored water to produce early leaves, as evidenced by decreases in stem diameter (as in Chapotin et al. 2006; see Chapter 6 in this thesis). Leaf retention in the dry season has also been used to suggest that trees use deep soil water (Walter 1971; Scholes & Archer 1997). The observation that trees absorbed little water below 20 cm implies that trees did not rely on use

of deep soil water. Trees and grasses showed, however, large differences in horizontal foraging. Trees actively foraged for soil water at least 5 m from the tracer addition area, a distance beyond the width of their crowns (mean radius of 3.9 m; Sternberg et al. 2005). Surprisingly, several grass samples also received tracer up to 5 m from the stem, but the concentration of tracer absorbed from these distances was small. As a result, >78% of tracer uptake by trees occurred outside the tracer addition area while <2% of tracer uptake by grasses occurred outside the tracer addition area. In summary, trees showed the potential to integrate resource-rich patches over wide areas while grasses foraged immediately under their stems. These results are consistent with previous measurements of root mass (Schenk & Jackson, 2002) and root activity in savanna systems (Sternberg et al. 2005).

University of Cape Town

CHAPTER 5

**STRESS OR STRATEGY? PLANT WATER STATUS
AND VULNERABILITY IN TWO TREE SPECIES WITH
ASYNCHRONOUS PHENOLOGY**

University of Cape Town

CHAPTER 5:

Stress or strategy? Plant water status and vulnerability in two tree species with asynchronous phenology

Introduction

The savanna biome covers approximately 20 percent of the earth's land surface (Scholes & Hall 1996) and 60 percent of sub-Saharan Africa (Scholes & Walker 1993). A defining feature of this biome is the co-dominance of trees and grasses. How these different life-forms maintain long-term coexistence has been the subject of continuous debate among savanna ecologists for the past decades (Sarmiento 1984; Scholes & Archer 1997; Higgins et al. 2000; Sankaran et al. 2005; Baudena et al. 2010; Midgley 2010). Recent studies have shown that trees and grasses are rooted in the same soil horizons and that there is considerable competition for resources between these two life forms (February & Higgins 2010; Kulmatiski et al. 2010). A further key characteristic of savannas is the strongly seasonal nature of the rainfall, with a dry season that may last several months (Frost et al. 1986). This strong seasonality renders this biome to be dominated by deciduous plant species, although evergreen species do occur. Variations in the timing of leaf out and leaf fall have often been observed in semi-arid and mesic savannas, not only between trees and grasses, but also between deciduous trees (Williams et al. 1997; Duff et al. 1997; Shackleton 1999; Sekhwela & Yates 2007; Archibald & Scholes 2007). Where the leafing out of the grass component is closely related to rainfall (Prins 1988; Seghieri et al. 1995; Scanlon et al. 2005), deciduous trees can extend the growing season by displaying leaves up to several weeks before the first rains (Monasterio & Sarmiento; Williams et al. 1997; Archibald & Scholes 2007).

An alternative hypothesis to the root niche separation hypothesis of Walter (1971) suggests that niche separation by leaf phenology may be a possible mechanism facilitating the co-

dominance of trees and grasses in savannas (Ovalle & Avendano 1987; Scholes and Walker 1993; Seghieri et al. 1995; Sala et al. 1997; Scholes & Archer 1997; House et al. 2003). Leaf phenology is an important indicator of plant responses to environmental conditions, and is thought to be affected by factors such as photoperiod, temperature, relative humidity, evaporative demand, (ground) water availability, etc. (Borchert 1994a; Do et al. 2005; Singh & Kushwaha 2005; Seghieri et al. 2009). It has been hypothesized that vegetative development is timed to maximise nutrient availability to plants (van Schaik et al. 1993). By leafing out immediately after or even before the first rains, trees can monopolize resources such as N and P, and reach maximum production rates early in the growing season, thereby avoiding competition with grasses for resources. Trees may also retain leaves well into the dry season, when grasses have already senesced, thereby maximizing photosynthetic potential (Jolly & Running 2004).

Several hypotheses have been formulated to explain how savanna trees are able to leaf out while moisture is still below wilting point (Scholes & Archer 1997). One of these suggests that at the end of the wet season, trees retract nutrients such as nitrogen and phosphorus from the leaves before abscission (Aerts 1996). These resorbed nutrients are stored by the tree to facilitate leaf formation before the rains at the beginning of the new growing season (Scholes and Archer 1997, February unpublished data). Nutrients in the soil only become available to the plant after rainfall amount is high enough to facilitate the mobilization and mineralization of nitrogen (Scholes & Walker 1993, Scholes & Archer 1997). Once the rains arrive, tree species adopting this strategy would already have their leaves in place. This would give them a clear advantage over grasses and other trees in the competition for resources. The water necessary for leafing out before the rains may either be taken up from deep soil layers (Borchert 1994a, Scholes & Archer 1997) or from stored reserves in the stem (Borchert 1994a, Chapotin et al. 2006).

Two common deciduous tree species in the Kruger National Park with very different leaf phenological patterns are *Sclerocarya birrea* subsp. *caffra* and *Terminalia sericea* (see Fig. 5.1). *S. birrea* displays leaves several weeks before the rains, whereas *T. sericea* starts leafing out only with the onset of the first rains. *S. birrea* sheds all its leaves in a relatively short period (a few weeks), almost immediately after the last rains, while surface soil moisture is still high and the trees are still well-hydrated. *T. sericea* on the other hand, retains its leaves much longer into the dry season than *S. birrea* and only gradually sheds them.

Understanding the different patterns requires some knowledge on the mechanism of long-distance transport of water through the xylem. The widely accepted cohesion-tension theory (Dixon & Joly 1895) suggests that the main force driving the anti-gravitational ascent of water through the xylem is generated by surface tension at the evaporating surfaces of the leaf. This tension is transmitted to the roots via the cohesion of water, so that Ψ of the roots is lower than Ψ of the soil water. Water is subsequently taken up from the soil, transported through the xylem conduits in the direction of the more negative water potentials to replace the water lost by transpiration (Tyree 1997). The water moves through the xylem in a metastable condition (maintained by cohesion and adhesion), meaning that the xylem pressure is lower than the vapour pressure of water (Tyree 1997). During the transport process, the increasingly negative pressures in the liquid water inside the xylem conduits can lead to cavitation: the sudden transition from metastable liquid to gas (Zimmermann 1983). If the continuous water column is interrupted by an air bubble, the plant's hydraulic conductivity declines. Increasingly low xylem pressures are associated with a decrease in xylem conductivity as an increasing proportion of the conduits become cavitared (Domec et al. 2006). The main consequence of embolism formation in the conducting tissue is an increase of resistance to water flow along the water pathway, which affects the plant's water relations, leading to desiccation and decreased photosynthesis (Cochard et al. 1992). The vulnerability to drought-induced embolism depends on the species and the conditions under which it grows. This vulnerability can be determined by relating decreasing levels of xylem pressure

potentials to remaining xylem conductivity and plotting the relationship in what is called a vulnerability curve (Tyree et al. 1994).

To prevent water from being lost more rapidly at the leaf level than can be replaced by uptake from the soil (leading to cavitation), trees have a few mechanisms at their disposal, among which are stomatal closure and leaf abscission. Stomata can be opened and closed by plants in rapid response to sudden changes in environmental conditions (evaporative demand). In times of prolonged drought, e.g. after the rains of the previous growing season have ceased, the more rigorous measure of leaf abscission may be needed to prevent water loss. For the two above-mentioned tree species I determine the extent to which soil moisture is a driver for leaf abscission or leaf flush. I do this by monitoring the seasonal changes in plant water stress, vulnerability to dysfunction and leaf phenology. I also relate plant water stress to leaf phenology along a rainfall gradient. The objective is to test the hypothesis that extending the growing season, either before or after the rains, is a trade-off between early exclusive access or late exclusive access to water. Species that use stored water for the following seasons' growth, would need to prevent water loss at the end of the wet season. Species that do not store water may leaf out only after the first rains, but can retain their leaves until very late in the dry season.

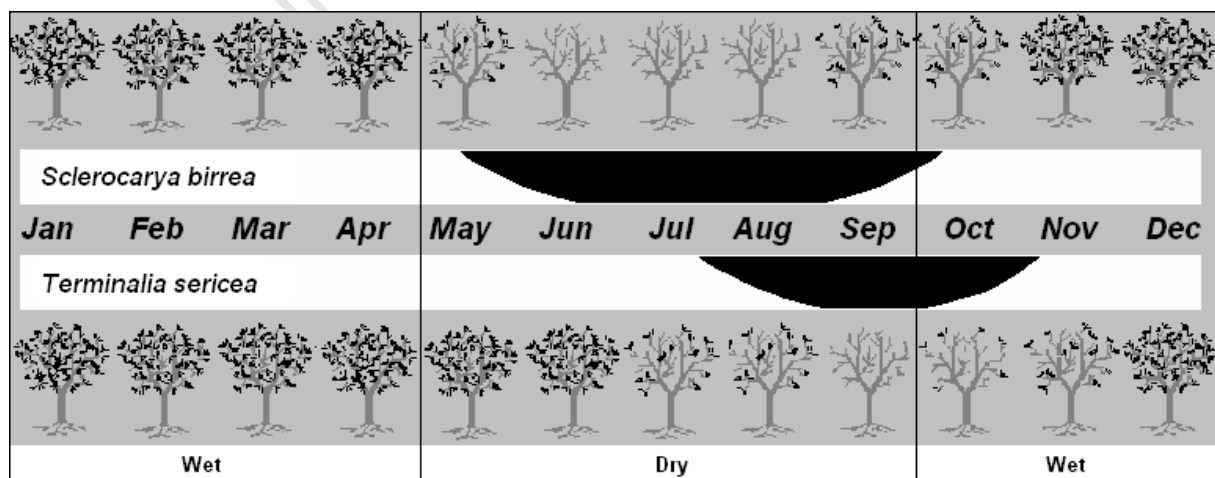


Fig 5.1: Leaf phenology of *S. birrea* and *T. sericea* (schematic). This diagram is based on canopy fullness data collected monthly from August 2005 - August 2006.

Materials and Methods

Study area

Part of the study was conducted at a study site located in the southern section of the Kruger National Park, near the Pretoriuskop rest camp (25°07'S; 31°13'E). The vegetation of the region is dominated by the trees *Terminalia sericea*, *Sclerocarya birrea* and *Dichrostachys cinerea* with grasses such as *Hyperthelia dissoluta* and *Setaria sphacelata*. The moderately deep (>1m) sandy soils are derived from the underlying granite (Barton et al. 1986). Average daily maximum and minimum temperatures are 30.6°C and 19.0°C for the hottest month (January, average: 24.8°C) and 23.8°C and

9.3°C for the coldest month (June, average 16.5°C), respectively. Average annual rainfall is 746mm, with January being the wettest (129mm) and June the driest (9mm) month (Zambatis 2006). The study was conducted through the growing season from September 2006 to May 2007. Rainfall for the year of study was (with 531mm for Pretoriuskop) below average, with a mid-season drought from January to March and no rain in May.

The other part of the study was performed along a transect comprising of the Albasini and Doispane Roads, which cover a rainfall gradient of close to 200mm on average, over a distance of approximately 50km between Pretoriuskop (736mm) and Skukuza (31°36'S, 24°59'E; 553mm). At the end of May 2007, *S. birrea* showed striking within-species variation in phenophases, although no obvious spatial patterns were observed along the rainfall gradient.

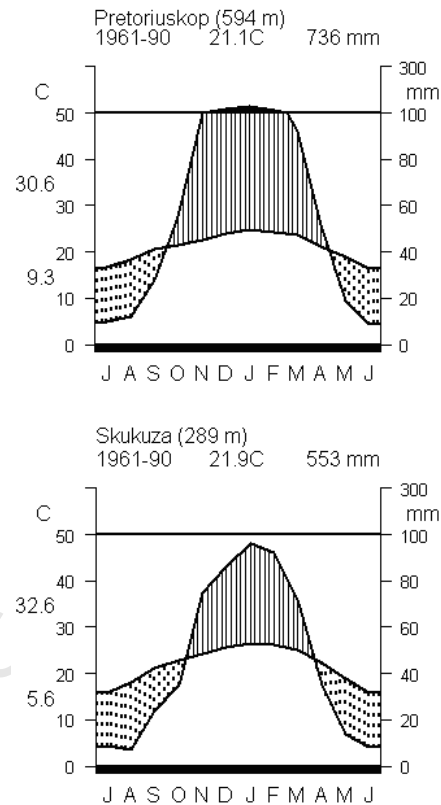


Fig 5.2: Climate diagrams for Pretoriuskop and Skukuza.

Leaf abscission for *S. birrea* trees occurred relatively soon after the last rain event (April 22nd), though not in absolute synchrony. While both *S. birrea* and *T. sericea* do grow along the entire gradient, *T. sericea* is more prevalent at the wetter end. Especially towards the dryer end it was difficult to find recognizable individuals along the road sides (important with regard to the wildlife, especially at night). It was therefore decided to only consider *S. birrea*.

SEASONAL PLANT WATER STATUS

At the study site near Pretoriuskop, both pre-dawn and mid-day xylem pressure potentials (XPP) were determined as indicators of plant moisture stress (Scholander et al. 1965). Plant moisture stress, defined as the demand for water in a plant, is established through a determination of the amount of tension the water column is under, using a Scholander-type pressure chamber (PMS Instrument Company, Albany, OR, USA). The more tension on the water column, the less water is available to the plant, the higher the plant moisture stress. Xylem pressure potentials were determined for 6 trees of *S. birrea* and 6 trees of *T. sericea* at the study site near Pretoriuskop. For more information on the procedure, see Chapter 3. The measurements were made on 8 separate occasions for *T. sericea*, and on 6 occasions for *S. birrea* from mid-September to the end of May of the 2006-2007 growing season.

WATER STATUS ALONG RAINFALL GRADIENT

To determine the extent to which plant available water induced leaf abscission in *S. birrea*, a once-off measurement (May 31st, 2007) of both pre-dawn and mid-day xylem pressure potential (XPP) of *S. birrea* was carried out along the rainfall gradient between Pretoriuskop and Skukuza. Using a Scholander pressure chamber (PMS Instrument Company, Albany, OR, USA), XPP was measured for one leafy twig from each of the 71 selected individuals located along the transect (Table 5.1). Selection of individuals was based on both location on the rainfall gradient and phenological stage. The gradient was divided into three rainfall

classes using the distance-interpolated rainfall map of the Kruger National Park (KNP Scientific Services 2006, Fig 5.3). These rainfall classes were: 501-600, 601-700 and 701-800mm. Within these rainfall classes, phenological stage was defined as percentage crown covered by leaves, and was divided in three classes (80-100%, 25-75% and 0-20%). I aimed for an even spread of the different phenology categories over the rainfall classes.

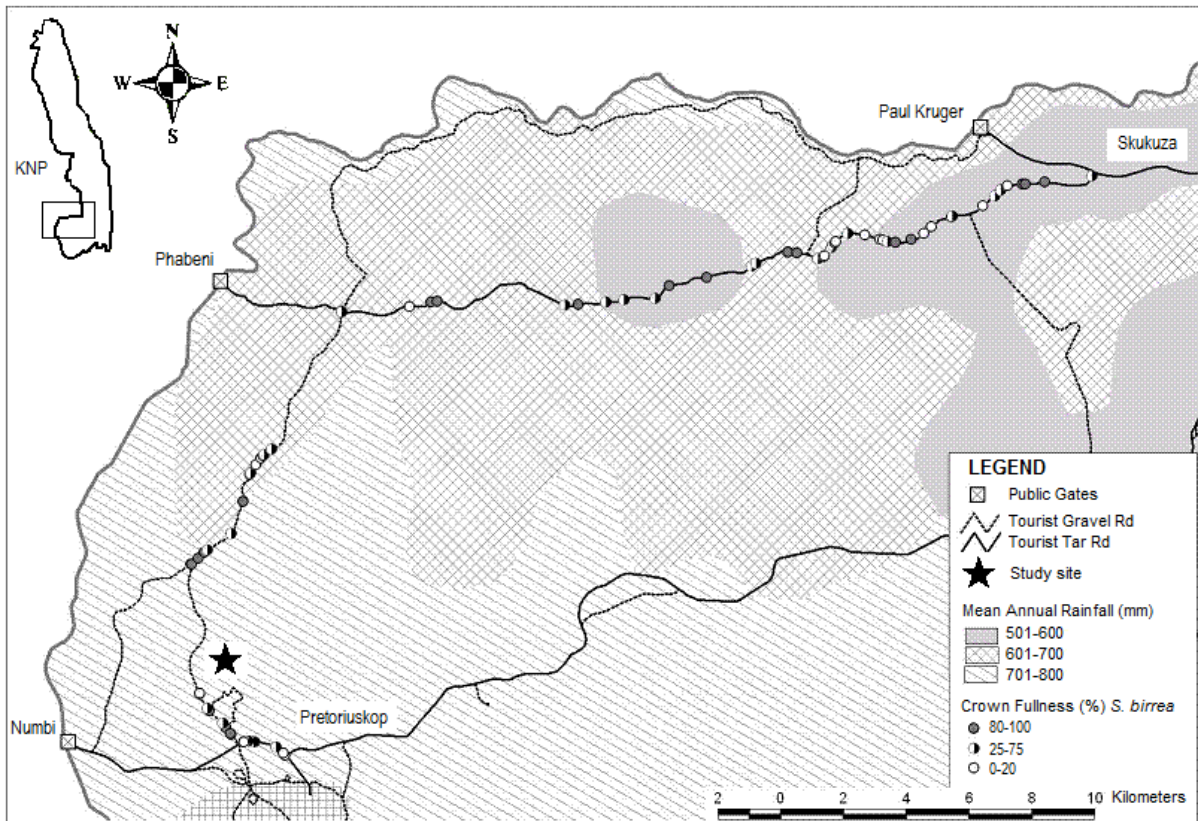


Fig. 5.3: Rainfall map of the study area in the southern Kruger National Park, South Africa, showing the location of the study site and the location of the individual *S. birrea* trees along the rainfall gradient between Pretoriuskop and Skukuza.

Table 5.1: Distribution of selected trees over the phenological stage classes and rainfall classes

Rainfall (mm)	Phenological stage (% crown cover)			n
	80-100	25-75	0-20	
501-600	9	9	6	24
601-700	7	10	7	24
701-800	8	7	8	23
n	24	26	21	71

VULNERABILITY CURVES

To quantify the relationship between XPP and plant moisture stress, I constructed vulnerability curves for *S. birrea* and *T. sericea* using an adjusted version of the air-injection method of Sperry & Saliendra (1994). A vulnerability curve is a plot of the percentage loss of conductivity and the pressure that induced that loss of conductivity. Such a plot would establish the degree of water stress the two study species are able to withstand before total hydraulic failure. The methodology used here, was previously described in detail in Chapter 3. In summary, the conductivity through a stem segment was progressively reduced by forcing air into the vascular system with increasing pressures in steps of 0.5 MPa using a Scholander-type pressure chamber (PMS Instrument Company, Albany, OR, USA). The procedure was repeated until the cumulative loss of conductivity was more than 95%. Cumulative percent loss of conductivity plotted against applied pressure resulted in a vulnerability curve for the individual tree. The vulnerability curves were constructed from 10 individuals of *S. birrea* and 5 individuals of *T. sericea*.

STATISTICAL ANALYSES

The XPP data (rainfall gradient) were analyzed using One-way ANOVA. The vulnerability curves for *T. sericea* and *S. birrea* were fitted with a Weibull function (Neufeld et al. 1992). Statistical analyses were done using R version 2.7.0 (R Development Core Team 2008).

Results

Xylem pressure potentials for the two study species at my study site over the 2006-2007 growing season are shown in Figure 5.4. *S. birrea* maintains relatively high XPPs throughout the season, whereas *T. sericea* has overall lower values, especially so in dry conditions. The measurement taken during the mid-season drought, 8 March, shows high levels of water

stress (predawn: -2.0 to -1.5 MPa; midday: <-2.5 Mpa) in *T. sericea*, whereas *S. birrea* is not affected (predawn: >-1.0 MPa; midday: >-1.5 MPa).

Fitting the Weibull function (following Neufeld et al. 1992) to the vulnerability data estimated using the air injection method reveals that a 50% loss of conductivity (P_{50}) occurs at -2.0MPa in *T. sericea*, and at -1.0MPa in *S. birrea* (Fig 5.5). The overall steeper curve indicates that of these two species, *S. birrea* is most vulnerable to drought-induced cavitation. These vulnerability curves, in combination with the respective seasonal water status trends (Fig 5.4), show that *S. birrea* keeps XPP's above –and therefore water stress below– the level where conductivity decreases by 50%, whereas *T. sericea* operates (i.e. transpires, photosynthesizes) under much higher stress levels. However, both species stay well within the limits of XPP's causing total hydraulic failure.

The results for the predawn XPP measurements on the rainfall gradient for *S. birrea* are shown in Fig 5.6, for the three phenological stages (80-100, 25-75, 0-20 %crown cover; Fig 5.6a), and the three rainfall classes (501-600, 601-700, 701-800 mm/y; Fig 5.6b). The vulnerability curve for *S. birrea* was used to translate the found XPP's into percent loss of conductivity. No significant differences were found in predawn %loss of conductivity between the phenological stage classes (ANOVA, $F_{2,68} = 1.8515$, $p = 0.1648$; Fig 5.6c), the same is true for the mid-day %loss of conductivity (ANOVA, $F_{2,68} = 0.8966$, $p = 0.4127$; not shown). The relationships between %loss of conductivity and rainfall class were also not significant, neither for predawn (ANOVA, $F_{2,68} = 2.5812$, $p = 0.08309$; Fig 5.6d), nor for mid-day (ANOVA, $F_{2,68} = 1.528$, $p = 0.2243$;not shown).

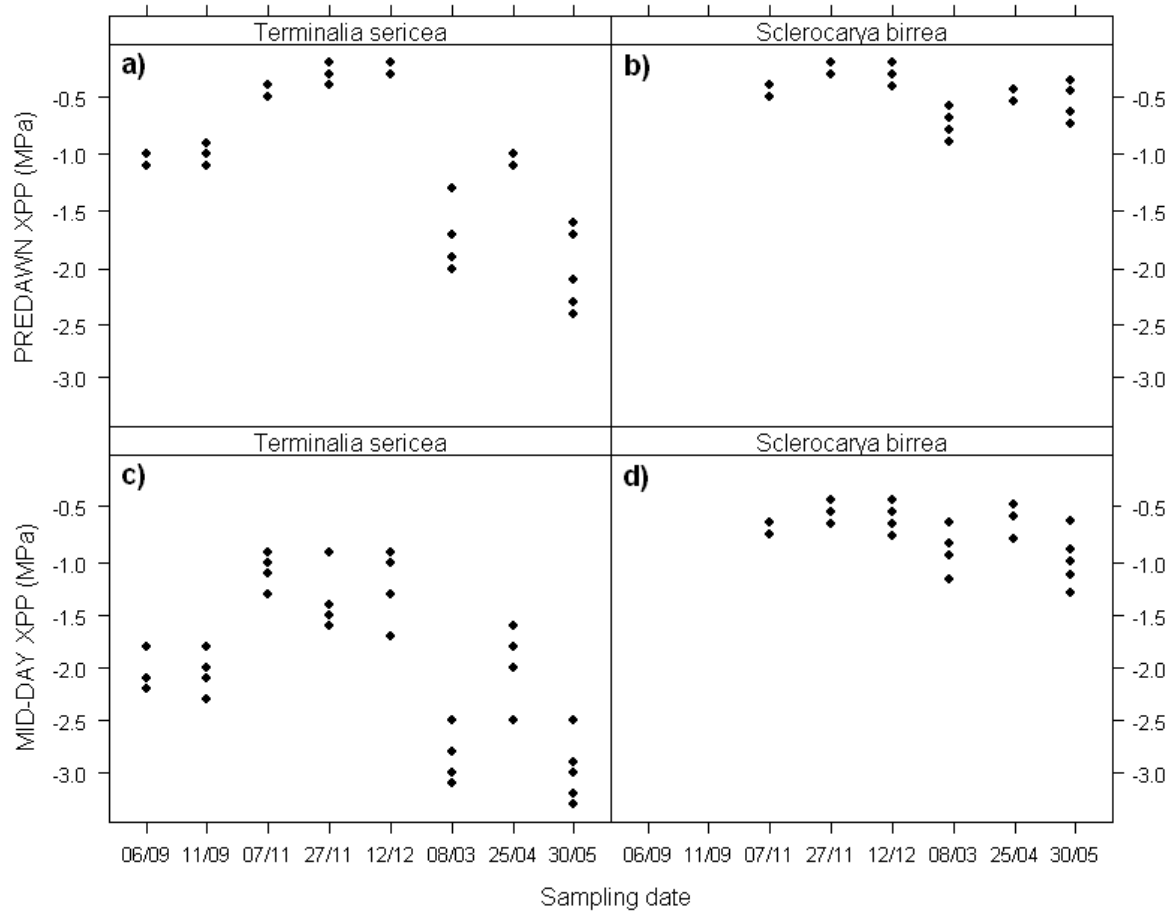


Fig 5.4: Xylem pressure potentials of *T. sericea* and *S. birrea* over the 2006-2007 rainy season. XPP of *T. sericea* was measured on two occasions in the late dry season, while *S. birrea* was not. On subsequent dates, XPP of both species was determined.

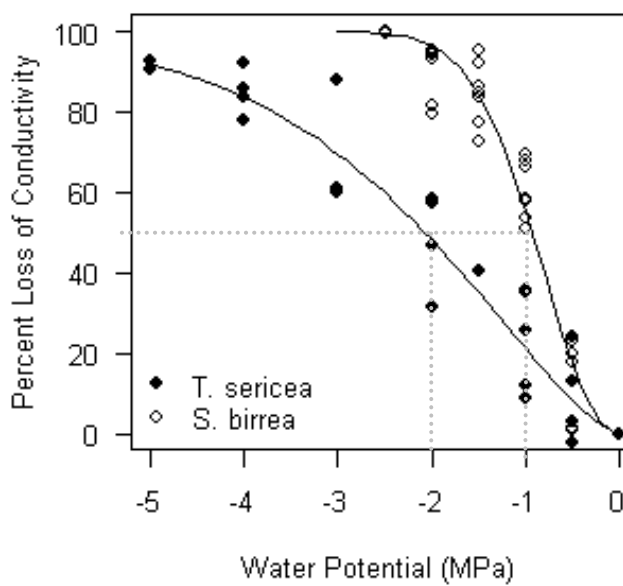


Fig. 5.5: Vulnerability curve for *T. sericea* and *S. birrea* as constructed with the air injection method. Conductivity is plotted as percent loss from initial value (PLC). The P_{50} value (dotted line), the pressure at which conductivity dropped to 50% from the initial value, lies at -2.0 MPa for *T. sericea* and at -1.0 MPa for *S. birrea*.

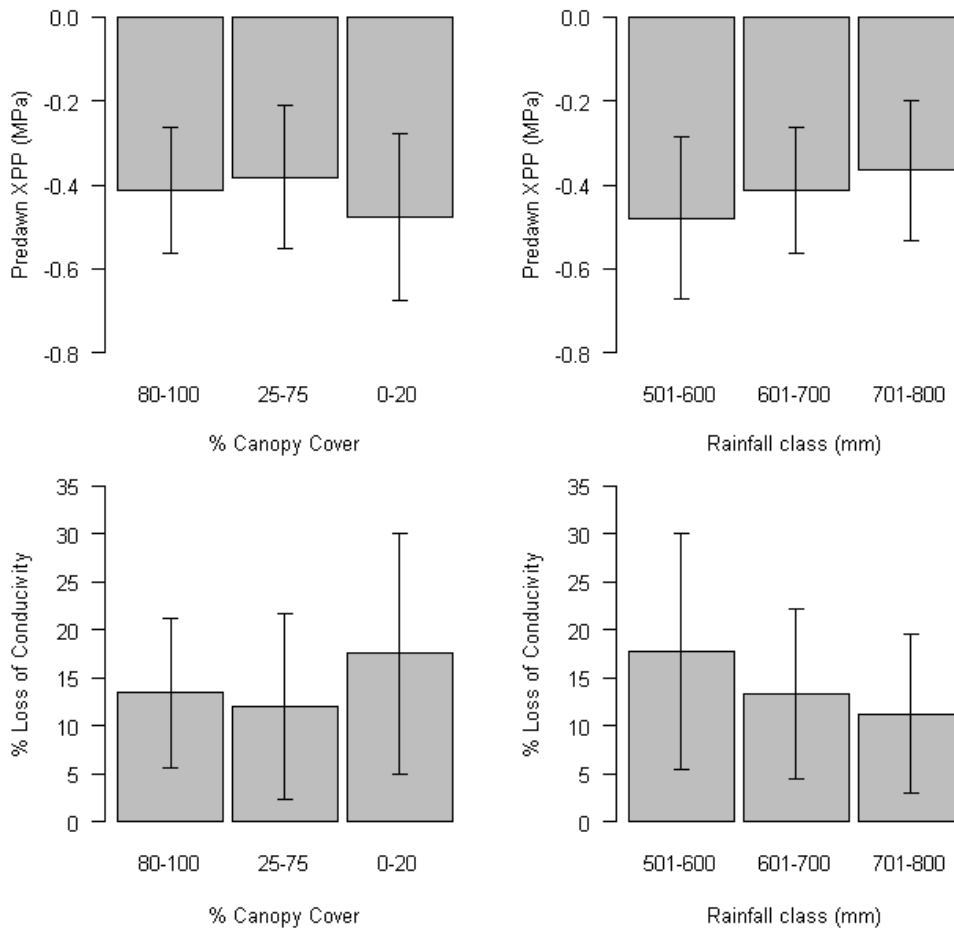


Fig 5.6: Means of the predawn xylem pressure potentials and % loss of conductivity for the different phenophases of *S. birrea* (% canopy cover) and for the different rainfall classes on the rainfall gradient. Error bars represent standard deviation. No significant relationships were found in predawn XPP, midday XPP (not shown), or loss of conductivity, neither for the phenophases, nor for the rainfall classes.

Discussion

The two species used for this study exhibit very different phenological responses to rainfall. *S. birrea* loses its leaves while soil moisture is still very high and XPP's are well below the 50% loss of conductivity values. To avoid intolerable levels of xylem cavitation (i.e. causing branch die-back), *S. birrea* may respond to moisture stress through stomatal closure. As it is more vulnerable to cavitation, it will do so quicker than other species, such as *T. sericea*.

Where *S. birrea* maintains high XPP's, the seasonal water status patterns suggest that *T. sericea* continues to transpire, even under dry conditions, hence operating under higher

levels of water stress. Contrasting to the drought-avoidance strategy of *S. birrea*, *T. sericea* is clearly the more drought tolerant of the two species. As a result, *T. sericea* is able to retain its leaves for longer extending its growing season substantially over that of *S. birrea*. The consequence, however, is that it does not have any stored water to leaf out early and is therefore reliant on the first rains (support for this statement is presented in Chapter 6, where the lack of stem shrinkage during the dry season suggests that no water is stored).

The relatively early leaf abscission in *S. birrea* prevents loss of water, which is stored in the stem. This water can be used to sustain the forming of leaves before the first rains of the next growing season. The benefit of this strategy is that the early leaf flush enables the tree to take full advantage of the available nitrogen following the first rains (Scholes & Walker 1993). Clearly, the water stored in the stem is not enough for the formation of a full crown of leaves, but it does enable *S. birrea* to obtain a head-start over the grass layer. Chapotin et al. (2006) found that water stored in Baobab trees was enough to support pre-rain green-up, but not stomatal opening before the first rains.

Xylem pressure potentials of *S. birrea* in the early dry season along the rainfall gradient are generally very high, and show no differences between phenological stages. When plotted on the vulnerability curve for *S. birrea*, XPP levels found in the field shortly after the rains, are well below the 50% loss of conductivity values, suggesting low levels of water stress at the time of leaf abscission. This could be an inherent attribute of the drought avoiding strategy that *S. birrea* is following: water potentials are kept high at all times. The tree may keep water potential in branches high by replenishing lost water with reserves in the trunk (Borchert 1994b). The shrinkage of the stem in *S. birrea* towards the end of the dry season (see Chapter 6) is a further indication of this mechanism.

There were also no significant differences in XPP detected between the different rainfall classes. This suggests that the trees do not experience water stress and that leaf abscission is triggered by another environmental factor. These findings are coherent with Do et al.

(2005), who found that models based on upper soil water availability or rainfall did not predict inter-annual variation in either leaf flush or leaf fall. Duff et al. (1997) conclude that predawn water potential is not a good indicator for phenological responses, such as flushing or leaf loss.

The phenological strategy and the associated seasonal water status pattern in *S. birrea* suggests that early leaf abscission at the end of the growing season may facilitate early green-up in the following growing season, and that phenology seems to be decoupled from the actual start and end of the rainy season. Other environmental cues, possibly day length or temperature, are probably responsible for the observed patterns.

Concluding, these different responses in leaf phenology suggest different strategies for avoiding competition for resources with grasses. The observation that trees have different phenological strategies may also suggest inter-tree competition avoidance. Following the first rains, nitrogen is mineralized through microbial activity and becomes available to the plant (Keretetse 2009). *S. birrea*, because it already has leaves deployed, is able to utilize this nitrogen as soon as it becomes available and before it becomes immobilized by grasses, other trees or microbes. *T. sericea* is only able to leaf out with the first rains and is therefore competing directly with microbes and grasses for nitrogen at the mineralization peak.

CHAPTER 6

**RELATING PLANT TRAITS TO PLANT FUNCTIONAL
TYPE: TRADE-OFFS IN WATER USE AND GROWTH**

University of Cape Town

Chapter 6:

Relating plant traits to plant functional type: trade-offs in water use and growth

Introduction

Trees in savannas have evolved physiological mechanisms to tolerate and/or avoid prolonged periods of resource stress. Plants can adapt to environmental conditions over evolutionary time, but can also adjust their shape and structure in response to temporal and spatial fluctuations in external stresses (Read & Stokes 2006). An example of phenotypic plasticity in trees is the timing and duration of leaf flush and leaf fall, i.e. foliar phenology, which may change in response to changing environmental conditions (e.g. climate change; Nord & Lynch 2009). With regard to adaptation to seasonal drought by means of foliar phenology, one can identify a number of plant functional types. The obvious distinction in leaf habit is 'evergreen' versus 'deciduous' (Givnish 2002). Evergreen species invest in longer lived leaves, whereas deciduous species support shorter-lived leaves with high leaf photosynthetic capacity (Hutley & Setterfield 2008). There is a multitude of interpretations possible in the usage of the term 'deciduous', because a quantitative connotation is often lacking (Singh & Kushwaha 2005). Adding information on the duration of leafnessness is useful when attempting to explain phenological patterns and the trees' biomechanical properties associated with it. Based on the findings described in the previous chapters, I conclude that *Sclerocarya birrea* should be classified as a "stem succulent" tree. In stem succulents -after rapid leaf abscission- the trunk rehydrates and maintains high water potentials throughout the dry season (Reich & Borchert 1982). Trees remain leafless for several months; bud break of vegetative bud in trees of this functional type has been found to be induced by increasing photoperiod (Rivera et al. 2002, Singh & Kushwaha 2005). A large portion of the stem succulent trees' biomass is composed of tissues that neither participate in gas exchange, nor in water uptake, but transport and store water below and above ground.

This stored water may be used to support the forming of the new leaves of the following growing season (Chapotin et al. 2006).

According to the previously described phenological and eco-hydrological data of *Terminalia sericea*, this species seems to adopt aspects of both the “(fully) deciduous” and the “brevi-deciduous” functional types as defined by Williams et al. (1997). The definitions were based on tree species growing in savannas. Fully deciduous species are species in which each individual loses its leaves for a period of at least one month during the dry season. Leaves are shed early in the dry season followed by a strong dehydration of stems, with bud break occurring after the first rains (Reich & Borchert 1984; Borchert 1994a; Williams et al. 1997). Brevi-deciduous species shed their leaves synchronously, and after a brief leafless period, bud break is induced by increasing photoperiod (Rivera et al. 2002). The savanna species *T. sericea*, sheds leaves gradually as soil moisture declines rather than in a synchronous sudden leaf-fall event. Although xylem pressure potentials decrease sharply after the last rains, leaves are shed very late in the dry season. Hence, the completely leafless period is relatively short (a few weeks). Contrasting with *S. birrea*, leaf formation was -during the study period- only observed to occur after the first rains, not before. This makes soil moisture the most probable trigger for bud-break in *T. sericea*.

The objective of this Chapter is to further investigate the phenological strategies of *S. birrea* and *T. sericea* by assessing whether seasonal patterns of plant growth and water use do match the profile of the plant functional types as I assigned them to the species. I do this by measuring stem circumference increment, seasonal transpiration (sap flow) and water use efficiency. The questions I ask are: 1) what do the observed water use patterns in the two study species tell about hydraulic constraints, 2) can the differences in phenology be explained in terms of differences in biomechanics, and 3) what are the implications for the strategies displayed by the study species? I hypothesize that there is a trade-off in having the ability to flush new leaves in the late dry season and the ability to extend the full-crown phenophase well into the dry season.

Methods

STUDY SITE

The research was conducted within an enclosure along the Shabeni Experimental Burn Plots near Pretoriuskop, Kruger National Park, between February 2006 and June 2008. The enclosure was constructed to protect stationary equipment from damage by animals. The site was burned in 2001, but protected from fire afterwards. The site can be described as a broad-leaved open woodland savanna dominated by *Terminalia sericea*. Dominant grasses are *Hyperthelia dissoluta*, *Setaria sphacelata*, and *Hyparrhenia hirta*. The region experiences hot summers and mild, mostly frost-free winters. Average annual rainfall at Pretoriuskop is 736mm (for measured rainfall during study period, see Chapter 2). The weathered, sandy soils are moderately deep. Elevation is approximately 600m.

STEM GROWTH

Dendrometer bands (Agricultural Electronics Corporation, Tucson, AZ, USA) were fitted around the trunks of a total of 24 trees: 8 adults and 4 saplings of both *T. sericea* and *S. birrea*. Saplings (or Gulliver trees) were defined as trees of less than 7 cm DBH, with part of the crown within the grass layer, i.e. prone to fire coppicing. Dendrometer bands are metal bands fitted around a tree and are held in place under tension by a spring-loaded vernier gauge. The material from which the band is constructed has a rated coefficient of thermal expansion of $11.2 \mu\text{m m}^{-1} \text{ }^{\circ}\text{C}^{-1}$. Changes in radial diameter are read manually from the vernier gauge at a resolution of 0.1 mm. The dendrometer may show swelling as well as shrinking of the stem, due to changes in water content and/or increases in diameter from new wood. Manual readings of the vernier gauge were made at the end of each month from February 2006 to the end of May 2008. For the first and last six weeks of the two growing seasons, the dendrometer bands were read bi-weekly.

WATER USE EFFICIENCY: THE BALL-BERRY STOMATAL MODEL

In order to determine the water use efficiency of both study species, gas exchange measurements were done using the LI-COR-6400 (LI-6400; LI-COR, Lincoln, Nebraska, USA). The LI-6400 utilizes gas exchange principles to measure the photosynthesis rates of plants. Net photosynthesis rates are expressed as rates of CO₂ uptake ($\mu\text{molCO}_2 \text{ m}^{-2} \text{ s}^{-1}$). Gas exchange in the LI-6400 is measured in an open-mode design, thus the CO₂ level of the air is maintained steady-state. Since CO₂ intake and H₂O release share the same pathway – the stomata-, photosynthesis measurements with the LI-6400 instrument include the estimation of photosynthesis, stomatal conductance, and transpiration.

A model capable of integrating the effects of several environmental factors on stomatal conductance, was introduced by Ball et al. (1987). This empirical law describes the behavior of stomatal conductance as a function of environmental conditions and net photosynthetic rate. The Ball-Berry equation calculates stomatal conductance (g) for water vapor in C3 plants as a function of net assimilation (A), relative humidity at the leaf surface (RH), CO₂ concentration at the leaf surface (C_a), minimum conductance (g_0) and the slope (m):

$$g = g_0 + (m \cdot ARH / C_a)$$

The Ball-Berry parameter m is believed to change little across biomes and environmental conditions. The slope m has been found to be unaffected by water stress or leaf age (Xu & Baldocchi 2003). The relationship between stomatal conductance and photosynthetic capacity is found to be linear (Xu & Baldocchi 2003). Stomatal sensitivity can be inferred from the slope of the relationship, as photosynthetic rate corresponds to stomatal aperture, so that m is a measure of stomatal control, or WUE of the species. Comparing Ball-Berry slopes for different plant species provides valuable insights in differences in WUE, making m

a fundamental ecosystem descriptor (Wolf et al. 2006). A steeper slope, m , indicates that for a similar photosynthetic rate, more water is lost through the stomata.

MEASURING SAP FLOW WITH THE THERMAL DISSIPATION METHOD

A custom-made xylem sap flow measuring system was used to quantify whole-plant water use in two dominant tree species in the study area. A widely-used empirical method for measuring sap flow velocities in trees was developed by Granier (1985, 1987). The major advantages of the thermal dissipation method are that it is relatively easy to install compared to other methods, it has straightforward requirements for recording sensor outputs and has lower costs (Smith and Allen 1996). The system consists of two sensor probes, which are radially inserted into the tree, 15cm apart from each other (see Fig 6.1). I constructed sap flow sensors as described in a sap flow probe construction tutorial composed by Hamilton (2004). Constant power (0.2W) is applied to heat the upper (downstream) probe; the lower (upstream) reference probe is left unheated. The constantan ends of the two copper-constantan thermocouples are connected to each other, to measure the temperature difference between the two probes at the ends of the copper wires. This temperature difference is influenced by the heat dissipation effect of sap flow in the vicinity of the heated probe. The Granier system directly measures the electrical potential difference (ΔV) between the two thermocouples. Sap Flux Density (F_d) – the sap flow per unit sap wood area ($\text{kg m}^{-2} \text{h}^{-1}$) – can be directly calculated from the voltage measurements with an empirically derived formula (Granier 1985):

$$F_d = 118.99 * 10^{-6} [(\Delta V_{\max} - \Delta V) / \Delta V]^{1.231}$$

Total sap flow (F) (kg h^{-1}) can be calculated by multiplying F_d with the sapwood cross sectional area. However, in this study I have not succeeded in properly determining sapwood area, as wood cores did not reveal clear differences in colour. Cutting down the trees on

which I did the sap flow measurements could have provided me with the necessary information, but permits to do this are understandably not easily issued in protected areas. The gains in knowledge and its practical implications were weighed against the adverse consequences that cutting the stems would have for the trees. I therefore decided to report the sap flow velocities per unit sapwood area, rather than total sap flow.

To convert raw Granier sensor data (ΔV) into sap flow readings, I used BaseLiner version 2.4.2 (© Y. Parashkevov, Duke University). As mentioned above, sap flow is calculated based on the difference between measured ΔV and expected ΔV_{\max} . The challenge in converting raw Granier data is to establish an appropriate baseline, as ΔV_{\max} is not constant. BaseLiner accomplishes this by identifying no-flow conditions (ΔV_{\max}) and setting baseline points at those times. The baseline is then calculated by linear interpolation between the points. I assumed that a no-flow condition occurred every night at pre-dawn. I report the data as sap flow velocities per unit sapwood area (see Chapter 2). An example of what the data looks like when plotted is given in Figure 6.2.

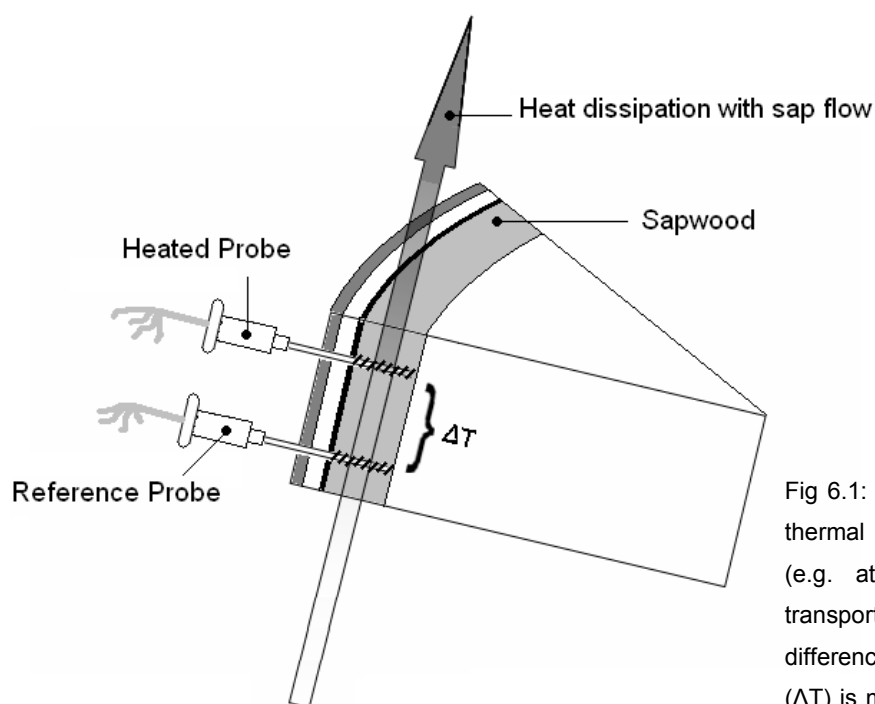


Fig 6.1: Schematic illustration of the principle of the thermal dissipation method. If there is no sap flow (e.g. at night), then the heat does not get transported away from the heated probe, i.e. the difference in temperature between the two probes (ΔT) is maximal. As sap flow rates increase, heat is dissipated more rapidly and so (ΔT) decreases. Adapted from Motzer (1998).

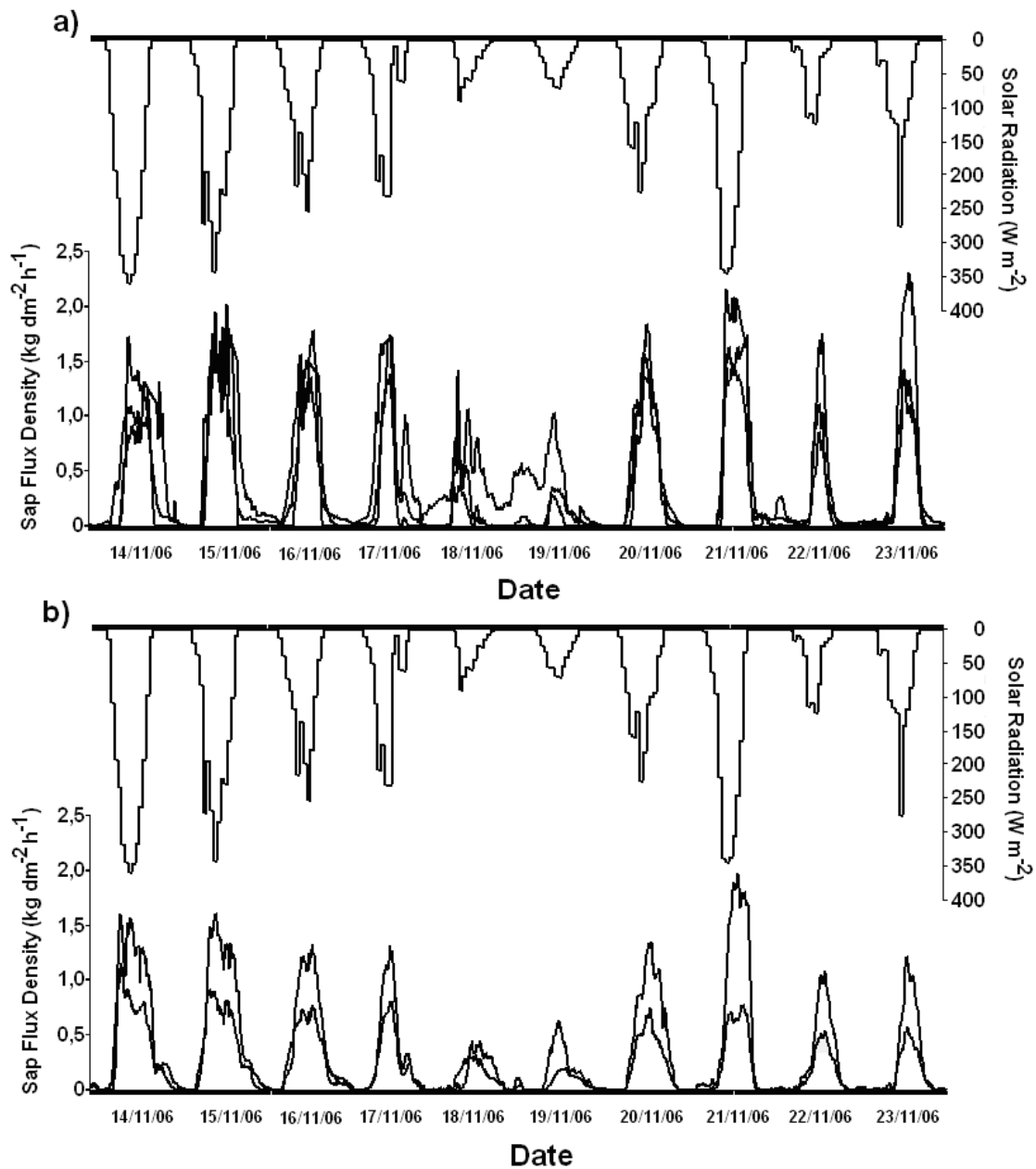


Fig 6.2: Cave plot, depicting the relationship between daily courses of sap flux density and solar radiation during a randomly chosen sequence of 10 days in the early growing season (November 2006), for a) *T. sericea* and b) *S. birrea*.

Results

Stem circumference increment over the two years of study was approximately 17mm and 10mm for adult trees of *T. sericea* and *S. birrea*, respectively, which would be an average of 8.5mm (*T. sericea*) and 5mm (*S. birrea*) per year. Saplings increased 20mm (*T. sericea*) and 52mm (*S. birrea*), with an average annual rate of 10 and 26mm, respectively.

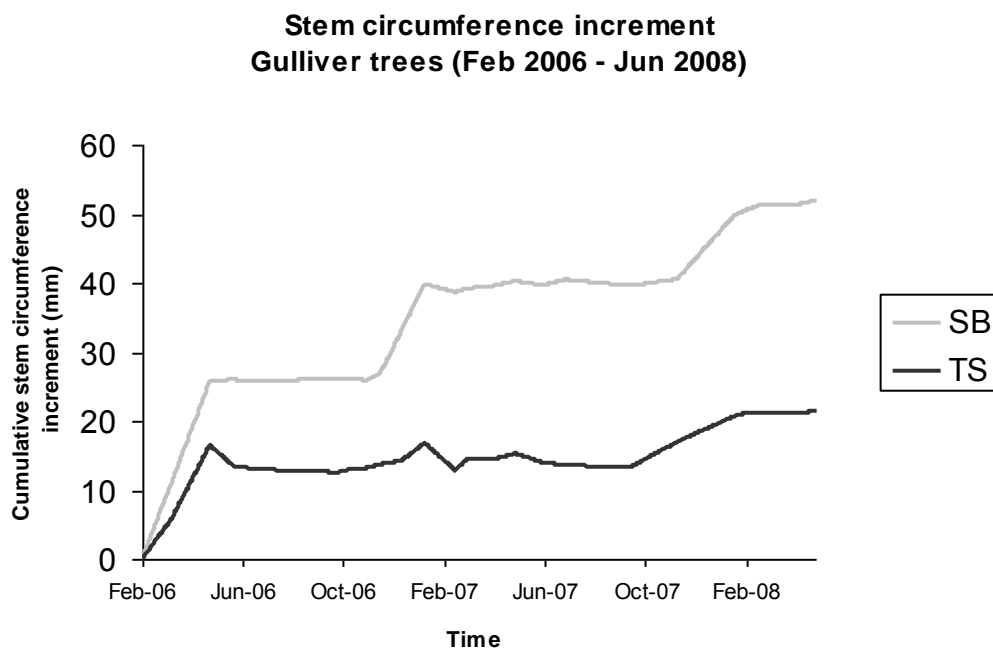
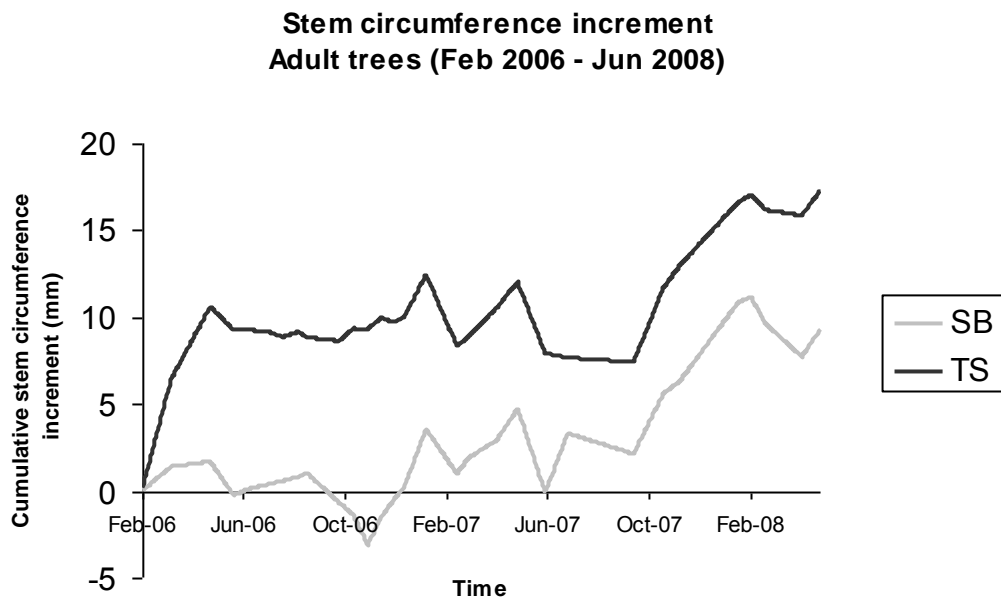


Fig 6.3: Stem circumference increment in *S. birrea* and *T. sericea* for a) adult trees and b) saplings (“Gulliver trees”, *sensu* Bond & Van Wilgen 1996).

Where adult trees of *S. birrea* realized a striking decrease in stem circumference, before the onset of the 2006-2007 growing season, *Terminalia sericea* did not. In the midseason dry periods in both growing seasons, both species decreased in stem circumference.

The Ball-Berry relationship for the study species is shown in Figure 6.4. As the Ball-Berry model underperforms when atmospheric CO₂ concentrations are too low below ambient (Wolf et al. 2006), I only used data when C_a > 360 μmol mol⁻¹. The linear regression equation for *T. sericea* is $g = 7.077 \text{ ARH}/C_a - 0.0024$ ($R^2 = 0.87$, $p < 0.0001$) and for *S. birrea* this is $g = 16.188 \text{ ARH}/C_a - 0.0034$ ($R^2 = 0.89$, $p < 0.0001$). This shows that *S. birrea* has the steepest slope, meaning it is less water use efficient than *T. sericea*. The difference in sample size is due to the logistical challenges faced when measuring photosynthesis using the IRGA in such tall trees as *S. birrea*.

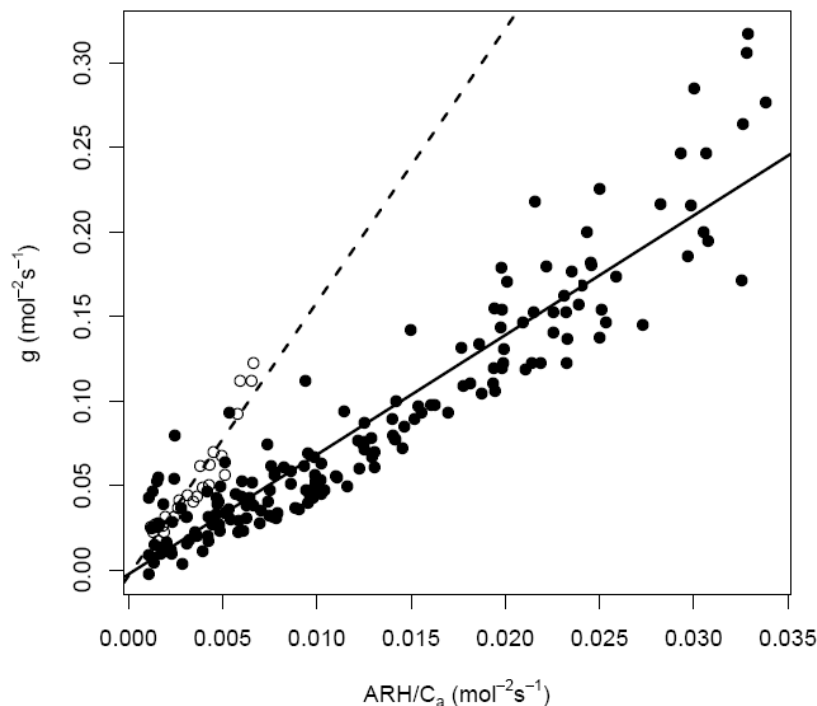


Fig 6.4. Relationship between measured stomatal conductance (g) and the product of net photosynthesis (A ; $\mu\text{mol mol}^{-1}$) and relative humidity (RH) divided by external CO₂ concentration (C_a ; $\mu\text{mol mol}^{-1}$), as determined by the Ball-Berry equation for *S. birrea* (open circles, dotted line) and *T. sericea* (closed circles, solid line).

Sap flow velocities per unit sap wood area per day were averaged per month in the growing season, and are shown in Fig. 6.5. There is a remarkable difference in average velocities between *T. sericea* and *S. birrea* in the months December and January, with *T. sericea* having the highest relative sap flux density. For *T. sericea*, values for the months March and April were pooled. For *S. birrea*, there were not enough data for this period.

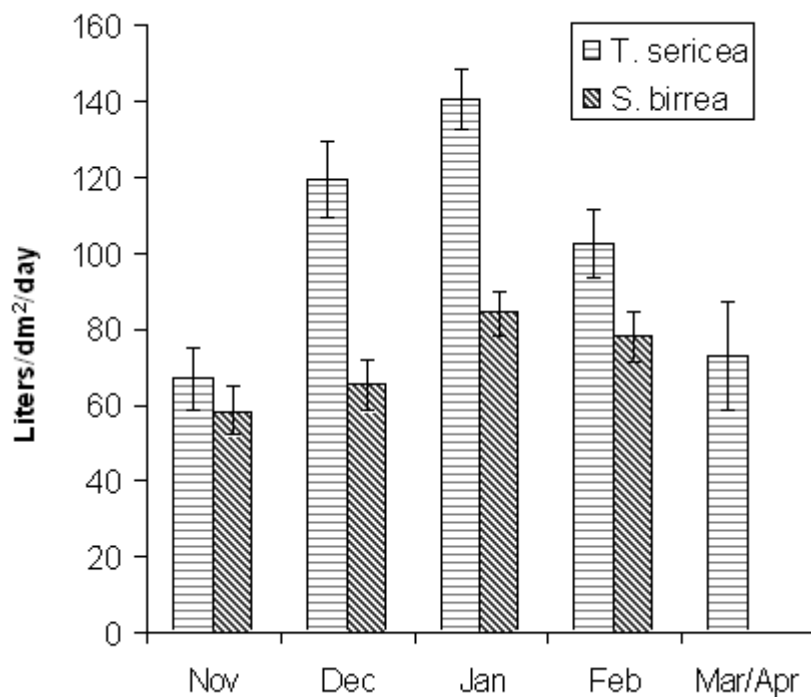


Fig 6.5: Average monthly water use per unit sap wood area for the 2006-2007 growing season.

Discussion

Both *S. birrea* and *T. sericea* showed higher stem circumference increment rates as saplings than as adult trees. Stem diameter increment rates for *S. birrea* were higher than *T. sericea* in Gulliver trees, but lower in adult trees. The realized increment rates are within the same range as found in a study by Scogings (2010), who reported stem circumference increments for six studies in the Kruger National Park (not including my study species). Changes in stem circumference may include shrinking and swelling of the stem, with changes in water content as well as increases in diameter from new wood (February et al. 2007). Adult *S. birrea* trees realized a sharp decrease in stem circumference (and produced leaves, Chapter 5) before the onset of the 2006-2007 growing season. *T. sericea*, on the other hand, did not realize a decrease in stem circumference and did not produce leaves before the rains. However, both *S. birrea* and *T. sericea* realized a decrease in stem diameter during the midseason drought in the 2006–2007 season. Neither tree species realized a decrease in stem diameter at the beginning of the 2007–2008 growing season, which included early rains (see Chapter 2). Again, both species realized a decrease in stem diameter during a dry period in February 2008. Assuming that the decrease in stem circumference can solely be attributed to water loss, the observed pre-growing season decrease is evidence that *S. birrea* uses stored water to produce leaves in the absence of rain. The early leaf deployment may confer an advantage over grasses in the competition for nitrogen, as the first rainfall after the dry season triggers a peak in nitrogen mineralization (Scholes & Walker 1993).

The results further suggest that *S. birrea* has lower water use efficiency than *T. sericea*, but – from the sapflow data- seems to transpire less water per unit sap wood area per day than *T. sericea* when averaged over longer periods (months) within the growing season.

In Chapter 5, I showed that *S. birrea* is more vulnerable to xylem cavitation. Although I did not measure and compare xylem vessel diameter of both tree species, I suppose that –given the vulnerability curves and the general association of wider vessel diameter with increased vulnerability to drought-induced cavitation (Sperry & Saliendra 1994) - assuming wider

vessels for *S. birrea* compared to *T. sericea* is not far-fetched. In general, a rule of thumb is that large diameter vessels are an advantage in times of high soil water availability. Water can then be transported to the leaves faster, increasing photosynthesis and tree growth (Tyree et al. 1994). In dry times, species with large xylem vessel diameters are more prone to hydraulic failure and need to prevent excessive water loss. If *S. birrea* does indeed possess a hydraulic architecture that would allow for rapid water transport, then I would anticipate high wet season sap flow rates. Instead, the sap flow data for *S. birrea* suggest conservative sap flow rates compared to *T. sericea*, which is quite the opposite of what I expected to find. Difficulties associated with determining the depth of the sap wood makes that one cannot exclude the possibility that sap flow in *S. birrea* was underestimated. This may happen if the heat dissipation probes come into contact with inactive xylem (Clearwater et al. 1999). Future sap flow measurements in *S. birrea* may need to be done using the heat pulse velocity (HPV) method, as with this method it is easier to determine the decrease in sap flow velocities with increasing depth of the inserted probe. On the other hand, sap velocities per se may not mean lower water use in *S. birrea*, as stems of *S. birrea* ($98.3\text{cm} \pm 47.2\text{cm}$) on average were wider than *T. sericea*. ($89.3 \pm 21.0\text{cm}$).

CHAPTER 7

SYNTHESIS

University of Cape Town

CHAPTER 7:

Synthesis

In this thesis I investigated the classic question of how trees and grasses manage to maintain long-term coexistence in savannas from the niche perspective. A longstanding, well-documented hypothesis -the two-layer hypothesis- has lost ground in recent years as an increasing number of studies demonstrated below-ground niche overlap rather than niche differentiation. Researchers have therefore moved away from this hypothesis and have formulated alternative hypotheses for tree-grass co-dominance, most of these based on stochastic mechanisms, such as climatic variability and variability in fire frequency and intensity (e.g. Jeltsch et al. 1996; Higgins et al. 2000; Beckage et al. 2009). This may be a manifestation of a recent general trend in community ecology to rethink the classical paradigm based on the niche perspective, or, in the words of Chase & Leibold (2003) “a decline in interest in the niche theory”. Lacking direct support for niche partitioning, non-equilibrium models have gained attention as explanations for species diversity in general and tree-grass coexistence in particular (Menaut et al. 1990; Higgins et al. 2000; Gardner 2006). Support for these hypotheses has also been mixed, resulting in a consensus that both resource availability and disturbance interact to determine the structure and function of savanna communities (Adler et al. 2007; Scheiter & Higgins 2007). In fact, there is a wide recognition among ecologists that simultaneous mechanisms are at work in a so-called niche-neutral continuum (Gravel et al. 2006; Adler et al. 2007; Mutshinda & O’Hara 2010). Future work in community ecology should focus on making this continuum concept operational and measurable .

I believe that although niche separation along environmental axes on spatial and/or temporal scales is unlikely to be the only mechanism of coexistence in savannas, it may play a more significant role than has been appreciated recently (Silvertown 2004; Riginos 2009).

Questions related to resource use by trees and grasses, and the role of the niche concept in savannas and other ecosystems are, in my opinion, far from exhaustively and satisfactorily answered. With this thesis I hope to have contributed to filling the hiatus in our understanding, or at the least have provided leverage for further research.

TWO-LAYER HYPOTHESIS

Plant access to soil resources is a fundamental aspect of terrestrial life that remains poorly understood. According to the two-layer hypothesis for tree-grass coexistence in savannas, woody plants use deep soil compartments to avoid competition with grasses, which dominate the upper soil layers. The direct measurements of root activity needed to test this hypothesis are rare as a result of difficulties associated with below-ground research. In two separate experiments I managed to directly test root activity. In the first experiment (Chapter 3), deep and shallow roots of *Terminalia sericea* saplings were severed, six weeks before the onset of the new growing season, using a novel technique. During the course of the growing season, physiological responses to the root severing were measured. Severing lateral (shallow) roots resulted in rapid abscission of the old leaves, smaller leaves after the onset of the following growing season and lower xylem pressure potentials –indicating higher water stress– compared to the other treatment groups. Effects of severing tap (deep) roots were small, demonstrating the importance of shallow roots for maintaining the trees' water balance, notably in drier times. The results of the root severing experiment show that trees do take up significant amounts of resources from the surface soil layers, despite the presence of grasses, and that exclusive access to deeper soil layers does not enhance their performance. The results further suggest that *T. sericea* manages to coexist with grasses without avoiding competition through spatial root separation. The results of my study therefore do not support the two-layer hypothesis as the explaining model of tree-grass coexistence in this mesic savanna. The coarse resolution (0-50cm versus 50-100cm) poses a potential problem in the interpretation of the results. The main conclusion that can be

drawn is that the two-layer hypothesis is not applicable in the study area in the sense that trees do not rely on resources beyond the vertical reach of grasses.

By quantifying the location of water uptake by different plants, the depth-controlled tracer technique (as presented in Chapter 4) allowed a critical step to be taken towards understanding plant access to soil resources. In the described experiment, deuterated water ($^2\text{H}_2\text{O}$) was injected into four soil depths, at four times of the year, to measure the vertical and horizontal location of water uptake by trees and grasses. Trees absorbed 24, 59, 14 and 4% of tracer from the 5, 20, 50, and 120 cm depths, respectively, while grasses absorbed 61, 29, 9 and 0.3% of tracer from the same depths. Trees absorbed tracer under and beyond their crowns, while 98% of tracer absorbed by grasses came from directly under the stem.

The results showed that plants can partition soil resources over small spatial scales and that the tracer technique can be used to detect these differences. This partitioning, however, did not reflect the ability of trees to access deep soil water that was unavailable to grasses, as suggested by the two-layer hypothesis. The results stress the importance of precise root activity measurements. Future research that combines the tracer technique with species-level estimates of transpiration offers the potential to produce quantitative and spatially explicit species-level water budgets. These water budgets are needed to predict how changes in precipitation and temperature are likely to change plant growth and feedback to influence hydrologic cycles (Casper et al. 2003; Li et al. 2007).

The abundance and overwhelming relative activity of tree roots relatively close to the soil surface, with neither trees nor grasses accessing considerable quantities of water deeper than 50 cm, competition for resources between trees and grasses may not be focused on water, but on nutrients (Mordelet et al. 1997). Nitrogen concentrations in the soil decrease significantly with depth with the greatest concentrations in the top 20 cm (Gebauer & Schultze 1991; Nadelhoffer & Fry 1994; Fynn et al. 2003). The results of Chapter 4 suggest that -possibly in the competition for N- grasses outcompete trees in the top 5 cm of the soil

(where N content is highest), whereas trees are only able to access resources below this (at 20 cm) where N concentrations are lower. Possibly, the niche partitioning should be seen as grasses being superior competitors, with trees rooting as close to the soil surface as possible (instead of growing deep) and wherever grass roots are not (or less) present. The wider foraging by trees seems necessary for them to persist in the system. The emphasis in this modification of the two-layer hypothesis is still on spatial avoidance of competition, but in a horizontal rather than vertical direction. The wide foraging for water and/or nutrients may also invoke tree-tree competition, possibly contributing to the persistence of the tree-grass mix in savannas through self-thinning mechanisms (Sea & Hanan 2011).

PHENOLOGICAL NICHE SEPARATION

Alternatively, the phenological niche separation hypothesis proposes that trees may avoid this competition for resources (both intra- and inter-life form) on a temporal dimension, by leafing out before the rainy season begins. When nitrogen mineralization peaks at the onset of the rains, trees such as *S. birrea* (by having their full photosynthetic potential in place), are temporarily able to monopolize the nitrogen resource until grasses display a significant leaf area (Scholes & Walker 1993). It may be this temporal access to available resources with grasses out-competing trees in the upper layers of the soil that can explain the considerable structural diversity for savanna from arid shrublands through lightly wooded grasslands to deciduous woodlands and dry forest (Scholes & Walker 1993; Sankaran et al. 2004). Scholes and Archer (1997) describe the manner in which temporal niche partitioning may explain the variation in tree-grass ratios across rainfall gradients as follows:

“Using a model that included the grass rooting-depth niche entirely within tree niche, we would predict dominance by trees wherever seasonality is strong, protracted and predictable. In arid unpredictable environments dominated by small rainfall events, the opportunistic strategy of grasses would be favored [...], while in continuously moist environments (such as those occupied by forests), a continuous growth, overtopping strategy

is favored. Within its area of applicability, the model predicts greatest advantage for trees where there is greatest potential for between-season carryover of resources, which is in the moister savannas and on deeper, sandier soils” (Scholes and Archer 1997).

In Chapters 5 and 6, I showed that two dominant savanna tree species display different strategies to cope with seasonal drought and competition with grasses.

Insights into the mechanisms enabling the early green-up of some savanna tree species will contribute to the understanding of temporal resource use and competitive interactions with grasses. Scholes & Archer (1997) suggested that trees may use stored resources from the previous growing season. My results suggest that the water needed to support the pre-rain leaf flush may indeed come from storage in the stem of *S. birrea*. Just before the onset of the 2006-2007 growing season, *S. birrea* realized a decrease in stem diameter and produced leaves before the rains, in contrast to *T. sericea* (Chapter 6).

Contrary to other tree species in the region, *S. birrea* sheds its leaves in the early dry season, while still well-hydrated. This observation led me to hypothesize that *S. birrea* needs to be conservative with water, especially at the end of the growing season, in order to have enough water in store at the onset of the following growing season. An important outcome of the study presented in Chapter 5, is that water stress does not seem to be driving leaf abscission in *S. birrea* at the onset of the dry season, supporting the water saving strategy hypothesis. Seasonal xylem pressure potentials and vulnerability curves indicate drought-tolerance in *T. sericea* and drought-avoidance in *S. birrea*. Results of the water status measurements of *S. birrea* revealed that leaf abscission is neither related to location on the rainfall gradient nor to phenological stage, i.e. remaining percent canopy cover, in the early dry season.

As a pilot study, the water status measurements for *S. birrea* in different phenophases is valuable. The main limitations of this study, however, are that 1) it does not supply evidence

for an alternative trigger for leaf abscission and 2) the rainfall classes were based on a distance-interpolated rainfall map constructed from long-term averages for the study area. Although long-term averages suggest that there is a rainfall gradient, assuming a proportional relationship along the transect, with distance from Pretoriuskop to Skukuza as a proxy for a decline in rainfall, is risky. As rainfall events in the southern Kruger National Park tend to be very local, the classification may not be precise enough to draw far-reaching conclusions about the relationship between rainfall and leaf phenology. Between season variation in localized rainfall may render trees that fall into a certain rainfall class on the map to fall into a different class in the field, depending on the rainfall patterns of the season. Rainfall could also be too poor a proxy for plant water availability. Therefore, the way forward is an approach in which a number of weather stations along the rainfall gradient measure the local conditions, including soil moisture, and where a number of trees in the proximity of these weather stations are selected for phenology and water status monitoring. Other potential triggers for leaf flush and leaf abscission, such as temperature and day length, could then also be taken into account.

Conclusion

In conclusion, I have found that trees and grasses did partition soil resources, but this partitioning did not reflect, as suggested by the two-layer hypothesis, the ability of trees to access deep soil water that was unavailable to grasses. With regard to the phenological niche separation hypothesis, I conclude from my work that tree species have different ways to overcome seasonal drought and competition for the scarce water resources, one of the possible ways being a different timing of phenophases. I have linked these patterns of water use to physiological traits of the study species and discussed involved trade-offs in Chapter 6. More work is needed to find out whether the observed patterns actually are the reflection of different niche occupations, and the importance of this mechanism in explaining long-term coexistence of trees and grasses in savanna systems.

CHAPTER 8

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