Ecophysiology of encroaching *Acacia mellifera* in intra- and inter-specific interactions

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Supervisors: David Ward
Michael D Cramer

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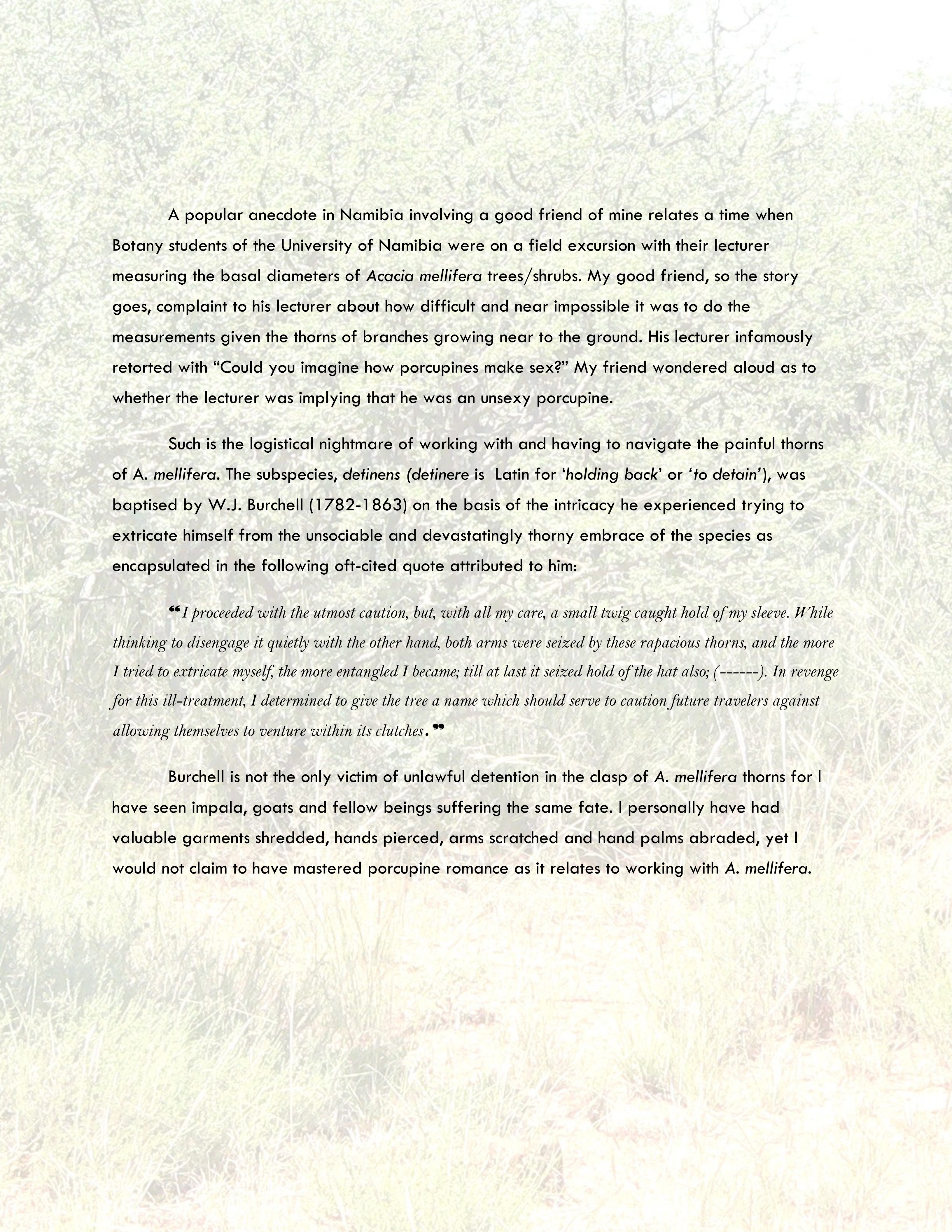

A popular anecdote in Namibia involving a good friend of mine relates a time when Botany students of the University of Namibia were on a field excursion with their lecturer measuring the basal diameters of *Acacia mellifera* trees/shrubs. My good friend, so the story goes, complaint to his lecturer about how difficult and near impossible it was to do the measurements given the thorns of branches growing near to the ground. His lecturer infamously retorted with “Could you imagine how porcupines make sex?” My friend wondered aloud as to whether the lecturer was implying that he was an unsexy porcupine.

Such is the logistical nightmare of working with and having to navigate the painful thorns of A. *mellifera*. The subspecies, *detinens (detinere* is Latin for ‘*holding back*’ or ‘*to detain*’), was baptised by W.J. Burchell (1782-1863) on the basis of the intricacy he experienced trying to extricate himself from the unsociable and devastatingly thorny embrace of the species as encapsulated in the following oft-cited quote attributed to him:

> “*I proceeded with the utmost caution, but, with all my care, a small twig caught hold of my sleeve. While thinking to disengage it quietly with the other hand, both arms were seized by these rapacious thorns, and the more I tried to extricate myself, the more entangled I became; till at last it seized hold of the hat also; (------). In revenge for this ill-treatment, I determined to give the tree a name which should serve to caution future travelers against allowing themselves to venture within its clutches*.”

Burchell is not the only victim of unlawful detention in the clasp of *A. mellifera* thorns for I have seen impala, goats and fellow beings suffering the same fate. I personally have had valuable garments shredded, hands pierced, arms scratched and hand palms abraded, yet I would not claim to have mastered porcupine romance as it relates to working with *A. mellifera.*

"The universe is like a safe to which there is a combination. Bit the combination is locked up in the safe."

Peter de Vries *(1910-1993)*

American editor and novelist

Quote sourced from Proverbia.net

http://en.proverbia.net/citastema.asp?tematica=373

Preface

The research presented in this thesis was carried out at the School of Biological and Conservation Sciences (SBCS) of the University of KwaZulu-Natal (UKZN) at Pietermaritzburg, South Africa under the supervision of Professor David Ward and co-supervision by Associate Professor Michael D. Cramer of the University of Cape Town (UCT).

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

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Jack Ratjindua Kambatuku (**Candidate)**

15 December 2010

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Prof David Ward (**Supervisor)**

15 Decemberr 2010

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Ass. Prof Michael D Cramer (**Co-Supervisor)**

15 December 2010

Faculty of Science and Agriculture

Declaration 1: Plagiarism

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

1. The research reported in the thesis, except where otherwise indicated, is my original research
2. This thesis has not been submitted for any degree or examination at any other university
3. The thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
    a. Their words have been rewritten but the general information attributed to them has been referenced.
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4. This thesis does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the thesis and in the Reference sections.

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Date: 15 December 2010

Faculty of Science and Agriculture

Declaration 2: Publications

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

Publication 1: Published in *Plant Ecology*

Kambatuku, JR, Cramer MD, Ward D (2011) **Intraspecific competition between shrubs in a semi-arid savanna.** *Plant Ecology* **212**: 701-713. DOI: 10.1007/s11258-010-9856-0

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

Publication 2: Published in the *Journal of Vegetation Science*

Kambatuku, JR, Cramer MD, Ward D (2011) **Savanna tree-grass competition is modified by substrate type and herbivory.** *Journal of Vegetation Science* **22**: 225-237. DOI: 10.1111/j.1654-1103.2010.01239.x

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

Publication 3: Submitted to *Ecohydrology*

Kambatuku, JR, Cramer MD, Ward D (2010) **Overlap in soil water sources of savanna woody seedlings and grasses**

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

Publication 4: Prepared for submission to *Oecologia*

Kambatuku, JR, Cramer MD, Ward D (2010) **Nitrogen fertilisation reduces grass-induced N_2 fixation by tree seedlings of semi-arid savannas**

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

Signed: ....

Jack Ratjindua Kambatuku

Dedication

To Ursula Kavekundu Nguvauva, Hineha, Blessing & Evans Mudongo, Jogbeth Nokokure Katjatenja.

Abstract

The long-term economic viability and ecological integrity of savanna rangelands is being undermined by increasingly dense woody thickets at the expense of palatable herbaceous cover. This process is known as shrub- or bush-encroachment. Bush encroachment is a subset of a broader ecological riddle underlying the coexistence of woody and herbaceous vegetation that has been the subject of many ecological models. The ecophysiological mechanisms and interactions between trees and grasses on which most assumptions of ecological models are premised have seldom been tested empirically. This document synthesises the results of greenhouse and field-based investigations of the underlying ecological mechanisms and ecophysiological interactions between encroaching *Acacia mellifera* trees and grasses in a semi-arid environment.

In a greenhouse study, I determined the contribution of N_2 fixation to the N-budget of *Acacia mellifera* under conditions of both varying N availability and competition from grass. Tree seedlings had longer shoots and greater total dry mass in the absence of grass. The leaf $\delta^{15}N$ values were lower with grass than without grasses. Thus, trees were more reliant on N_2 fixation in the presence of grasses. N_2 fixation may enable the tree seedlings to survive competition with grass at critical and vulnerable developmental stages of germination and establishment.

In a field removal experiment, I monitored the growth rates, water relations and mortalities of shrubs around which neighbouring woody plants were removed (target) and control shrubs over three years. Results showed target trees to have benefitted from removal of neighbours, which was manifested in significantly faster growth rates, less negative predawn water potential and a relatively small degree of canopy die-back. Nonetheless, neighbouring trees appeared to prevent the whole plant mortalities resulting from severe environmental stress. Growing in close proximity with neighbours could therefore yield positive and negative ecophysiological effects.

In another greenhouse experiment, I tested the effects of the separation of moisture uptake with depth between tree seedlings and grasses on two common substrate types. I also examined the influence of repeated grass clipping on the persistence of soil moisture. Results indicated a three-tier rooting pattern with a top layer exclusively exploited by grasses, an intermediate layer occupied by both grass and tree roots and deeper layers exclusively tapped by trees. Tree seedling biomass was negatively affected by grass competition although the biomass of grass was enhanced in the presence of tree seedlings on sandy substrates only. The repeated clipping of grass benefitted tree seedlings on rocky substrate more than it did on sandy substrate. The effects of heavy grazing on soil moisture availability to woody shrubs and thus bush encroachment may be contingent on substrate type, being more acute on rocky terrains.

Grass competition suppresses tree seedlings but the removal of grass by grazing weakens this suppressive effect, particularly on rocky substrates. The insufficiency of space and soil resources on rocky substrates may necessitate increased investment in root biomass by plants. It is not known why grasses have lower densities on rocky substrates than on sandy substrates, but the obstruction by rocks disadvantages grasses against tree seedlings, leaving grasses vulnerable to grazing pressure. This may allow the woody plants on rocky substrates to benefit more from grass removal than on sandy substrates. Root restriction by rock barriers and, perhaps, sparse soil volume further lead to small tree sizes on the rocky substrate. Small shrubs are less likely to compete intensively for resources and cause density-dependent mortality. Intraspecific competition may maintain shrub sizes within the threshold that can be supported by available resource pools.

I conclude from my results that the two-layer hypothesis of niche separation between savanna vegetation is valid although there is an overlap in the grass and tree rooting depth/moisture uptake. An additional factor that affects the success of *A. mellifera* is the substrate.Trees are more dense on rocky substrates but grow larger on sand. I have further shown that *A. mellifera* trees fix nitrogen when competing with grasses but do not do so when grasses are absent. A mechanistic model of savanna dynamics will need to integrate water use patterns, substrate and nutrients to make effective predictions about encroachment patterns.

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It took immense faith in me and infinite patience as well as tolerance from my supervisor, Prof. David Ward not only to invite me to do a PhD, but to persist with me against all odds until the end. I would therefore like to extent my heartfelt gratitude to him for having stood by me when conventional wisdom would indicate otherwise. My indebtedness further extends to my co-supervisor Prof. Michael Cramer who equally had shown forbearance beyond the normal in among other things tackling the daunting task of taming my ‘fancy language use’ to the ‘dull and consistently boring’ (Sand-Jansen 2007) scientific style and unravelling my ‘overly complicated’ sentences. I thank both my supervisors for their constructive critique, advice, unwavering support, guidance and mentoring.

This work and the data presented herein would not have been possible without the generous financial assistance to Prof. David Ward from the National Research Foundation (NRF) and the International Fund for Science (IFS) to me. I am greatly beholden to them for the invaluable financial and material backing. Prof Ward also had to delve into his own research funds to see me through some financially trying times and I am genuinely gratified for this. My long-term friend, colleague and confidante Dr. Elias Charlie Shanyengana advanced a financial donation to ‘get me back to finishing’ when I had ran out of steam.

In my quest to develop, comprehend and master methodological techniques and approaches as well as comparatively interpret trends, I have consulted widely. The willingness of very busy professionals to sacrifice their valuable time and engage in correspondence or in person with a rookie scientist on topics and in fields of which they are leading world experts is very much appreciated and duly acknowledged. I attempted to construct, with the help our Technical Manager, an in-house apparatus for carrying out the soil moisture measurements I had in mind and sought technical advice

from various sources to whom I am deeply indebted for taking the time to advice and direct me. Dr. Stefan Schlaeger, (SCHLAEGER - Mathematical Solutions & Engineering, Universität Karlsruhe [TH], Forschergruppe Feuchtemesstechnik, Soil Moisture Group [SMG], Karlsruhe, Germany) exchanged long emails with me on the use of SISOMOP soil moisture probes that were in their initial phase of production and testing. He offered to develop a custom made TDR set-up for my experiment and forwarded technical details plus papers on SISMOP. Hervé Cochard (UMR PIAF, INRA Site de Crouel, Clermont-Ferrand, France) provided comprehensive advice on the essential components and cost implications for a prototype Xyl'em (xylem embolism meter) as well as offering to provide a DOS software that he uses with Mettler, Satorius or Ohaus balances. Ian Jackman (Local Agent for Analytico Environmental, Starplex 53 cc, Distributors for: Eijkelkamp Agrisearch Equipment, SoilMoisture Equipment Corporation and Controls Testing based in Durban, South Africa) also engaged in long discussions about available suitable equipment and their cost implications.

Prof. Mel Tyree (Chair of Tree Physiology, Department of Renewable Resources, University of Alberta, Edmonton, Canada) exchanged emails with me in which he sufficiently explained the futility of using the High Pressure Flow Meter (HPFM) to determine percentage loss of conductivity (PLC) in plants, testified to the influence of rooting competition on plant water relations based on his work in Panama which was at the time in preliminary draft form, and also let me in on unpublished data. Funny as it may be, Prof. Tyree introduced me to Prof. Norman Pammenter of the University of KwaZulu-Natal (Durban) who, together with his student Michael Cheek, subsequently devoted considerable amount of time in giving me HPFM 'driving lessons'. Prof. Pammenter also assumed the role of supervisor when my supervisor had to undergo an operation and take a leave of absence from work.

Juan M. Martinez of AGLUKON Spezialduenger GmbH & Co. company in Germany through a local partner company in Durban, Grovida of Vincent Lourenco (Snr.) donated slow-release nitrogen fertilizer free of charge for use in my experiments for which I am forever appreciative. Dr Mags Moodley (formerly with CSIR, Natural Resources and the Environment, Agrometeorology, University of KwaZulu-Natal, Pietermaritzburg, South Africa) granted permission for the use of the Frequency Domain Reflectometry (FDR) Diviner2000, got me in contact with local suppliers of access tubes and negotiated for my project to be incorporated in scheduled Diviner usage agenda of CSIR. Vivek Naiken took over the reigns after Dr. Moodley left and has been equally amiable and supportive regarding my use of the Diviner, providing the software for downloading data. Jessica Chetty played a significant role in facilitating my contact with CSIR staff and students. Melake Fessehazion (PhD student, CSIR) got the Diviner to work for my 'different experimental set-up', and was very helpful and considerate throughout. All CSIR students and staff who had to accommodate my needs and share the Diviner with me are herewith thanked for their tolerance and patience.

On numerous occasions when I could not access papers and publications or obtain the full text of papers that I came across in my online searches, I simply wrote emails to the corresponding author requesting a copy (either electronically as PDF or a hard copy print-out via surface mail) of the said paper. Authors have been very generous and promptly mailed me their papers. Dr. Jack Tessier (Department of Biology, Central Connecticut State University, New Britain, USA), Dr. Robert M Dunn (Institute of Grassland & Environmental Research, North Wyke, Okehampton, Devon, UK.), Dr. Andy Dougill (Senior Lecturer in Environment & Development, Deputy Head of School, School of Earth and Environment, University of Leeds, UK.), Prof. Ary Oliveira-Filho (Departamento de Ciências Florestais, Universidade Federal de Lavras, Lavras, Brazil), Dr. Thierry Ameglio (P.I.A.F., U.M.R. 547 [INRA-Univ. Blaise-Pascal], Centre INRA de Clermont-Fd-Theix, Site de Crouelle, 234, avenue du Brézet, 63100 Clermont-Ferrand Cedex 02, France), Dr. Robert M. Boddey (Research Scientist, Embrapa-Agrobiologia, Rio de Janeiro, Brazil), Dr. Lauchlan Fraser (Associate Professor, Thompson Rivers

University, Canada Research Chair in Community and Ecosystem Ecology and Interim Director of the Institute of Environmental Studies) all sent PDF files. Prof. Charlie Shackleton (Rhodes University, Grahamstown, South Africa) photocopied and mailed his paper to me. I am incredibly grateful for the assistance provided by all of the above-mentioned luminaries and sincerely acknowledge their assistance.

All of my isotope analyses were done at the University of Cape Town (UCT) by dedicated staff in the Archaeology Depertment. A word of gratitude is extended to Dr. Ed February for the permission to make use of his ‘private’ assembly line. Staff in the isotope laboratory, John Lanham (Principal Scientific Officer for the Stable Light Isotope Facility, UCT), Ian Newton and Shaamielah Davids are heartily thanked for their swift processing of samples for isotope analysis. A special mention ought to be made of Shaamielah Davids who had to process my colossal amount of plant, water and soil samples. Personnel in the Botany Department of the UCT had the unenviable task of having to put my crudely ground plant samples through the miller once more and I sincerely thank them for this. Nitrogen and phosphorus analysis was done by the Animal Science Department and I cherish the friendly and prompt facilitation provided by Deborah Davies and Susan Van Malsen in this regard. My ‘non-soil’ soil samples were analysed by UKZN soil science laboratory and Prof Jeffrey Hughes and Tad Veeramuthoo Dorasamy are due an acknowledgement for their constructive engagement in this regard. The South African Weather Bureau diligently supplied rainfall and temperature records. Nondumiso Mabe and Shouneez Chaka from the Department Water Affairs and Forestry (DWAF)’s Hydrological Services Directorate, Sub-Directorate: Geohydrological Information furnished detailed geohydrological and geochemical data on boreholes in and around the study from the database. My former colleague and long-time friend Fred Tego compiled and produce the maps in GIS which I did not use in the end.

An undertaking of this magnitude could not have been planned, executed and taken to its logical conclusion without the involvement and assistance in terms technical, logistical and manpower contribution by others. I unreservedly and sincerely credit and express my thankfulness to Vanessa Stuart for the pivotal role she assumed not only in my academic endeavours, but also my personal wellbeing in Pietermaritzburg well beyond her call of duty. Martin Hampton was not only my first stop whenever I came up against technical hitches with equipment and vehicles but his door was always open, even when he was not in, for me to confer with him on designing prototype apparatus, borrow kits or seek technical advice of any sort. This was in spite of perennial inflow of speeding tickets in my name which threatened to taint his reputation (as a proxy to the University vehicles). Alison Young untiringly had room for my disproportionate (relative to my body size) demands for space in the shade shed or greenhouses, supervised irrigation of experiments in my absence, arranged for the hiring of casual workers and proffered precious advice on use of pesticides. Rogan Roth tirelessly attended to computer snags and entertained the dullest queries concerning software. George Carelse always fixed whatever and whenever I broke something in addition to his entertaining tales. The crew in the Botanical Gardens specifically Merriman, Magic, Justice and Goodman including a sizable number of casual workers lended a hand when called upon especially with the filling of dustbins with sand, collection and offloading stones, translocation of the 'big experiment', re-installation of a sprinkler system and sorting of trillions of samples. To all of them I eagerly convey unadulterated gratiitude. Particular mention has to be made of Mduduzi Nene who, as a casual labourer at the time, has singlehandedly carried on and completed the harvesting of my dustbin experiment when I left midway through the harvesting.

In the field, exceedingly courteous hospitality by Errol Tegg, Barbara Tegg and Charles Hall always made the acerbic mission of waltzing with thorny acacias feel like an exotic vocation. I am not merely referring to the tranquil lodging, gratis unguided game drives and afternoon beers, but also their eagerness to assist with phone calls, faxing, vehicles and purchasing of essentials. Charles Hall further provided records on boreholes on Pniel Estates. Ben Stemmer had been a reliable hardworking field assistant with an exceptional knack for understanding equipment and procedures while being a delightful companion. As the ‘foreman’ he was very efficient in organising casual labour when needed. To all the people above, as well as the entire Pniel community into which I got adopted and the football teams for whom I was an ‘official photographer’ I want to express sincere gratitude and appreciation for having turned Pniel into my third ‘home-from-home’.

I will betray my conscience if I omit to acknowledge the moral, spiritual, psychological and material support from friends, relatives, fellow students and family that I immensely benefited from during the trying times of my studies. Ursula Kunduu Nguvauva has been a perennial spring of moral, emotional and material support for which I cannot imagine a just reward or fitting gratitude. Acknowledgements are due to Georges Bayonne Mboumba who initiated me into the ‘Pietermaritzburg campus life’ and smoothed my transition from a taxpayer to full-time student with no stipends. He lent a hand especially with the set-up of my dustbin experiment and installation of sprinklers in addition to showing me the hotspots of PMB nightlife. Desale Yosief Okubamichael who joined UKZN after George left has been more than a friend, not only introducing me to the ‘north-eastern African flavours of cuisines (ijara) but in the process nourishing me in addition to gap-stopping financially whenever the situation got out of hand. Drs Ndafuda Shiponeni, Mutjinde Katjiua, Charlie Shanyengana and Gurvy Kavei never failed to encourage and spur me on when I could see no tomorrow, as did Fred Tego. A myriad of friends on my e-mail circulation circuit, notably my former classmates in Bangor and fellow ‘hard-rockers’ from the Sida sponsored course at the Chalmers University of Technology, Göteborg, Sweden, *Advanced International Training Course in Groundwater Development and Management in Hard Rock Areas* in 2004 provided a receptive audience to my tales of toil and hardships. My sisters, Jagboth Nokokure Katjatenja, Jaqualine Mbahahiza Katjatenja and Mara Kauapirura as well as my mother, Angeline Ngaisiue Katjatenja were instrumental in reigniting, reviving and revitalising my desire to complete when I had all but thrown the towel and I savour their priceless counsel and care. An unexpected stanchion of psychosomatic and substance backing was my motor-mouthed long-term friend Ben Tuundjakuje Ngairorue whom I gracefully have to recognize.

The brunt of my PhD undertaking was borne by my children as it did not only deprive them of my physical presence but severely curtailed my ability to provide for their needs. As the eldest and the one who was at school during this unsavoury epoch, Hineha Mudongo felt my absence more than her siblings, yet she had shown an understanding way beyond her age. Rather than gratitude, I offer my children a solemn apology and promise to devote all my energy and the rest of my post-PhD time on earth in dedication to their wellbeing, needs and wishes.

Tradition dictates that an acknowledgement section eulogises and exalts all the immeasurable support, assistance and encouragements. There have however been times throughout the tortuous course of my research when I felt like cursing rather than praising a number of people. In fact there are two categories of these people upon whom I would have gladly cast a devilish curse at the time. The first category include all friends, colleagues, acquaintances and professionals who, based on their perceived familiarity with my intellect, all unrelentingly asked me when I was going to do my PhD. The second category, which may not be mutually exclusive of the first comprise individuals who, again on the premise of their supposed knowledge of my capabilities, were incessant in insisting that, '*for you, you could do it in a matter of three years*'. Well my alleged abilities did not turn my PhD into an easy sailing adventure nor did I 'merely take three years', but nonetheless it has been accomplished at long last and gratitude is thus due to these potential victims of curse for thrusting me into it.

Reference: Sand-Jensen K 2007. How to write consistently boring scientific literature. *Oikos* **116**: 723-727.

1. Chapter 1: Introduction

1.1. Introduction

An eighth of the global surface, amounting to a fifth of the terrestrial landscape and over 65% of land area in the tropical and subtropical regions of continental Africa is classified as savanna (Werner 1991, Scholes & Hall 1996, Sankaran et al. 2005). The physiognomic classification savanna covers tropical and subtropical vegetation communities naturally characterized by simultaneous occurrence of an uninterrupted cover of graminoid species of vegetation (C4 grasses, sedges and forbs) interspersed with variably spaced C3 woody shrubs or trees (Johnson & Tothill 1985, Frost et al. 1986a) which, while being important and prominent, do not form a closed canopy cover. Grasses and trees, two contrasting functional types of vegetation with markedly divergent life forms in paradoxical apparent stable coexistence while competing directly for soil resources usually tend to co-dominate savanna systems (Menaut 1983). An additional defining characteristic on which delineation of savanna ecosystems is based includes seasonality of especially the herbaceous C4 component (but also including deciduous woody plants) in tandem with water availability (Shackleton 2000) which delimits plant productivity and restricts the type of plant species and communities that survive alternating dry and wet periods (Werner 1991). Savannas are thus structurally constituted by co-occurring trees, herbs, forbs and grasses and climatically defined by seasonal moisture availability patterns (Johnson & Tothill 1985). Local variations in topography, geomorphology and rangeland management add complexity to the floristic constitution, structure and function of savannas (Solbrig 1996). In southern Africa, savanna ecosystems include the dominant vegetation in Zimbabwe, Botswana and Namibia while occupying a portion of Mozambique and most of the northern and north-eastern parts of South Africa (Rutherford 1997). Savanna thus constitutes the most prevalent biome in southern Africa, taking up just above three quarters of total land area on the subcontinent. In South Africa, savanna extends well into the lowveld (Mpumalanga and Limpopo provinces, as well as northern KwaZulu-Natal) and Kalahari regions covering about one third of total land area (Rutherford & Westfall 1986, Rutherford 1997).

Proportional predominance of either trees or grasses in savannas may fluctuate greatly over time or space, but neither plant life-form competitively excludes the other entirely. The underlying mechanisms and determining factors giving rise to, altering or maintaining the variation in relative proportions of trees to grasses in savannas continue to intrigue ecologists (Sarmiento 1984) and has formed the focal point of ecological debates, investigations, theories and models for long. This puts the coexistence of trees and grasses in savanna systems among of the most intensively investigated areas in terrestrial ecology. In arid to semi-arid ecosystems, moisture availability and thus vegetation dynamics are regulated by rainfall and temperature regimes (MacMahon 1980, Sala et al. 1989). The onset and termination of the growing season in savannas is largely dictated by the timing of the wet (rainy) season while the pools of plant available nutrients control the rates of growth by plants. Soil moisture and nutrient availability are thus the two key determinants of tree-grass relational dynamics in savannas. Water and nutrient interactions may in combination set the boundaries of savanna plant productivity or biomass output.

Primary production in plants is strongly influenced by the supply of the nutrients necessary for plant growth (Aerts & Chapin 2000). Soil nutrient availability in plant accessible forms determines the rates of growth achievable by plants with most frequent limitation resulting from nitrogen and phosphorus deficiencies (DeAngelis 1992, Larcher 1995, Marschner 1995). Competing interplay between grasses and trees kindled by their persistent coexistence in semi-arid savanna ecosystems also revolves around the uptake of surface, leached and decomposition-released soil nutrients (Walter 1939). Stimulation of plant growth and productivity by minerals in the soil is a function of site-specific soil conditions as well as the functional adaptations of the plants growing in a given environment (Chapin & van Cleve 1989). Nutrient availability to plants is controlled by and in turn regulates other environmental limitations (Bloom et al. 1985) on plant growth, productivity and reproduction. Adaptations by plants to an array of environmental resource availability patterns, involving the ability to effect phenotype modification are contributory determinants in the extent to which resources become limiting to plants (Gleeson & Good 2003).

Availability of combined nitrogen (NH4+, NO3-, NO2-) and organic N are widely considered to restrict terrestrial and aquatic primary productivity (Field et al. 1998, Raven et al. 2004). Inorganic N is the element most limiting to the productive output by plants growing under natural environmental conditions for a majority of terrestrial plant ecosystems (Clark & Rosswall 1981). Although elemental N is abundant in the atmosphere and soil, its plant available form is the scarcest nutrient at any given time. The pool of soil N in forms that are readily available for uptake by plants is often depleted over time (Whitehead 1995, Tate 2000). Volatility and high mobility of N in the soil exacerbates its shortage for uptake by plants (Vitousek & Howarth 1991) making the recycling of nutrients through litter production crucial to arid savannas. Requirements of N in relatively large amounts for incorporation into proteins, amino acids and nitrogenous organic molecules in the form of hormones and chlorophyll frequently renders it the most limiting factor to plant growth (Hopkins 1995). Competition for inorganic N by soil microbial communities (Jackson et al. 1989) worsens N scarcity and limitation to plant growth. Steady robust increases in plant biomass were induced by N addition and not other nutrients with N limitation diminishing when water became limiting (Tilman 1987, 1990). Within a semi-arid plant community, N addition was shown to be the only treatment to result in increased plant biomass production, doubling the biomass of the dominant grass component (Krueger-Mangold et al. 2004). Available combined N is lost to primary producers in the form of N_2 and N_2O via the process of denitrification. Frequent fires in savannas contribute to the emission of N through pyrodenitrification and its loss to decomposition through undegradable charcoals (Sanhueza & Crutzen 1998). Atmospheric N_2 can be converted to plant available form through biological N_2 fixation. A symbiotic association with rhizobial bacteria often represents a successful strategy by plants competing for limited N availability in nutrient poor sites (Smith & Read 1997). Plant competition for N is a critical factor in semi-arid environments, especially for grasses (Krueger-Mangold 2004).

Evidence for a degree of plant growth limitation by P has been found in grasslands (LeJeune & Seastedt 2001). Occurrence in low concentrations and its solubility in water within the soil matrix increase the potential of P as a limiting factor in many ecosystems (Sharpley 2000). P deficiency limits plant growth and demand for N leading to a reduction in nodulation by legumes (Almeida et al. 2000). The rate at which legumes symbiotically fix N_2 is also related to the availability of P (Munns & Mosse, 1980, Hartwig et al. 1996) such that N_2 fixation is limited under conditions of deficient P availability (Cadisch et al. 1989). When the growth of Acacia woody seedlings in savannas is not limited by water, competition for N by grasses may become the main limiting factor which is then overcome through N_2 fixation (Cramer et al. 2007), however the legume ability to fix N_2 may further be constrained by competition for P by the grass component (Cramer et al. 2010). Campillo et al. (2005) found a deficient P to lower legume productivity and increase the competitive advantage of native grasses in volcanic soils of Chile. Positive impact of P on N_2 fixation is not a mere secondary outcome of its effects on plant growth as suggested by Robson et al. (1981), but the mineral is additionally involved in the explicit initiation, growth and functions of rhizobial nodules (Israel 1987). Almeida et al. (2000) reported that the effect of lower P concentration was more pronounced on the mass of nodules produced than it was on total plant biomass or growth.

Grasses are often said to be competitively superior to woody plants especially at the seedling/sapling stage where they may outcompete woody seedlings for moisture, nutrients and light (Scholes & Archer 1997, House et al. 2003). The germination, survival, establishment and maturation of woody seedlings and saplings in fertile soils are indeed severely curtailed by grass competition (Sankaran et al. 2008, van der Waal et al. 2009). Recent work suggests the suppressive impact of grasses to affect all demographic stages of savanna woody plants in nutrient-rich environments (Riginos 2009). Grasses additionally provide the fuel for recurrent fires that further keep tree densities in check. The life form of grasses enables them to respond more quickly and more efficiently exploit seasonally pulsed resource availability such as moisture and nutrient fluxes than trees. Compared to woody plant investment in nutrient poor but carbon rich woody structures (Bond et al. 2003), grasses allocate a larger proportion of biomass to nutrient-requiring aboveground shoots, resulting in faster relative growth rates (Poorter & Nagel 2000). Moreover, a water-use efficient C4 photosynthetic pathway in grasses allows for production of relatively larger biomass per amount of water consumed compared to woody plants (Ehleringer & Monson 1993, van der Waal et al. 2009). Combined with a fibrous root system that is more effective at absorbing resources and a relatively higher rate of transpiration (Frost et al. 1986a) than woody plants, grasses can outcompete trees for both nutrients and moisture in the topsoil.

Trees persist and maintain a significant presence in savannas despite these grass-mediated restrictions on their survival and recruitment. While the understanding of the precise mechanisms enabling trees to continue coexistence with grasses in savannas remains inadequate (Scholes & Archer 1997, House et al. 2003, Sankaran et al. 2004, 2005), four attributes of woody plants have generally been invoked to explain their persistence. These are deep rooting depths that enable them to source separate layers of the soil profile for moisture (Walter 1971, Walker & Noy-Meir 1982), an ability to fix atmospheric N_2 through an association with rhizobial bacteria (Danso et al. 1992) in legumes and the storage of long-term reproductive potential in different overlapping generations of woody plants (Warner & Chesson 1985, Higgins et al. 2000) primed to exploit rare favourable occasions. A fourth aspect of woody plants put forward as playing a role in savanna tree-grass dynamics does not relate to overcoming suppressive grass competition by trees, but rather rationalizes why trees in savannas do not form closed-canopy woodlands. Inter-tree competition is postulated to intensify with increasing density to reach a stage where stronger individuals outcompete and eliminate weaker competitors thus opening up patches for the growth of grass cover (Wiegand et al. 2005) and maintain simultaneous tree-grass existence in savannas.

Individual trees can be randomly or regularly distributed over a landscape or grow in clumped clusters as a function of competing neighbouring individuals (Ward et al. 1996), patchy soil characteristics (Picard et al. 2005), tree vegetative reproduction (Peterson & Squiers 1995), seed dispersal over short distances (Peterken & Jones 1989) or the history of land management (Drew & Flewelling 1979). Maintenance of regular tree-tree distribution by intra-tree competition could be counterbalanced by fires which promote clumped densities. Fire regimes promote mosaics of short-term heterogeneity comprising open grass patches susceptible to frequent burning and areas with clumped trees devoid of grass and thus impervious to intense burning, thereby maintaining long-term stability of savannas at a regional scale (Menaut et al. 1985, Menaut et al. 1990). Random woody plant distribution patterns may however be a transient stage from clumped to evenly distributed patterns (Jeltsch et al. 1999). The shrub/tree component can be extremely sparse consisting of very few scattered, widely spaced individuals, or dispersed in clustered clumps or form very dense woodland in the savanna physiognomy (Walter 1971, Bourliere & Hadley 1983, Smith & Grant 1986).

Patterns of primary production in savanna are further modulated by the removal of plant biomass through herbivory and burning which constitute secondary influences on competitive plant interactions.

Fire effects also depend on fuel load and related fire intensities (Walker & Noy-Meir 1982, Frost et al. 1986b, Solbrig 1990, Scholes & Hall 1996). The evolution and speciation of natural African savanna vegetation were strongly driven by pressure from extant herbivores with diverse dietary preferences over time (Kelly & Walker 1976). Herbivore pressure on both the woody and herbaceous components of savannas in concert with frequent fires helped shape and sustain the open structures of savanna grassland and woodlands (Frost & Robertson 1987). Savanna structure, as determined by shrub/tree densities, is an outcome and function of multiple dynamic factors including climatic patterns, fire regimes, herbivore pressure and competition for resources interacting at different scales of time and space (Skarpe 1992, Scholes & Archer 1997). The perpetual effects and interactions of rainfall intensity, fires and herbivore pressures in keeping tree seedling recruitment in check and driving grass/fuel biomass may control overall savanna structure and composition (Higgins et al. 2000, Jeltsch et al. 1996).

Frequency of fire outbreaks and intensity in savannas is a function of moisture and nutrient content of the constituent vegetation (Higgins et al. 2000) varying as function of available fuel conditions (Trollope 1982, van Langevelde et al. 2003), air temperature, humidity, wind speed (Cheney et al. 1993, Cheney & Sulluvan 1997, Trollope 1998), soil moisture content and season during which the burning occurs (Trollope 1984, Menaut et. al. 1993, Higgins et al. 2000). Fuel load is primarily conditioned by the dry mass of the standing grass crop and its moistness (Webber 1997) which may in turn change spatially due to patchy grass production (Chidumayo 1997), herbivorous impact (Coughhenour 1991), the effects of woody plants (Mordelet & Menaut 1995) as well as the type and species of grass present (Vetaas 1992). The major role played by fires in savannas is thought to be through the topkill of trees seedlings, saplings and ‘Gullivers’ within the flame zones (Bond & van Wilgen 1996) thus preventing the woody component from reaching its maximal potential cover and forming a closed canopy (Hoffman & Solbrig 2002, Bond et al. 2005). While many savanna trees are resilient to fire-induced mortality through an ability to withstand flames by means of a thick bark (Gignoux et al. 1997) or by re-sprouting rapidly after burning (Rutherford 1981, Trollope 1996) frequent burning can compound overall tree deaths to have a significant effect on woody plant cover (Bond & Midgley 2000, Higgins et al. 2000). Savannas are thought to have coevolved with and adapted to frequent burning which serves to remove accumulated dead biomass, recycle nutrients and prevent the woody components from growing into dense thickets (Troloppe 1982, Walker & Noy-Meir 1982). Burning liberates carbon, nitrogen, phosphorus and other elemental nutrients held up in dead biomass which may be volatilised, lost with smoke or reincorporated into soil as ash (Menaut et al. 1993). Over the last 50 centuries, anthropogenic control and management of fire has either increased or decreased the frequency of burning and restricted the spatial scope of fires (Menaut et al. 1985). Interdependence and interactions among different factors shaping savanna structure and function mean that the influence of individual factors varies with spatial and temporal changes.

1.2. Woody plant encroachment in savannas

Savannas sustain the largest biomass per hectare of livestock and wild mammals worldwide (White et al. 2000). As a result, a large and growing proportion of the global human population inhabits and derives livelihoods in savannas (Scholes & Archer 1997). The expansive savannas of southern Africa support species rich, diverse and dynamic ecosystems in which abundant fauna thrive (Prins & Olff 1998, Olff et al. 2002) while presenting habitats for flora. This system has provided the base for agricultural enterprises, notably free-ranging cattle rearing under pastoral (Lamprey 1983, Lamprey & Field 1983, Lamprey & Reid 2004) or commercial ranching that constitute among the biggest primary production economic sectors for most of southern African countries. In South Africa, 84% of the savanna biome supports game and cattle ranching, sustaining dairy, beef, eco-tourism and hunting industries (Grossman & Gandar 1989). The quality, productivity and long-term economic viability of rangelands and thus livestock and game farming as well as the ecological integrity of inherently fragile savannas in most southern African countries is being endangered by increasingly dense invading thickets of woody vegetation (Archer 1995, van Auken 2000, Roques et al. 2001, Molele et al. 2002) often at the expense of palatable herbaceous cover, a process known as bush- or shrub encroachment (O'Connor & Crow 2000, Hoffman & Ashwell 2001). Bush encroachment has been recognized as a serious problem in the savanna biomes for over half a century (Walter 1939, Pole-Evans 1948). Recent reporting of the shift towards an increasing abundance of woody plant species in temperate or tropical grasslands and savanna biomes in literature indicate it to be a worldwide trend (Werner 1991, Archer 1995, McPherson 1997, Jeltsch et al. 2000, Roques et al. 2001, Ward 2005). Numerous mechanisms have been put forward as explanations for changing woody plant abundance and distribution in savannas and grasslands. These processes include historically altered atmospheric CO_2 concentrations with associated climatic changes, habitat fragmentation, distorted and suppressed fire regimes, altered herbivory pressure and rodent populations (Polley et al. 1996, McPherson 1997, Weltzin et al. 1997, Jeltsch et al. 2000). Seedlings of woody plants however appear less susceptible to fire induced mortalities (Archer et al. 1988, Hoffmann 1998, Meyer et al. 2005) than is expected or indicated in previous work (Jeltsch et al. 2000, van Langevelde et al. 2003). In resource-poor arid environments, the encroaching woody species are often but not always (Katjiua & Ward 2006) less palatable to domestic large stock, due to comparatively high investments in chemical (tannins) and physical (thorns) defences (Rohner & Ward 1997), compared to the grasses they suppress and outcompete. This ultimately leads to a significant reduction in the forage resources and carrying capacity of rangelands for livestock production (Fisher 1977, Higgins et al. 1999) and the shrinking of habitats for wildlife (Norton-Griffiths

1979, Ben-Shahar 1992) not only through displacement of the palatable herbaceous fodder (McNaughton 1993, Scholes & Archer 1997, Ward 2005), but also by formation of impenetrable thickets (Adams 1967) closing up rangeland and limiting the movement of livestock and game. An altered relative ratio of trees and grasses in savanna may also negatively transform vital facets of ecosystem functioning including surface hydrological and subsurface geo-hydrological processes, nitrogen and carbon cycles as well as the overall net primary production and dependent herbivore biomass output (Scholes & Archer 1997, Hoffman & Jackson 2000, Jackson et al. 2002, House et al. 2003, Riginos & Grace 2008).

Extremely severe thicket encroachment may lead to failure of rangelands and seemingly irredeemable degradation of land (Scheffer et al. 2001, Tobler et al. 2003) with dire implications for agricultural and economic performance. Given that the savannas of southern and central Africa support large and rapidly expanding human populations with a strong rural farming livelihood (Lamprey 1983, Scholes & Archer 1997, Lamprey & Reid 2004), a loss of rangeland productivity through bush encroachment is of great significance and consequence to the human population and security of food supply. The elimination of the herbaceous cover which is often more diverse in species richness (Solbrig et al. 1996) compared to encroaching woody plants and its replacement by stands of bushes represents a serious loss of biodiversity to the system.

The two-layered model of Walter (1939, 1964, 1971) postulates the utilisation of soil moisture and nutrients by both woody vegetation and herbaceous plant cover from two distinct layers of the soil profile as an explanation for grass and woody plant coexistence. Grasses are assumed to exclusively derive their supply of resources from the topmost layers while woody plants exploit both the top and subsurface layers of the soil for moisture and nutrients. Supposing soil moisture, primarily from precipitation input (Schwinning et al. 2002), to be the most critical limiting factor in the growth of both trees and grasses in semi-arid savannas, a vigorously growing grass cover in a stable open savanna ecosystem will be at an advantage by intercepting and trapping most of water from precipitation and infiltration at the surface. As the larger percentage of rain events in semi-arid environments is made up by light showers that do not infiltrate beyond the shallow soil layers, most precipitation is only available to shallow-rooted vegetation, chiefly the grass component (Knoop & Walker 1985, Frost et al. 1986a, Schwinning et al. 2002, Hipondoka et al. 2003). Grasses are able to take up and use rainwater at a much faster rate than woody trees (Frost et al. 1986a). Less or insufficient soil moisture will reach the deeper subsurface layers of the soil where the roots of woody plants dominate, thus putting the trees at a disadvantage.

The disturbance of such a situation through the selective removal of grass cover by heavy grazing allows the infiltration and percolation of water to deeper soil layers exploited by the roots of woody vegetation, conferring a competitive advantage unto trees. As a result, woody vegetation may then recruit en masse in the patches opened up by grazing disturbances. Coexistence of trees and grass as predicted by Walter's (1939) hypothesis was corroborated by models of Walker et al. (1981) as well as Walker and Noy-Meir (1982). Prevalence of shrub encroachment in areas with apparently heavy grazing pressure reinforced the view that bush encroachment is mainly caused by cattle farming (Skarpe 1990, Harrington 1991, Jeltsch et al. 1997, Scholes & Archer 1997, Roques et al. 2001). Some studies (e.g. Madany & West 1983, Van Vegten 1983) single out livestock grazing as the primary cause behind the conversion of savannas vegetation to dense woody stands. The introduction of cattle as the predominant grazers and near exclusion of browsing game and mixed feeders in savannas are said to have altered the tree-grass ratios to often impenetrable thickets of mainly Acacia species within a few decades (Van Vegten 1983). High grazing rates are seen as promoting encroachment by reducing or eliminating species of fodder grasses, diminishing its competitive effect on trees (Archer et al. 1988, Skarpe 1991, Archer 1995, Scholes & Archer 1997, Jeltsch et al. 2000, van Langevelde et al. 2003) and creating favourable light conditions for germination and survival of woody plant seedlings (Belsky & Blumenthal 1997, Hagenah et al. 2009) thereby allowing woody plans to recruit successfully in savannas (Walker & Noy-Meir 1982, Stuart-Hill & Tainton 1989, Jeltsch et al. 1997). In combination with prolonged droughts, heavy grazing pressure ensures an almost complete removal of perennial grasses and given a significant time lag before annual grasses compete for water, these factors generate conducive conditions for recruitment of woody trees (Westoby et al. 1989, Graz 2008). However, low tolerance for drought by woody tree seedlings (Higgins et al. 2000) may nullify the recruitment opportunity presented by a combination of drought and heavy grazing, especially as intense grazing creates parched microclimatic conditions which exacerbate seedling desiccation (Gerhardt 1996, Hagenah et al. 2009).

The effect of grazing pressure on shrub encroachment in savannas is thought to be indirectly related to the reduction of fire frequencies (Roques et al. 2001). When grazing pressures are light, it facilitates the outbreaks of sufficiently frequent fires capable of precluding the encroachment of shrubs (Roques et al. 2001). Reduced grass biomass due to high grazing pressure reduces fuel load and may minimise the frequencies and intensities of fires to allow seedlings and saplings an escape into adult classes (Higgins et al. 2000, van Langevelde et al. 2003). Dispersal of woody plant seeds in dung of herbivores (Brown & Archer 1987, Hoffman et al. 1989, Reyes et al. 1994) combined with the enhancement of seed germination probability after passing through ruminant guts, due to scarification (van Staden et al. 1994, Bodmer & Ward 2006), have also been advanced as a mechanism through which introduction of domestic livestock aided bush encroachment. However, the incidence of bush encroachment coinciding with the presence of high stocking rates and thus heavy grazing does not necessarily validate the causative link between cattle ranching and woody plant encroachment (Ward

2005). The widespread occurrence of the bush encroachment phenomenon in areas with minimal and infrequent grazing (Brown & Archer 1999, Ward 2005) or where two-layered soil profiles are nonexistent (Wiegand et al. 2005) do not support the two-layer model. Moreover, seedlings, saplings and young woody plants become established in the shallow subsurface layer used by grasses during their sensitive and critical early stages of growth and establishment (Ward 2005). Additionally, the rocky terrains of savanna ecosystems support sparse grass cover and appear to be more prone to bush encroachment than relatively deep sandy areas (Britz & Ward 2007).

There is a growing body of literature indicating that niche partitioning may be insufficient to account for tree-grass coexistence in savannas (Jeltsch et al. 2000, Ludwig et al. 2004, Sankaran et al. 2004) and it is therefore improbable that rooting niche separation alone can offer adequate explanation for initiation and proliferation of bush encroachment. Temporal separation of resource uptake by trees and grasses through non-overlapping timing of phenological responses to seasonal rhythms may allow the coexistence of the two contrasting and competing life forms (Harrington et al. 1984, Fowler 1986, Menaut et al. 1990, Scholes & Walker 1993, Wiegand et al. 2006). Phenological separation of break of dormancy, regeneration, duration of growth, resource (moisture and nutrients) uptake and utilization at different times of the season may allow coexistence through non-overlapping temporal niches of trees and grasses (Harrington et al. 1984, Sala et al. 1997, Scholes & Archer 1997, House et al. 2003). Conceptual and empirical knowledge of the mechanistic processes driving the increase in woody plant densities within savannas remain inadequate (Teague & Smit 1992, Smit et al. 1996, Molele et al. 2002, Ward 2005). The directional change from sparsely wooded ecosystems to densely vegetated landscapes is a subset of a broader ecological puzzle underlying the coexistence of woody and herbaceous vegetation (Graz 2008) and is indicated to become even more important with global warming, elevated CO_2 and atmospheric deposition of nitrogen (Wigley et al. 2010, Ward 2010).

Elevated atmospheric CO_2 levels over the past two millennia has often been linked to the expansion of woody plants in savannas (Idso 1992, Polley 1997, Polley et al. 1997) by favouring the trees/shrubs' C3 photosynthetic pathway over the grasses' C4 photosynthesis and leading to reduced rates grasses transpiration. These resulted in relatively higher biomass yield of trees than grasses and more water being available to percolation deeper to the benefit of establishing tree seedling and mature woody plants (Polley et al. 1997). Alternatively increased CO_2 levels may enhance the carbon storage of trees to enable them to resprout after a fire and grow fast enough for Gullivers to grow beyond the flame zone and escape height (Bond & Midgley 2000). However Körner (2006) has strongly argued that the standing crop and biomass production of natural ecosystems, particularly those limited by water and nutrients (such as semi-arid savannas) are less likely to be overly influenced by elevated CO_2 levels. The optimization of low CO_2 concentrations since the past 20-25 million years have largely shaped the evolution of terrestrial plants which have adapted to cope with low CO_2 levels. Carbon allocation by plants to the growth of structures depends on genetic, morphologic attributes and developmental stage as well as the supply of necessary resources of water, light and nutrients (Körner 2006). Moreover different plant organs and rhizobial symbionts demand carbon and hence there is no direct proportional translation of photosynthetic CO_2 response into growth. Long-term responses of plant biomass production in response to CO_2 enrichment is ultimately limited by cycles of soil nutrients and may depend on plant species (Körner 2006). While the responses of young trees to elevated CO_2 can accumulate and be compounded over time, terminate seasonal growth of grasses eliminates the propagation of a positive response with time (Körner 2006). Nonetheless savanna trees could still exploit resource niches previously occupied by C4 grasses in response to CO_2 enrichment.

1.3. Bush encroachment in semi-arid and arid environments

A hypothesis by Wiegand et al. (2005, 2006) holds that bush encroachment in many semi-arid and arid environments is a natural phenomenon of ecological systems governed by patch-dynamic processes. Wiegand et al. (2005) theorised that any form of disturbance (including grazing pressure) is capable of creating space and making water and nutrients available for tree germination. Under low soil nitrogen conditions, the nitrogen-fixing trees have a competitive advantage over other plants and, given enough rainfall, may germinate en masse in patches created by the disturbances. With time, tree growth and inter-tree competition will convert the bush-encroached patch to an open savanna through competitive elimination of weaker trees by dominant ones (Wiegand et al. 2006). This process may then be repeated in a dynamic cyclical succession of trees and grasses in patches without anthropogenic mediation.

Experiments indicated that immature trees are competitively inferior to grasses whereas grasses tend to lose out in competition with mature trees (Moore et al. 1988). Such age-driven asymmetrical outcomes of competitive interaction between trees and grasses generate volatility in the interplay between woody and herbaceous cover in savannas (Ward 2005). In nutrient-rich high rainfall areas, the suppressive effect of grasses on trees is not limited to seedling and sapling life-history stages (Riginos 2009). The most critical stage in tree-grass competition within arid, nutrient-poor savannas is the initial recruitment and establishment such that a well established grass component is able to exclude the emergence of tree seedlings (Harper 1977, Kraaij & Ward 2006) as much as a mature stand of trees in a water-limited ecosystem outcompetes grasses in terms of access to topsoil moisture (Ludwig et al. 2004). Selective grazing reduces the stifling effect of an established grass cover on tree seedlings, leading to the conversion of an open savanna patch into a tree-dominated thicket. It has been shown that the most important determinant in the growth of competing plants during the initial stages of establishment is root competition rather than shoot competition (Donald 1958, Wilson 1988). Root competition for soil moisture, and to an extent nutrients, between trees and grasses in arid zones is limited to the wet season when both grasses and trees rely on the same supply of available soil moisture in the upper soil layers while it is the only time the highly seasonal grass component grows actively. Once the transient wet season source of soil moisture is depleted in the dry season through evaporation from the surface or deep percolation, this competition ceases as trees rely on deeper sources for their water supply (Eggemeyer et. al. 2004). Frequency of rainfall, and accumulation of soil moisture (Foth 1990), emerges from experimental studies as the most critical factor primarily driving the germination, seedling vitality and survival of grasses (Evers & Parsons 2003) and trees (Kraaij & Ward 2006, Meyer et al. 2007), irrespective of soil type or nutrient levels. The role of herbivores, both grazers or browsers, in the interaction is but one of a multiple of secondary factors and process helping to drive the dynamics and pattern of changes in the ecosystem (van Langevelde et al. 2003, Sankaran et al. 2008) rather than being the single major cause of the spread and increased density of woody vegetation in formerly sparsely wooded grasslands of the semi-arid savanna. Other factors include climate, fire, availability of nutrients, plant interactions, light, energy and their varying magnitude at temporal and spatial scales (Skarpe 1992, Scholes & Archer 1997, Higgins et. al., 2000, Gillson 2004, van der Waal et al. 2009, Ward 2009). On a global scale, the role of elevated CO_2 and atmospheric deposition of nitrogen may assume greater significance in driving the increase of woody plants relative to grasses (Ward 2010, Wigley et al. 2010). It is largely the manifold interactions of a multitude of factors (Frost 1996, Childes & Walker 1987, Holdo 2005) at different spatial extents rather than the unimodal influence of a single factor that determines the condition of a savanna ecosystem at a particular temporal scale.

1.4. Encroachment by *Acacia mellifera*

The ecophysiological mechanisms and interactions between trees and grasses on which most of the assumptions and models are premised have seldom been tested empirically. The vertical separation of infiltrated soil moisture between upper and lower soil layers (Walter 1939) has not been conclusively established whereas direct competition for moisture in the upper 300 mm soil layer between woody plants and grasses has been shown by Smit and Rethman (2000). The ecophysiological effects of intraspecific woody plant interactions have rarely been quantified in the field. Intraspecific competition among trees is assigned a major role in creating open savannas by the patch-dynamic model of Wiegand et al. (2006). An additional component of the patch dynamic model is that nitrogen fixation by the trees gives them a competitive advantage over grasses under low soil nutrient conditions (Ward 2010). The addition of nitrogen fertiliser in greenhouse experimental trials conferred a significant competitive advantage to grasses over trees in the initial stages of recruitment (Kraaij & Ward 2006). Anecdotal data, from preliminary investigations that failed to detect nodules associated with nitrogen-fixing rhizomes in established *Acacia mellifera* trees (Ward and Cramer, pers. comm.), may point to an absence of nitrogen-fixing ability among mature trees of this species. The possibility exists of Acacia trees relying on nitrogen-fixing bacteria during initial stages of establishment and discontinuing the association at a mature age when a competitive advantage has been established over grasses. An ability to derive N from the atmosphere could thus help the persistence of woody plants on nutrient-deficient soils of semi-arid regions, possibly conferring a competitive advantage to woody plants over non-N_2-fixing herbaceous layer vegetation (Milton 1980, Ward 2005, Wiegand et al. 2006). This may be an important factor in the initiation of the processes of shrub encroachment in savannas. One of the most common encroaching species is *Acacia mellifera* which occurs as a widespread shrub on rangelands in

Botswana, Namibia and northern parts of South Africa (Skarpe 1991, Molele et al. 2002, De Klerk 2004, Kraaij & Ward 2006).

This document synthesises the results of greenhouse and field-based investigations of the underlying ecological mechanisms and ecophysiological interactions between encroaching *Acacia mellifera* ([M. Vahl] Benth) trees from the semi-arid Northern Cape province of South Africa and grass species as well as intraspecifically among *A. mellifera* trees. Key questions that this thesis addresses concern the sources of soil moisture available to trees and grasses to ascertain if indeed trees and grasses access water from different depths in the soil and whether such a separation, if established, is distinctly two-tiered in support of Walter's (1971) model of niche separation in resource acquisition. Investigations also covered the role of nutrient supply and limitation in the competitive interplay between grasses and trees as well as the function of N_2 fixation by *Acacia mellifera* seedlings in their interaction with grasses and conspecifics. *A. mellifera* is known to encroach on rocky and sandy substrates, but does so more frequently on rocky substrates (Britz & Ward 2007). Hence, the effects of these substrate typess on the probability of bush encroachment occurring are also considered. Other than the two-way tree-grass competitive interactions on biomass output and nutrient status under sustained grazing pressure on different substrate types, intraspecific tree competition and its effect on tree vitality, plant water relations, nitrogen content and water use efficiency (WUE) is also covered.

The working hypothesis of this dissertation was that savannas are shaped by a multitude of plant interactions with both tree versus grass and tree versus tree interactions playing major roles in the process. The overarching hypothesis postulated competition from grass and conspecific neighbours for nutrients and soil moisture to negatively influence the growth, biomass, nutrient content and ecophysiology of *A. mellifera* so as to limit its proliferation into thickets, with disturbance of the grass component favouring trees. It is also the underpinning assumption of this thesis that both inter- and intra-specific competition is critical during tree seedling establishment stages and a competitive advantage established by grass over trees, trees over grasses or individual trees over cohorts is perpetuated thereafter until disrupted by disturbance. Given sparse grass cover on rocky terrains that formed part of our field study sites and the results from field experiments in the same area that indicated fire and herbivory to have reduced effects on *A. mellifera* recruitment (Kraaij & Ward 2006, Ward and Esler 2010), the focus of the investigations described here was on the mechanistic ecophysiology of inter and intraspecific competition. Chapter 2 tested the effect of intraspecific competition among field plants on the growth, mortality, water relations, nutrient concentration and WUE as key factors undepinning the theory of savanna dynamics. The influence of repeated grass herbivory and substrate type (rocky, sandy) in the competitive interactions between *A. mellifera* and grasses relative to biomass accumulation, nutrient concentration (Chapter 3) and soil moisture uptake with depth by either growth form (Chapter 4) were also investigated. Naturally limited N availability in savannas and competition for nutrients by grass may enhance a reliance on fixing N_2 (Cramer et al. 2007) and confer a competitive advantage to woody legumes over grasses. The nitrogen-fixing capacity of *A. mellifera* and its significance in conferring competitive advantage to tree seedlings over grasses was experimentally tested by evaluating the biomass accumulation, ^{15}N isotopic signatures and WUE of *A. mellifera* plants growing with and without grass competition at different concentration levels of fertilizer N in the greenhouse (Chapter 5).

The work undertaken under this doctoral research is intended to establish the main ecophysiological factors and feedback mechanisms or processes in the competitive interplay between *Acacia mellifera* trees and grasses in semi-arid savannas as well as the intra-tree competitive influences on plant ecophysiology. The most important expected outcome of this research was the establishment of a mechanistically appropriate representation of the factors causing a drastic increase in woody plant density that lead to bush encroachment in savanna ecosystems in the Northern Cape and Northwest provinces of South Africa, as well as in Botswana and Namibia. This will ultimately help the development and use of both predictive and management models or tools to control and limit the phenomenon of bush encroachment in order to maintain or increase agricultural productivity and biodiversity of rangelands.

The synthesis has four major thematic areas of focus, all of which seek to contribute to the understanding of bush-encroachment processes/mechanisms in the context of tree-grass coexistence paradigm as well as tree-on-tree interactions. Consequently, four manuscripts were prepared based on the investigations and experiments undertaken under the current study. Different ecophysiological methodologies, protocols and techniques were followed in addressing the different research questions and these are elaborated in each chapter. Chapter 2 covers a field study investigating the effects of tree-on-tree interaction on growth, partial canopy die-back, mortality, water relations, nitrogen and carbon isotopic signatures of mature shrubs from a neighbour removal experiment. Chapter 3 focuses on tree biomass production and nutrient concentration when grown with and without grasses. Niche separation of soil moisture uptake by tree seedling and grasses with depth in a greenhouse experiment is the subject matter of chapter 4 whereas chapter 5 investigates the nitrogen isotopic signature of trees and grasses as a function of intra-specific competition. The ecological significance and implications of the results from this work is synthesised in the concluding chapter 6. Chapters 2-5 were prepared for submission to particular peer-reviewed journals and therefore follow the format of the target journals.

All experimental set-ups, sample collection, direct measurements and data analysis as well as interpretation and write ups were done by the candidate. The field tree removal experiment was set-up by David Ward (see e.g. Meyer et al. 2008). Mass spectrometry and isotopic composition of samples, including nitrogen and carbon concentration, were analysed at the Stable Isotope Laboratory in Cape

Town. Nitrogen and phosphorus nutrient composition analyses were carried out by the Animal Science Department of the University of KwaZulu-Natal.

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2. Chapter 2: Intraspecific competition between shrubs in a semi-arid savanna

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2.1. Abstract

Tree-on-tree competitive interactions may be more important in affecting the distribution of the tree components of savannas than inter-specific competition with grasses. The presence of intraspecific competition is expected to negatively affect inter-tree spacing, individual size distributions and plant physiology as well as survival/mortality. In this field removal experiment on *Acacia mellifera*, one of South Africa's most common encroachers on nutrient-poor soils, the growth, water relations and mortalities of shrubs where all neighbouring woody competitors were removed (target) were monitored three times during each of three growing seasons. After three years, the nitrogen and carbon isotopic ratios of the study plants were analyzed. Target shrubs benefitted from removal of neighbours, resulting in greater growth, less water stress, a relatively small degree of canopy dieback and reduced reliance on N_2 fixation. Target shrubs grew by 25 ± 4% in height relative to 7 ± 4% for controls, with the targets suffering a maximum of less than 15% canopy dieback compared to up to 60% in the controls. Severe environmental stress is known to affect neighbour-interactions among shrubs and competition may constrain shrub sizes and avoid density-dependent mortality. In contrast, release from competition in our study may have allowed greater growth of target plants, increasing their total evapotranspirational leaf surface areas and leaving them vulnerable to drought and water stress. Intra-tree competition on shallow nutrient-poor soils in savannas may thus aid the persistence of bush encroachment by regulating the sizes of individual shrubs below the threshold of drought vulnerability.

Keywords: *Acacia mellifera*, bush encroachment, mortality, near-neighbour removal, water potential

2.2. Introduction

Intraspecific competition between woody plants plays a significant role in savanna structure and productivity (Smith and Grant 1986; Bonan 1991; Teague and Smit 1992), but has been less frequently cited as a factor determining the function of savannas than competition between grasses and woody vegetation (Stuart-Hill and Tainton 1989; Teague and Smit 1992; Scholes and Archer 1997). Recent work indicates that intraspecific competition between trees plays a major role in opening up encroached savannas and creating grassy patches in a cyclical succession (Wiegand et al. 2006). In infertile low-rainfall savannas, the effect of grasses on the performance of mature trees is considered negligible (Knoop and Walker 1985). Laterally extensive roots and a marked effect of woody plants on soil moisture (Knoop and Walker 1985; Wiegand et al. 2006; Meyer et al. 2007) may elevate the importance of inter-tree competition relative to interspecific competition with herbaceous plants in structuring the spatial distributions of trees in savannas. Competition among trees may limit or reduce their densities, sizes and spatial distribution (Smith and Walker 1983; Jeltsch et al. 2000), but positive intraspecific interactions may lead to aggregation and increased densities (Scholes and Archer 1997; Bruno et al. 2003; Miriti 2006). Semi-arid savanna vegetation dynamics are primarily driven by competition for soil moisture derived from rare but critical precipitation events (Ward 2005; Wiegand et al. 2005; Meyer et al. 2009). Rates of growth and the maximal stem heights achievable by savanna trees in semi-arid ecosystems are strongly influenced by moisture and nutrient availability (Shackleton 1997; Sankaran et al. 2008). Shackleton (2002) found that intra-specific competition, mostly within *Acacia* species, was more common than inter-specific interactions (see also Smith and Grant 1986; Wiegand et al. 2006; Meyer et al. 2007; Moustakas et al. 2008).

The presence/absence of competition between woody plant species is inferred from the spatial distribution of individuals and is associated with reduction in size of one or both neighbours (Bonan 1991; Shackleton 2002; Meyer et al. 2007). Individual plants within a community may exploit distinct regions for resources delineated by the vertical and lateral extent of their roots (Walter 1971). When rooting zones and areas of resource acquisition by trees coincide, local competition for resources may ensue (Walter 1971; Stoll and Weiner 2000). This competition intensifies with increasing densities of neighbouring plants, leading to density-dependent tree mortality (Harper 1977; Antonovics and Levin 1980; Slatkin and Anderson 1984; Ward 2005). This process has been invoked by Wiegand et al.'s (2006) patch dynamics model which postulates *en masse* cohort recruitment resulting in intense intraspecific competition and subsequent self-thinning, which re-opens encroached patches (Wiegand et al. 2006) and reduces tree densities (Peet and Christensen 1987; Cade and Guo 2000). Resource partitioning due to exploitation of different rooting zones among savanna tree species has been invoked to explain tree-on-tree competition avoidance (Knoop and Walker 1985). However, resource uptake by conspecific neighbouring trees with identical rooting patterns and phenological rhythmns will overlap in time and space. Thus, intra-specific competition probably can not be avoided through spatial/and or temporal niche partitioning (Goldberg and Novoplansky 1997).

We tested the hypothesis that individual shrubs not competing with neighbours experience relatively less water stress, have higher nutrient status and grow bigger (in height and canopy volume) than shrubs that are forced to compete with their neighbours. To do this, we examined the growth, mortality, water relations and isotopic composition of nitrogen and carbon in individual *Acacia mellifera* shrubs with and without neighbours in an encroached arid savanna (Kraaij and Ward 2006; Meyer et al. 2008) over three growing seasons between 2006 and 2008.

2.3. Methods

2.3.1. Study area

The study site was Pniel Estates in the Northern Cape province of South Africa, 35 km northwest of Kimberley (28° 34' , 24° 25') at ± 1000 m a.s.l. This site is on the southernmost fringe of *A. mellifera* distribution in Africa. The climate is semi-arid (388 mm rainfall p.a.), dominated by variable mean summer rainfall (Coefficient of Variance = 39%), hot summers (32.8°C max) and cold (3.2°C min) dry winters. Frost is experienced on an average of 22 d per annum (Kraaij and Ward 2006). The study area falls within the arid savanna biome (Mucina and Rutherford 2006) and encompasses a mosaic of predominantly two main vegetation units; the Kimberley Thornveld and the Vaalbos Rocky Shrubland. The Kimberley Thornveld is found on undulating sandy plains characterised by irregular to well-developed tree cover dominated by isolated *Acacia erioloba* and *A. tortilis* trees with *A. mellifera*, *Tarchonanthus camphoratus* and *Grewia flava* predominating the shrub layer. The Vaalbos Rocky Shrubland on isolated rocky hillsides and ridges comprises an open shrubland co-dominated by *A. mellifera* (the main encroaching species) and *T. camphoratus* L (Mucina and Rutherford 2006). The plants at the study site were among large dolerite boulders on rocky outcrops where mean shrub height was 0.75 m (Meyer et al. 2007). Grass cover was dominated by perennial *Schmidtia pappophoroides* and *Eragrostis lehmanniana*.

2.3.2. Climate and weather

Long-term precipitation records covering the study period (2006 - 2008) from the Kimberley (28^0.80 S; 24^0.77 E) and Barkly West (28^0.53 S; 24^0.53 E) weather stations were obtained from the South African Weather Service. The Kimberley weather station (1 204 m.a.s.l) is 35 km from the study site while the Barkly-West station (1 198 m a.s.l.) is 5 km from the field experimental site. On site ambient air (dry bulb) and relative humidity (RH) were obtained every 15 min during the collection of water potential data using a sling psychrometer and accompanying psychrometric chart (S. Brannnan & Sons Ltd., Cumbria, England). Precipitation at Barkly-West in the past 124 years was (mean ± S.E) 380 ± 14 mm yr^{-1}. Most of the rain (44 ± 1 % of total annual precipitation) fell towards the end of the rainy season in autumn (March-April). The total annual amount (109.5 mm) in 2003 just prior to the establishment of the experiment in 2004 was the lowest annual rainfall on record. Since the start of the experiment in 2004 by Meyer et al. (2008) until the end of the present study in 2008, above-average annual rainfall was recorded only once in 2006 (Fig. 2.1). Differences among the different stages of the growing season in ambient temperatures as well as RH were recorded (Fig. 2.1).

2.3.3. Field study

The same site and plants reported in Meyer et al. (2008) were used for the current study. Briefly, the mean lateral extent of near-surface roots of 30 randomly-selected *Acacia mellifera* shrubs was determined and a distance of double the length of roots (7.5 m) was measured from each shrub. All other woody vegetation within the circumference of the measured distance from the target shrub was mechanically removed in February 2004 and the remaining stumps poisoned with Garlon 4 (Efekto, Silverton, South Africa; emulsified with diesel in a volumetric ratio of 1:50 and sprayed onto the cut surfaces) to eliminate tree-tree competition for the particular individual (target) shrubs. The current study was initiated after two complete growing seasons without intraspecific competition. *Acacia mellifera* shrubs (controls, n = 30) of similar heights and canopy sizes were identified, tagged and mapped in the vicinity of each shrub for which neighbours had been removed. The positions of control shrubs relative to target plants in distance, direction and slope were varied at random. Measurements of canopy height and dimensions by Meyer et al. (2008) since March 2004 formed the baseline data for continued monitoring of shrub canopy heights and volumes for the current study (2006 - 2008). Occasional cutting and poisoning of re-sprouting neighbours were repeated during the study.

Shrub height and canopy volume were measured at the beginning (November), middle (February) and end (April) of each growing season. Whole tree mortalities and partial canopy dieback were also assesed from branch/twig mortalities by estimating the percentage of dead parts (following Bowers and Turner 2001). *Acacia mellifera* frequently grows as a multi-stemmed shrub, particularly in rocky areas. Shrub mean height (*h*) was determined by measuring the highest point above the ground at three different positions around the shrub. Canopy diameter and radius was estimated from the mean of the major horizontal axis (*a)* and the axis perpendicular to the major axis (*b*). The shape of *A. mellifera* shrubs approaches that of an inverted cone; thus, the formula of a cone ($V = \pi/3\ [a+b/2]^2 x\ h$) was used to estimate canopy volume. The growth of shrubs in height and canopy expansion over the seasons was calculated using $log(X_2\text{-}X_1)/t_2 - t_1$ where X_1 denotes the canopy height or volume measured by Meyer at al. (2008) in 2004 (t_1) and X_2 is the height or volume at the subsequent date (t_2) of measurement.

2.3.4. Plant water potential

To determine the effects of neighbour competition on plant water relations, a Scholander pressure chamber was used to measure xylem pressure potentials of both control and target shrubs. Three leafy twigs were excised with a razor blade from three separate parts of the shrub canopy and immediately inserted into the pressure chamber. Xylem pressure was measured at pre-dawn (Ψ_{PD}) and at midday (Ψ_{MD}) in November, February and April between 2006 and 2008. Shrub water potential deficit (Ψ_{DF}), was calculated from the difference Ψ_{MD} - Ψ_{PD}.

2.3.5. Nitrogen and carbon isotopes

Leaves were sampled at the end of the growing season in April 2008 and milled to a fine powder using a Culatti Type MFC micro-fine pulverizing electrical grinder (Janke and Künkel GmbH, Staufen, Germany) fitted with a 1 mm mesh. Samples were weighed (± 1 µg) into tin cups on a Sartorius (Sartorius, Göttingen, Germany) micro-balance and combusted in a Flash EA 1112 series elemental analyzer (Thermo Finnigan, Italy). The gases were passed to a Delta Plus XP IRMS isotope ratio mass spectrometer (Thermo electron, Germany), via a Conflo III gas control unit (Thermo Finnigan, Germany). The in-house standards used for expressing $\delta^{15}N$ isotopes were Merck Gel (Merck KgaA, Darmstadt, Germany) and dried lentils. All the in-house standards were calibrated against International Atomic Energy Agency (IAEA) standards. Carbon isotope ratios are expressed as $\delta^{13}C$ (Ehleringer and Rundel 1989) relative to Pee-Dee Belemnite (PDB) while nitrogen isotopes are expressed as $\delta^{15}N$ (Evans 2001) relative to the natural abundance signature of N in atmospheric air.

2.3.6. Data analysis

Differences among between-subject factors (phases of the growing season in different years) in terms of Ψ, height and canopy size (growth) were analysed using general linear repeated measure MANOVA, with a full factorial model with Type III Sums of Squares. Linear comparisons of group means and pair-wise multiple comparisons were subjected to Bonferroni *post hoc* tests. The main effects were compared with Bonferroni confidence interval adjustments at a significance level (p) of 0.05.

Growth of canopy height and volume at each date of field measurement was calculated relative to the original height/sizes measured in 2004 by Meyer et al. (2008) and the results compared via repeated measures MANOVA. Repeated measures MANOVA was also used to analyse partial canopy dieback (% shrub canopy that was dead) in study plants as estimated during every field visit. A few plants died during the experiment and this resulted in missing data which created a statistical bias of temporal autocorrelation. Fractional canopy mortalities were arcsine square-root transformed to conform to ANOVA test assumptions. Whole plant mortality was evaluated using the Kaplan-Meier estimate (Kaplan and Meier 1958). Only shrubs that died during the current study were considered. Completely dead shrubs were excluded from analysis of partial canopy dieback while branch death was not factored into the Kaplan-Meier procedure. Once-off comparisons of target and controlshrubs of %N, δ^{15}N‰, %C and δ^{13}C‰ were analysed using one-way ANOVA. One-way ANOVA was also used to compare Ψ of target and control trees in each season.

To relate rainfall amount to Ψ, height and canopy volume, the cumulative rainfall amount over 90, 60, and 30 d preceding field recordings was regressed against the means of these parameters. The strength of correlations of Ψ and sizes to rainfall over a given period indicated whether shrub Ψ and growth were responsive to precipitation or more reactive to longer-term soil moisture conditions. Given the inherent high variability of precipitation in the study area, extreme weather conditions can give rise to rainfall amounts outside the range of normal distribution (outliers). The leverage exerted by outlier cases on the predicted value of the regression line was screened using Cook's D statistic (Cook 1977; Newton and Rudestam 1999).

2.4. Results

2.4.1. Shrub sizes and growth

Both target (18.4 cm) and control shrubs (6.6 cm) canopy heights increased significantly over the five years of monitoring. Similarly, significant expansions were also found in canopy volumes (Table 2.1). All the plant canopies increased in height and volume over the study period despite expansion and contractions between seasons (Fig. 2.2). Decline in canopy height was largely noted over winter for these deciduous shrubs between the end of the wet season (April) when they lose leaves and the onset of the next (November) when the shrubs grow new leaves. For control shrubs, the first negative canopy height growth (-5.2 cm) was measured in November 2006 while it occurred a year later (November 2007) in target shrubs (-5.0 cm). There was no significant difference between target and the control shrubs in March 2004 when measured by Meyer et al. (2008). The target shrubs were significantly taller than the control plants in April 2008 (Fig. 2.2a).

While there was significant variation among seasons in canopy height and volume, the season x treatment interaction was not significant for height (albeit nearly significant) or volume (Table 2.1). Significant differences were found in the growth of shrub canopy volume but not in height. Season x treatment interactions showed no significant effects on growth in canopy height or volume (Table 2.1).

Target shrubs had significantly greater canopy volume expansions than control shrubs (Fig. 2.2b). Comparing the final (April 2008) measurements to the initial, target shrubs showed an average of 25 ± 4% canopy height growth and 357 ± 128% in volume over the study period. The final height of control shrubs was 7 ± 4 % greater than the initial measurements, and the volume was 164 ± 125 % larger than the original measure.

2.4.2. Plant mortality

Fewer individual target shrubs (6 out of 30) compared to 17 of 30 control shrubs suffered partial canopy dieback in a given season ($\chi^2 = 8.53$, d.f. = 1, P = 0.003). Individual target shrubs experienced 5-15% canopy dieback whereas most control shrubs suffered 25-60% partial canopy dieback. Control shrubs showed a significantly higher propensity toward partial loss of canopies compared to target shrubs. There were significant seasonal variations in partial canopy dieback but no significant treatment x season interaction (Table 2.1). However, 13.3% of target shrubs died (i.e., did not resprout) while no control shrub suffered complete mortality during this experiment, resulting in a significant difference between total shrub mortalities of target and control shrubs ($\chi^2 = 4.08$, d.f. = 1, P = 0.043). Partial and whole plant dieback were especially noticeable at the onset and end of the growing season. All but one of the dead shrubs were discovered in April 2007, the other individual having died in November 2006.

2.4.3. Plant water potential

The most negative water potentials (both Ψ_{PD} and Ψ_{MD}), possibly indicating water stress, were measured in February 2007 by control shrubs (Fig. 2.3), which was also the date on which large Ψ_{DF} comparable to late season values was found in both sets of study plants (Fig. 2.3c). The early rainy season (November) showed the least negative Ψ_{MD} in all study plants (Fig. 2.3b). Significant differences were found in seasonal variations of pre-dawn (Ψ_{PD}), midday (Ψ_{MD}) water potentials and the difference between the two (Ψ_{DF}). There were significant season x treatment interactions for Ψ_{PD}, Ψ_{MD} and Ψ_{DF} (Table 2.2).

Control shrubs had significantly more negative Ψ_{PD} than target shrubs in all seasons, other than November and February 2008 when the values did not differ significantly (Fig. 2.3a). Significant differences between Ψ_{PD} of control and target shrubs were measured in April 2006 ($F_{2,54}$= 33.27, $P \leq 0.001$), February 2007 ($F_{2,56}$= 71.49, $P \leq 0.001$) and November 2007 ($F_{2,51}$= 8.79, $P = 0.005$). Thus, when soil moisture availability was limiting or temperatures were high during hot and dry rainy seasons, control shrubs experienced higher predawn water stress than target shrubs, but in seasons with favourable soil moisture availability and low minimum temperatures, there were no significant differences. November 2006 received and was preceded by, above-average autumn (April) precipitation and February 2008 benefitted from heavy early-season precipitation, with low temperatures recorded in both these seasons (Fig. 2.1). However, when soil moisture content was high and the maximum and minimum temperatures were significantly lower than the mean, as in April 2006, target shrubs had significantly less negative $\Psi_{PD.}$

Midday water potential (Ψ_{MD}) values also varied in accordance with seasonal precipitation, with control shrubs having significantly more negative Ψ_{MD} in February ($P \leq 0.001$) and November 2007 ($P = 0.048$) when rainfall was below average (Fig. 2.3b). Target shrubs had significantly more negative Ψ_{MD} in April ($P \leq 0.001$) and November 2006 ($P = 0.049$) when precipitation was above average (Fig. 2.3b). The Ψ_{MD} differed significantly during April 2006 ($F_{2,54} = 28.39$, $P \leq 0.001$), February 2007 ($F_{2,56} = 76.71$,$P \leq 0.001$) and November 2007 ($F_{2,50} = 4.11$, $P = 0.048$). Thus, when soil moisture was not limiting, target trees had significantly more negative Ψ_{MD}. Midday Ψ values in April 2007 and February 2008 were not significantly different between the target and control treatments (Fig. 2.3b). The least negative Ψ_{PD} was registered in the late season (April) by all shrubs, possibly reflecting adequate plant water status. The late season was also the only time when significant differences were found between treatments in terms of $\Psi_{DF,}$ with target shrubs recording larger deficits (Ψ_{DF}) than control shrubs on both occasions (Fig. 2.3c). Significant differences between Ψ_{DF} were found during April 2006 ($F_{2,54} = 45.93$, $P \leq 0.001$) and in April 2007 ($F_{2,53} = 7$, $P = 0.011$).

2.4.4. Influence of cumulative precipitation

Shrubs invested in increased height of the canopy in response to long-term rainfall trends. Positive correlations of target shrub canopy heights to cumulative precipitation (Fig. 2.4) occurred over 60 d ($r = 0.93$, $F_{2,5} =31.72$, $P = 0.002$) , 90 d ($r = 0.89$, $F_{2,6} =22.47$, $P = 0.003$) and 120 d ($r = 0.80$, $F_{2,6} =10.7$, $P = 0.017$) when exceptionally low and high precipitation amounts (one of each) were removed (Fig. 2.4). The canopy heights of control shrubs showed significant positive correlations with cumulative rainfall for the full range of recorded rainfall amounts over the period of 90 d ($r = 0.77$, $F_{2,7} = 10.32$, $P = 0.015$) and 120 d ($r = 0.73$, $F_{2,7} = 7.25$, $P = 0.026$). Canopy volume showed a significantly positive correlation ($r = 0.89$, $F_{2,5} = 18.79$, $P = 0.007$) to total rainfall after 60 d for both target and control shrubs (Fig. 2.4).

Canopy predawn water potential (Ψ_{PD}) was not correlated to the short- or long-term accumulation of rainfall, but showed a strong positive correlation to medium-term rainfall over 30 d ($r = 0.86$, $F_{2,10} = 18.15$, $P = 0.002$) and 60 d ($r = 0.94$, $F_{2,10} = 29.14$, $P = 0.001$). The predawn water potential of target shrubs showed stronger correlations to additive rainfall amounts compared to control shrubs (Fig. 2.4c), indicating that shrubs had a more robust response to soil moisture accumulation without neighbours. The difference between predawn and noon water potential (Ψ_{DF}) in target shrubs showed a strong negative correlation to accumulated rainfall over the long-term of 60 ($r = -0.93$, $F_{2,4} = 24.45$, $P = 0.008$) and 90 ($r = -0.87$, $F_{2,4} = 12.26$, $P = 0.025$) d (Fig 2.4).

2.4.5. Tissue nitrogen and carbon and their isotopes

Target shrubs had significantly higher δ^{15}N‰ mean values than control plants (Table 2.3), indicating a stronger dependence on biological nitrogen fixation among control plants relative to target shrubs. Target shrubs exhibited significantly higher foliar N concentrations than the control shrubs (Table 2.3). The low foliar N resulting from competition for N by neighbours induced nodulation and thus increased reliance on N_2 fixation by controls. No significant difference was found between the carbon isotope discrimination (δ^{13}C) of target and control shrubs ($F_{2,53} = 0.078$, $P = 0.781$) five years after near-neighbour shrub removals. The δ^{13}C of control (-27.2 ± 0.2 ‰) and target (-27.1 ± 0.1 ‰) shrubs were nearly identical, possibly indicating little influence of neighbouring *A. mellifera* shrubs on each other's water use efficiency (WUE). Target shrubs had comparable ($F_{2,53} = 3$, $P = 0.089$) carbon content (%C) in their leaves (47 ± 0.19%) to control shrubs (46.5 ± 0.22%).

2.5. Discussion

The removal of neighbours had a marked effect on the growth and water relations of the shrubs. The increase in canopy height of target shrubs over five years between the first measurement in 2004 (recorded by Meyer et al. 2008) and the final measurement for this study in 2008 as well as their average growth between seasons were almost threefold that achieved by control shrubs. While more control shrubs suffered partial canopy-dieback than target shrubs, with greater percentages of the control shrub canopies being affected relative to target shrubs, a few target shrubs died whereas no control shrubs died. Control shrubs had significantly more negative predawn water potential (Ψ_{PD}) than target shrubs in dry and hot or wet and cool times of the season, but target shrub had more negative midday water potential (Ψ_{MD}) and larger differences between Ψ_{MD} and Ψ_{PD} (Ψ_{DF}) when soil moisture availability was non-limiting. Plant sizes responded positively to cumulative rainfall amounts over the long-term whereas Ψ_{PD} was more responsive to precipitation accumulated over an intermediate period. Control shrubs had lower foliar N concentration and showed increased reliance on N_2 fixation relative to target shrubs but there was no difference in their WUE.

A marked expansion in shrub size following the above-average precipitation of 2006 was immediately followed by contraction during the subsequent dry season (i.e. prior to November 2006) for control shrubs. However, it took the exceedingly hot and dry conditions of 2007 to bring about a decline in target shrub canopy sizes a year later (November 2007). Augmented availability of soil moisture during or following above-average rainfall may bolster extensive root production and accelerated canopy growth (Zegada-Lizarazu et al. 2007). When soil moisture decreases to or below average levels, mortalities often occur, especially on underdeveloped soils (Hamerlynck and McAuliffe 2008). It is probable that a limited volume of soil and low water-retention capacity of stony underdeveloped horizons found in our study site exposed rooting zones to rapid desiccation. A relatively high degree of soil porosity would allow rapid uptake of moisture by roots (Archer et al. 2002) and downward percolation. Infiltrated moisture is thus either rapidly consumed by root uptake, evaporation or percolation deeper into the soil, resulting in reduced surface soil moisture availability to plants, despite transitory subterranean storage of moisture beneath rocks (Nobel and Zutta 2007). Relatively greater increases in canopy size (Figs. 2.1, 2.2) showed that the absence of intraspecific competition enabled *A. mellifera* to take advantage of above-average precipitation. The removal of neighbouring individuals thus conferred benefits in terms of aboveground growth of target compared to control shrubs, indicating that intraspecific compeition could indeed be important in semi-arid environments. Canopy size contractions during the early summer were attributable to mortality of crown branches in the dry season, possibly as a consequence of water stress resulting from low rainfall, high temperatures and low humidity in the preceeding season (e.g. April 2007).

The tree removal experiment was preceded by the driest season on record (2003) and the study area persistently experienced below-average precipitation for most of the study period. Such an extreme drought may possibly have caused significant dieback of both the crown branches and some roots, thus reducing the competitive interactions among shrubs. This may explain why the effect of near-neighbour removal on plant sizes and growth was not immediate, but took until 2007 to manifest itself (Fig. 2.2). Moreover, the drought may have resulted in low or depleted soil moisture storage, such that much of the water accessed by shrubs during the study was derived from recent precipitation as indicated by the correlation of sizes andΨ_{PD} to cumulative rainfall. When soil moisture availability was limiting or evaporative demand was high, target shrubs had better access to soil moisture than control shrubs. This could have resulted from reduced/absent sub-surface competition for soil moisture among target plants. Water stress associated with strongly negative Ψ, as measured in our study plants, may cause branch or whole plant mortality (Zimmermann 1983; Tyree and Sperry 1988). The protection of individual plants by intact vegetation during extreme drought (DeSteven 1991; Gill and Marks 1991; Berkowitz et al. 1995) may have facilitated the partial survival of control shrub canopies, but death of a number of target shrubs. This could be attributed to either a shielding effect of neighbours and the exposure of neighbourless shrubs to extreme conditions or shrubs benefiting from shared hydraulic lift. Target shrubs were exposed to direct sunlight, radiation and wind at canopy and ground level which may have increased evapotranspiration relative to control plants shielded by conspecifics. During seasons of non-limiting soil moisture availability, target shrubs had more negative Ψ_{MD} than the controls and this reverse trend (relative to Ψ_{PD}) partially supports the beneficial effect of neighbours. It is more likely however that increased growth of target shrubs predisposed them to greater sensitivity to extreme dry conditions through increased evapotranspirational surfaces. Ansley et al. (1998) attributed fourfold higher evapo-transpirational water loss in individual honey mesquite (*Prosopis glandulosa*) trees without neighbours relative to trees at high density to either resource limitation or increased water conservation. High water loss by target shrubs experiencing minimal resource limitation during the wet season probably rendered them vulnerable to harsh conditions. Partial canopy dieback exceeding 30% of the total canopy was observed to affect discrete segments of shrub crowns in our study. Partial demise of the canopy could be a means of reducing the total photosynthetic surface area by shrubs. Shrubs with dead parts of their canopies exhibited relatively improved water relations compared to other seemingly healthy shrubs from similar treatment that suffered little or no branch mortalities. This may suggest hydraulic segmentation (Zimmermann 1983) by *A. mellifera* to minimise water stress and ensure the survival of the individual. We note that, for the spatial scale studied as well as the temporal scale of the experiment, the influence of tree size on both growth and water potential was not significant.

Relatively smaller $\delta^{15}N‰$ values in control plants may indicate a reliance on N_2 fixation to have been induced by intraspecific competition. Previous studies have shown interspecific competition with grass to induce N_2 fixation in acacias (Cramer et al. 2007) with belowground competition for N generating such competitive responses (Cramer et al. 2010); competition from conspecifics may have a similar effect. Interestingly, a reliance on N_2 fixation by control shrubs did not lead to higher foliar N concentrations in control relative to target shrubs, but target shrubs achieved more growth. Removal of neighbors may have increased the access of target shrubs to N while competition from neighbours caused a low tissue N concentration, inducing a reliance on fixation of atmospheric N_2 by control shrubs. Cramer et al. (2009) argue that a function of water acquisition by plants is to facilitate the mass flow of nutrients through the soil. Thus, the availability of soil nutrients may influence the rate of transpiration, increasing transpiration when soil N is limited (Cramer et al. 2009). Limited soil N may have been partially responsible for negative Ψ in control shrubs with limited access to N due to intraspecific competition. Tree-on-tree competition for co-limiting N and water resources may regulate the sizes and pattern of tree aggregation in savannas.

Boulders in our site may have acted as conduits for the percolation of rainwater, while shielding the soils beneath them from evaporative losses (Nobel et al. 1992), allowing persistence of soil water (Nobel et al. 1992; Hamerlynck and McAuliffe 2008). Rocks could thus aid plant establishment through transient soil moisture and yet constrain rooting biomass via physical impediment, thus limiting plant growth and leading to small shrubs aggregating at high densities (Hamerlynck and McAuliffe 2008; Ward and Esler 2010). Physical limitations presented by rocks, shallow soil horizons and limited resources such as N probably put a threshold on the maximum size attainable and thus the extent of plant resource capture zone. Plants growing in a water-limited nutrient-poor environment show reduced leaf growth (Van Volkenburgh and Boyer 1985; Munns and Cramer 1996) which ultimately leads to reduced plant size. The small stature of plants on rocky areas in arid environments (Schmiedel and Jurgens 2004; Ward and Esler 2010) and less robust competition among small individuals (Schenk et al. 2003) may allow for compact aggregation of individuals, as occurs during *A. mellifera* encroachment. Ward and Esler (2010) found that *A. mellifera* seedling densities were higher on rocky soils but these plants grew larger on sandy soils at the study site. Even so, a degree of tree-on-tree competition may maintain the individual plant sizes within the threshold that can be supported by the available resource pool. The removal of near-neighbour competition may in turn release an individual from the competition-imposed size limit and allow it to grow beyond such a threshold which exposes it to drought-induced mortality. The implications of our findings for bush encroachment control and management is that in shallow resource-poor soils, it may not be necessary to eliminate all plants in order to reduce shrub density. Retaining some large individuals may also benefit the soils in terms of N_2 fixation (Wiegand et al. 2005).

2.6. Acknowledgements

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Table 2.1 Results of Repeated Measures MANOVA General Linear Model (GLM) for monitoring of the height, volume (including data reported by Meyer et al. 2008) and partial dieback of the canopies of shrubs with and without neighbours. Differences in d.f. result from the fact that growth could only be calculated from the second measurement onwards as well as partial dieback (branch mortality) not monitored by Meyer et al. (2008)

Source of Variation (Effect)	**Wilks' λ**	**F**	**d.f**	**Error d.f.**	**Significance (P)**
Canopy Height					
Seasonal Canopy Heights	0.584	7.73	8	44	<0.001
Season x Neighbour Competition	0.263	1.97	8	44	0.074
Growth in Height	0.864	1.02	7	45	0.433
Seasonal Height Growth x Competition	0.855	1.09	7	45	0.384
Canopy Volume					
Seasonal Canopy Volumes	0.552	4.46	8	44	0.001
Season x Neighbour Competition	0.800	1.38	8	44	0.233
Growth in Volume	0.704	2.70	7	45	0.020
Seasonal Volume Growth x Competition	0.890	0.80	7	45	0.593
Seasonal Partial Canopy Dieback	0.520	7.38	6	48	<0.001
Seasonal Partial Dieback x Competition	0.789	1.10	6	48	0.376

Table 2.2 Results of Repeated Measures MANOVA General Linear Model (GLM) for shrub water potential (Ψ) over the course of the growing season between April 2006 and February 2008

Source of Variation (Effect)	Wilks' λ	F	d.f	Error d.f.	Significance (P)
Predawn Water Potential (Ψ_{PD})					
Seasonal Ψ_{PD}	0.034	356.26	5	62.00	<0.001
Seasonal Ψ_{PD} x Neighbour Competition	0.164	10.58	15	171.56	<0.001
Midday Water Potential (Ψ_{MD})					
Seasonal Ψ_{MD}	0.196	42.53	5	52.00	<0.001
Seasonal Ψ_{PD} x Neighbour Competition	0.172	8.55	15	143.95	<0.001
Water Potential Deficit (Ψ_{DF})					
Seasonal Ψ_{DF}	0.206	40.15	5	52.00	<0.001
Seasonal Ψ_{DF} x Neighbour Competition	0.297	5.31	15	143.95	<0.001

Table 2.3 One-way ANOVA comparisons of δ^{15}N isotopic composition and foliar tissue N concentration (mean ± SE) of control and target shrubs at the end of the experiment in 2008

Measured Parameter	Treatment		F	Error d.f.	Significance (P)
	Control	Target			
δ^{15}N isotope (‰)	0.30 ± 0.42	1.9 ± 0.54	5.67	53	0.021
Foliar N concentration (%)	2.93 ± 0.07	3.13 ± 0.05	5.31	53	0.025

Figure Captions

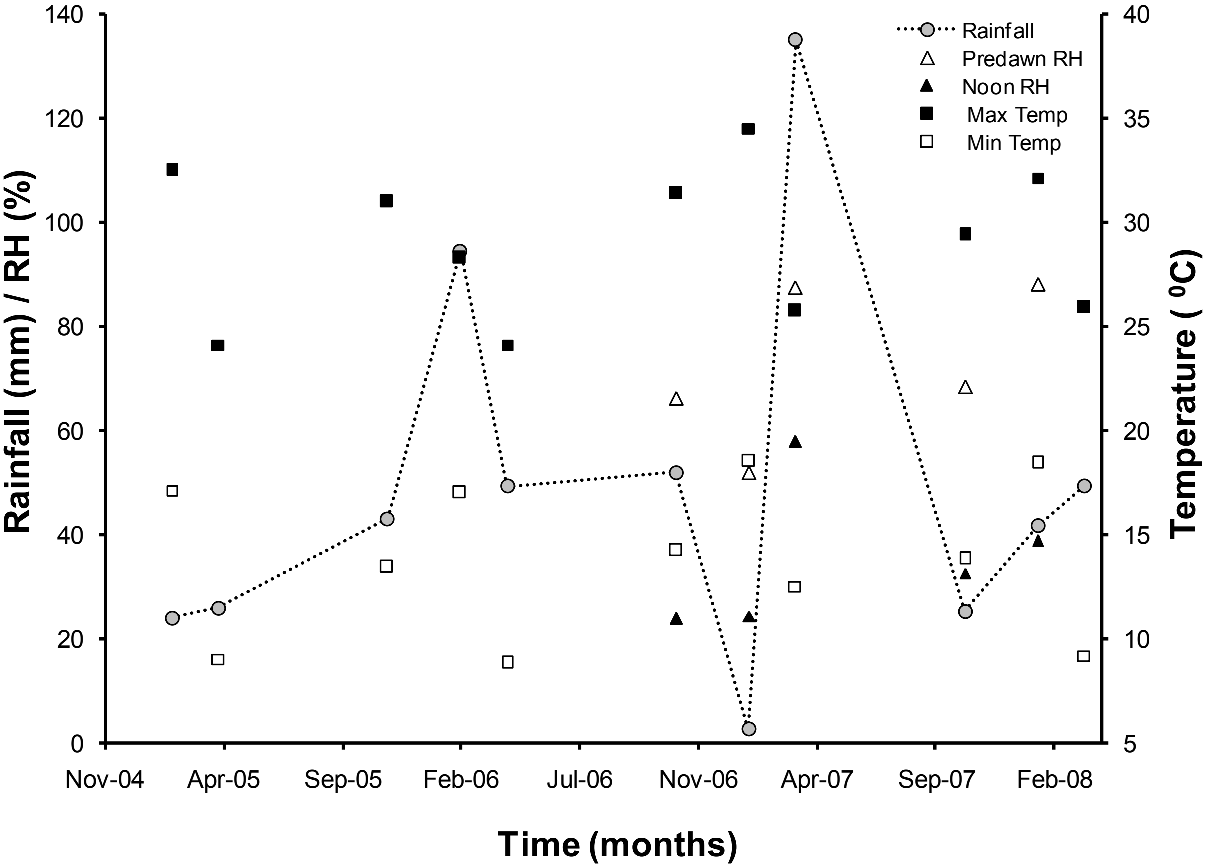
Fig. 2.1 Rainfall and temperature recorded at nearby weather stations as well as on-site relative humidity (RH) during the study period.

Fig. 2.2 Sizes of target and control shrubs (mean ± SE) over time; (a) height and (b) canopy volumes. Following above-average rainfall in February 2006 and November 2007, the size of target trees became significantly larger than those of control shrubs.

Fig. 2.3 Plant water potential (Ψ) (mean ± SE) of control and target shrubs recorded in different months over the growing season between 2006 and 2008 at (a) predawn and (b) midday as well as the (c) difference between the two values. Water potentials were measured twice in each month of the growing season of February (2007/8), April (2006/7) and November (2006/7). Differences in error d.f. resulted from some trees not having leaves or dying during the course of the study.

Fig. 2.4 Linear regression of control and target shrubs canopy height (a), volume (b), predawn water potential (c) and water potential deficit (d) against cumulative rainfall recorded over 60 d prior to field measurements. For sizes (height and volume), it was found that Cook's D value for two outliers (i.e. exceptionally high [157.5 mm] and low [8 mm] rainfall amounts over 60 days) recorded in April 2006 and February 2007 respectively was greater than 1, indicating that these amounts were influential leverage points. These data points were removed and a corrected correlation performed.

Fig. 2.1



Rainfall
Predawn RH
Noon RH
Max Temp
Min Temp
Rainfall (mm) / RH (%)
Temperature (^{0}C)
Time (months)
0
20
40
60
80
100
120
140
5
10
15
20
25
30
35
40
Nov-04
Apr-05
Sep-05
Feb-06
Jul-06
Nov-06
Apr-07
Sep-07
Feb-08

Fig. 2.2

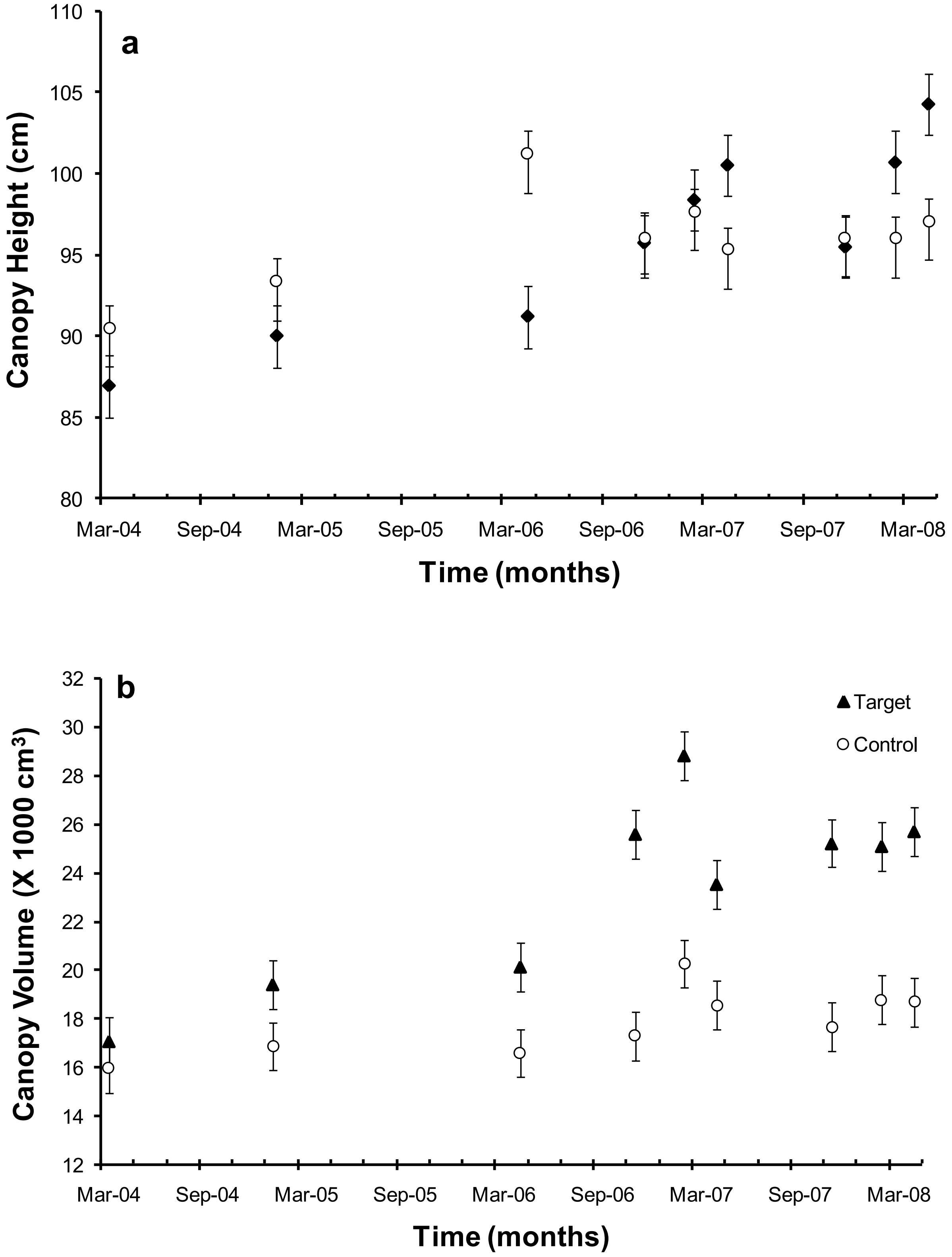
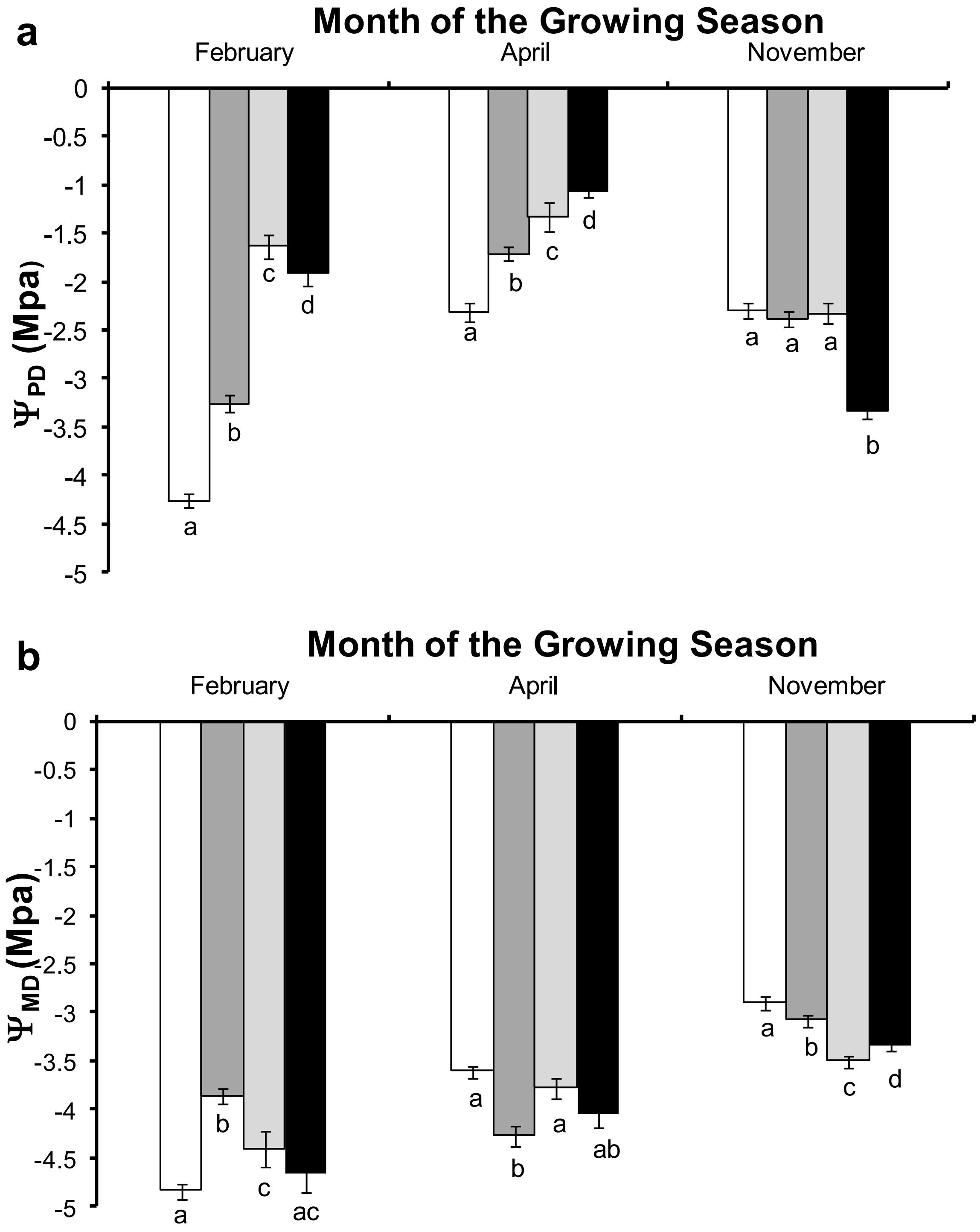

a
Canopy Height (cm)
110
105
100
95
90
85
80
Mar-04
Sep-04
Mar-05
Sep-05
Mar-06
Sep-06
Mar-07
Sep-07
Mar-08
Time (months)
b
▲Target
○Control
Canopy Volume (X 1000 cm³)
32
30
28
26
24
22
20
18
16
14
12
Mar-04
Sep-04
Mar-05
Sep-05
Mar-06
Sep-06
Mar-07
Sep-07
Mar-08
Time (months)

Fig. 2.3


a
Month of the Growing Season
February
April
November
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
Ψ_{PD} (Mpa)
a
b
c
d
a
b
c
d
a
a
a
b
b
Month of the Growing Season
February
April
November
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
Ψ_{MD}(Mpa)
a
b
c
ac
a
b
a
ab
a
b
c
d

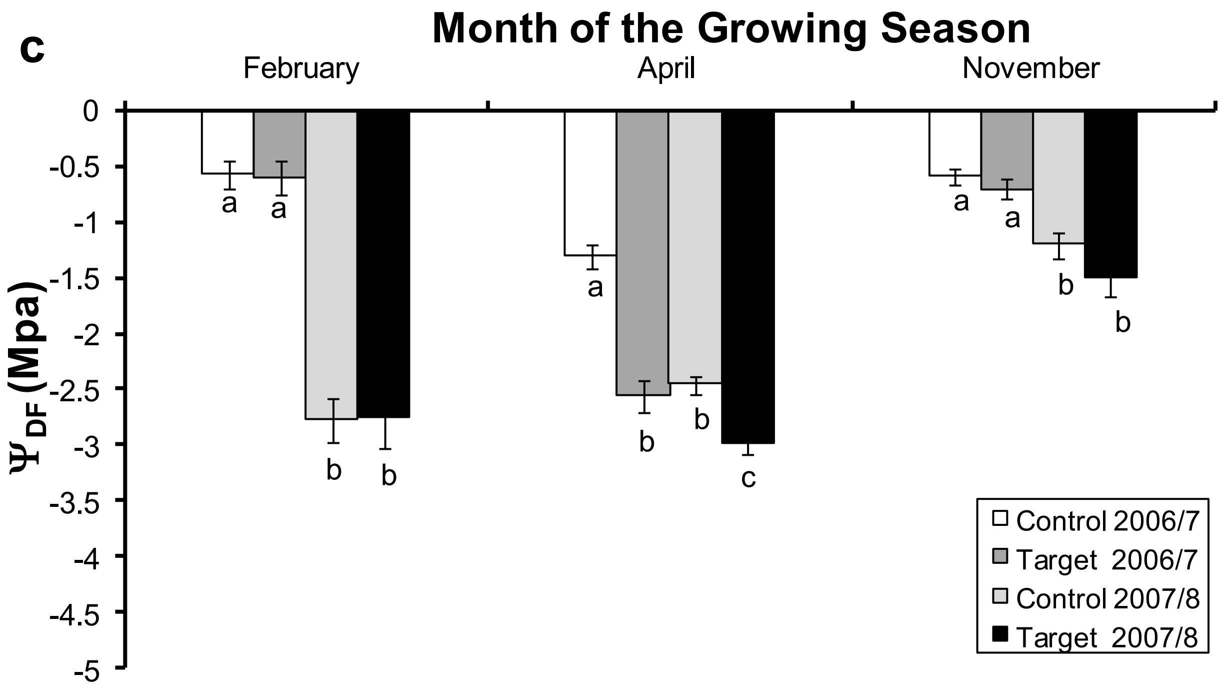
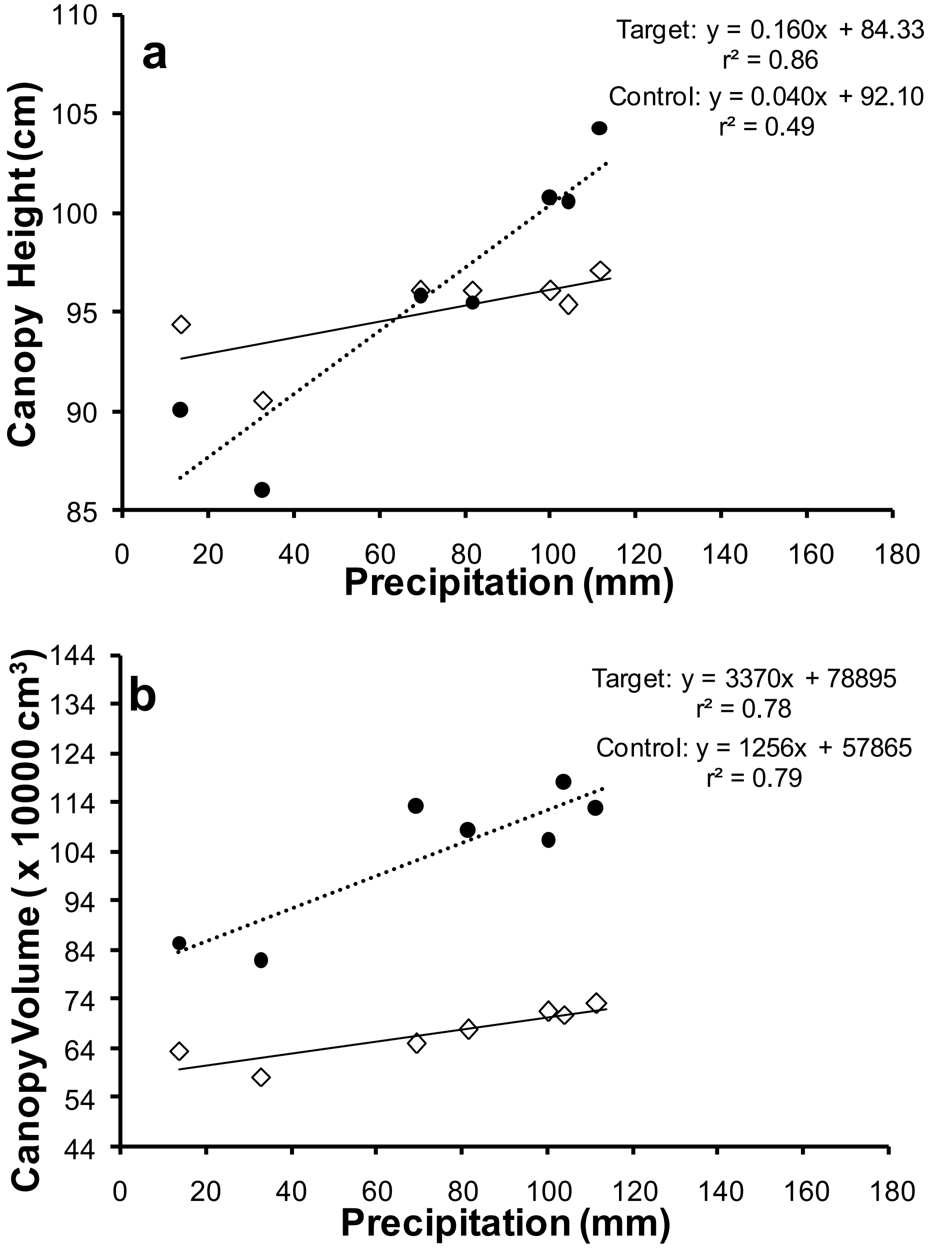
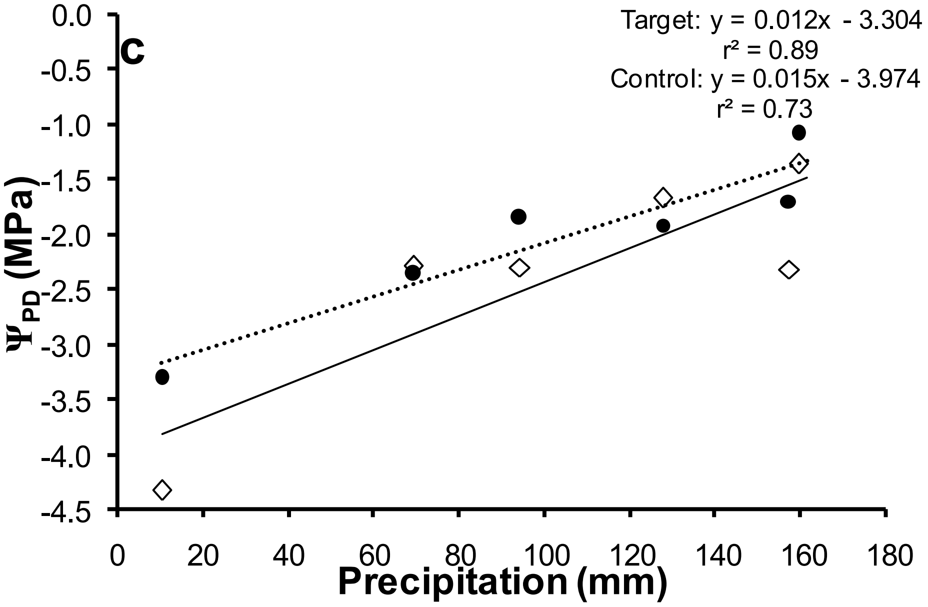
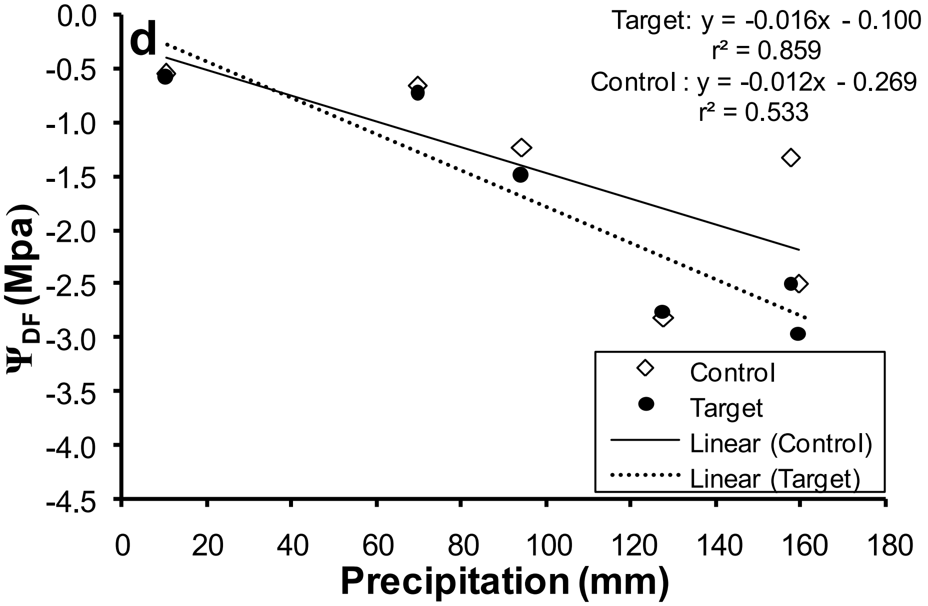

c
Month of the Growing Season
February
April
November
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
Ψ_{DF} (Mpa)
a
a
b
b
a
b
b
c
a
a
b
b
☐ Control 2006/7
▨ Target 2006/7
☐ Control 2007/8
■ Target 2007/8

Fig. 2.4


a
Target: y = 0.160x + 84.33
r² = 0.86
Control: y = 0.040x + 92.10
r² = 0.49
Canopy Height (cm)
110
105
100
95
90
85
0
20
40
60
80
100
120
140
160
180
Precipitation (mm)
b
Target: y = 3370x + 78895
r² = 0.78
Control: y = 1256x + 57865
r² = 0.79
Canopy Volume (x 10000 cm³)
144
134
124
114
104
94
84
74
64
54
44
0
20
40
60
80
100
120
140
160
180
Precipitation (mm)


c
Target: y = 0.012x - 3.304
r² = 0.89
Control: y = 0.015x - 3.974
r² = 0.73
Ψ_PD (MPa)
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
-4.0
-4.5
0
20
40
60
80
100
120
140
160
180
Precipitation (mm)


d
Target: y = -0.016x - 0.100
r² = 0.859
Control : y = -0.012x - 0.269
r² = 0.533
Ψ_DF (Mpa)
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
-4.0
-4.5
0
20
40
60
80
100
120
140
160
180
Precipitation (mm)
◇ Control
● Target
Linear (Control)
Linear (Target)

3. Chapter 3: Savanna tree-grass competition is modified by substrate type and herbivory

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3.1. Abstract

Question: Woody plant and grass interactions in savannas have frequently been studied from the perspective of the response of one growth form on the other and seldom evaluated two-way interactions. What causes woody plant encroachment in semi-arid savannas and what are the competitive responses of tree seedlings and grasses on rocky and sandy substrates?

Methods: In this greenhouse study, we investigated the influence of substrate and grazing on the responses to interspecific competition by tree seedlings and grasses. We measured competitive/ facilitative responses on biomass and nutrient status by tree seedlings and grasses grown together.

Results: Interspecific competition suppressed the growth of trees and grasses. Tree seedlings and uncut grass accumulated double the biomass when grown without competition relative to when they competed. Competitive responses varied on different substrates. Grass biomass on rocky substrates showed no response to tree competition, but appeared to be facilitated by trees on sandy substrates. Grass clipping resulted in greater tree seedling biomass on rocky substrates, but not on sandy substrates. There was a positive response of grass nutrient status to competition from tree seedlings.

Conclusion: Selective grass herbivory in the absence of browsing or the suppression of shade-intolerant grasses by trees are commonly-cited reasons behind bush encroachment in savannas. We show that grazing may confer a competitive advantage to tree seedlings and promote bush encroachment more readily on rocky substrates. This may be due to the imposed sharing of the soil-depth niche on rocky substrates whereas possible niche separation on sandy substrates minimises the advantage conferred by reduced competition.

Keywords: *Acacia mellifera*, biomass, bush encroachment, interspecific competition, nutrient status, semi-arid savanna, substrate

Nomenclature: Gibbs Russell et al. (1991), Van Oudtshoorn (1999), Coates Palgrave (2005)

3.2. Introduction

The co-occurrence of trees and grasses in savannas is associated with reciprocal competitive interactions between the two growth forms (Walker & Noy-Meir 1982; Tainton & Walker 1992; Scholes & Archer 1997). Tree-grass interactive dynamics are primarily driven by the availability of soil moisture and nutrients (Frost et al. 1986; Wiegand et al. 2006; van der Waal et al. 2009). Reported interspecific competitive responses and effects of coexisting savanna trees and grasses on either growth form vary from positive to neutral to negative interactions that sometimes change over time (Belsky et al. 1992; Scholes & Archer 1997). For example, grass production is either enhanced (Belsky et al. 1989) or hindered (Mordelet & Menaut 1995; van der Waal et al. 2009) by the presence of trees. Suppression of tree seedling growth, survival and establishment by grass competition has also been shown (Cramer et al. 2007; Riginos & Young 2007), although grasses may facilitate the germination of trees by moderating microclimatic conditions (Aide & Cavelier 1994).

By directly competing with grasses for light, mineral nutrients and soil moisture, trees cause a reduction in the growth of grasses (Scholes & Archer 1997; House et al. 2003; Ludwig et al. 2004a; van der Waal et al. 2009). Trees may also reduce soil moisture availability through the interception of precipitation by the canopy. Beneficial effects of trees on grasses include the use of water in the surface soil hydraulically lifted by deeper roots of trees (Caldwell *et* al. 1981; Ludwig et al. 2004a), cooler temperatures with accompanying minimized evapotranspiration under the shade of trees (Scholes & Archer 1997; Ludwig et al. 2004b) and enhanced nutrient availability due to leaf litter and possible N_2 fixation (Scholes & Archer 1997) which increase the nutrient content of grasses (Treydte et al. 2007; Ludwig et al. 2008). For example, augmented grass production below tree canopies has been attributed to increased soil fertility (Callaway et al. 1991; Anderson et al. 2001), although this does not always lead to higher grass biomass.

For woody plants, competition with grasses may impede seedling and sapling survival and establishment (Weltzin & McPherson 1997; Jurena & Archer 2003; Riginos & Young 2007). Shade intolerance has been reported for many savanna woody plants, especially leguminous trees (Smith & Shackleton 1988; Belsky 1994). Thus, a high standing grass biomass may suppress the growth of woody seedlings (Brown & Booysen 1967; Walker et al. 1981; Knoop & Walker 1985; Harrington 1991). High grass biomass may also suppress woody plant growth for reasons other than shading (Brown & Booysen 1967; Walker et al. 1981; Harrington 1991). For example, Riginos (2009) recently showed that the suppressive effect of grasses on savanna trees affects all demographic stages of woody plants in a wet and nutrient-rich savanna, although the applicability of this finding to nutrient-poor low-rainfall savannas (e.g. Knoop & Walker 1985) is unclear. While instances of facilitation of tree seedling establishment by standing grass biomass have been reported (e.g. Holmgren et al. 1997; Davis *et al.* 1998), there is strong evidence that competition with grasses in arid ecosystems together with the increased fire risk limits the invasion of grasslands by *Acacia* trees irrespective of soil type (Nano & Clarke 2010). High grass productivity that results in high biomass accumulation together with slow decomposition rates and dry season senescence of grasses (Ehleringer & Monson 1993) combine to accumulate combustible fuel (Knapp & Seastedt 1986). This results in fires that are detrimental to tree seedlings and saplings (Higgins et al. 2000; Bond 2008). Fires are harmful to trees but not to the herbaceous/grass layers, which regrow readily (Wolfson 1999; Riginos 2009).

The main drivers of grass production in addition to water and light are soil physical properties and nutrient availability (Scholes & Walker 1993). For example, augmenting the availability of nutrients through addition of nitrogen (N) and phosphorus (P) resulted in increased grass production outside and beneath tree canopies, respectively (Ludwig et al. 2001). Natural increase in grass N concentration beneath the canopies of savanna trees relative to grasses in open areas often exceeds the magnitude of increase in P, especially under *Acacia* species (Callaway et al. 1991). Increased grass nutrient content (Belsky 1992) and high protein concentration (Laclau 2008; Treydte et al. 2008) underneath *Acacia* trees have been reported. These trends could be attributed to higher concentrations of nutrients under woody plant canopies due to animal activities (Belsky 1994; Scholes & Archer 1997), or grasses benefitting from N_2 fixed by leguminous *Acacia* trees (Bernhard-Reversat 1982; Tolsma et al. 1987). However, competition for soil moisture and shading by trees may also lead to reduced grass biomass (Ludwig et al. 2008), resulting in higher concentrations of nutrients per unit grass biomass (Laclau et al. 2008).

Tree-grass interactions in savannas have mainly been considered as competition for soil moisture and nutrients or avoidance thereof (e.g. Walter 1971; Walker *et al.* 1981; Walker & Noy-Meir 1982; Knoop & Walker 1985). This is based on Walter's (1939) two-layer hypothesis of vertical niche separation in rooting depth and thus differentiation of soil resource use between the herbaceous layer and the woody component. Grasses with their fibrous root systems are more effective at exploiting soil resources in the upper layer than trees which they tend to outcompete while trees dominate resource capture from deeper soils (Pärtel & Wilson 2002). Defoliation of grasses by grazers is thought to diminish their competitiveness against tree seedlings by limiting grass carbon assimilation and root production (Chapin & Slack 1979). The outcome is reduced density of above- and below-ground grass biomass (Pandey & Singh 1992) which reduced the competitive effect of grass on trees (Walker & Noy-Meir 1982; Skarpe 1991; Jeltsch et al. 1997) and increased the availability of soil moisture to woody plants (O' Connor 1995; Weltzin & McPherson 1997). Consequently, reduced grass competition and low magnitudes of fires due to a diminished fuel load may lead to bush encroachment (van Langevelde et al. 2003; Graz 2008). Low frequencies of fires have been associated with shrub encroachment by enabling the establishment of tree seedlings (Van Auken 2000). Woody plant encroachment in arid environments is thought to be principally driven by grazing pressure, above-average precipitation events and anthropogenic alteration of fire regimes (Kraaij & Ward 2006; Nano & Clarke 2010). Browsers play a role in keeping bush encroachment in check by browsing on young tree seedlings, minimizing their establishment and curtailing regeneration of woody plants (Prins & Van der Jeugd 1993; Ripple & Beschta 2007, 2008). Displacement of browser populations from savanna ecosystems by grazers (predominantly cattle) has reduced herbivory on woody plants, altered the timing and intensity of grass defoliation as well as its recovery periods, often contributing to bush encroachment (Van Vegten 1983; Jeltsch et al. 1997; Graz 2008).

Both bottom-up resource limitations and top-down disturbance regimes (herbivory, fire) are important in suppressing woody plant recruitment, but their relative significance varies across environmental gradients and regions (Bond 2008; Nano & Clarke 2010). Bush encroachment has been observed to be most prevalent on rocky outcrops and/or areas with shallow soil horizons (Ward 2005; Wiegand et al. 2005; Kraaij & Ward 2006; Britz & Ward 2007). Britz & Ward (2007) showed a relatively constant (but high) tree-density on rocky areas that has changed little over many years whereas they found that there has been a recent increase in bush encroachment on sandy substrates.

Grass defoliation may promote the growth of already established seedlings as opposed to seedlings in the initial stages of establishment (O'Connor 1995). We used a manipulative greenhouse experiment to test the effects of grass defoliation and soil substrate type (rocky versus sandy) on *Acacia mellifera* tree seedling biomass and nutrient concentration. *A. mellifera* (Vahl) Benth is a drought-adapted woody shrub commonly occurring in arid to semi-arid environments where it is often known to be among the most frequent encroacher species (Skarpe 1991; Kraaij & Ward 2006; Wiegand et al. 2006; Joubert et al. 2008). It commonly grows as a multi-stemmed shrub up to 3 m in height, but may reach up to 7 m (Smit 1999). We predicted that tree seedling sizes and nutrient status would respond negatively to grass competition and that frequent clipping of the grass to simulate herbivory would negatively affect the suppressive competitive influence of grass. Rocky substrates are likely to impede and restrict tree seedling rooting depth (Savory 1963; Rutherford 1983), thus limiting the scope for niche separation. We predicted that the advantages conferred by grass defoliation to tree seedlings would be greater on rocky substrates than those on sandy soils due to a greater overlap in rooting zones of tree seedlings and grasses, resulting in a greater influence of competition. We also tested the responses of grass biomass and nutrient status to competition with tree seedlings. We predicted that grass grown with tree seedlings would have higher forage quality (i.e. higher nutrient concentration) due to nitrogen derived from tree seedling N_2 fixation.

3.3. Methods

Plants were grown in the greenhouse in 90 bins (95 L) which were 0.45 m in diameter and 0.60 m in height in a completely randomised experimental design. Bins were filled with an alluvial sandy-gravel aggregate. This aggregate is 17% gravel (≥ 2mm) and 83% soil (≤ 2 mm) of which 1.7% was clay and 4% silt with 53% of the sand portion being coarse (0.5-1 mm), 25% medium (0.25-0.5 mm), 14% fine (125-2550 µm) and 3% very fine (62.5-125 µm) particles with a low nutrient content. This sand aggregate mix was mixed with cobbles (≥ 64 ≤ 256 mm) and boulders (≥ 256 mm) in 45 of the bins. All bins were filled with sand up to 0.05 m below the rim resulting in a sand column of *ca.* 55 cm depth in the bins.

The set-up consisted of 45 bins with sandy and 45 with rocky substrate. Each of the two groups of 45 bins was made up of 9 bins with trees only, 18 bins with grass only and 18 tree + grass combination bins. A total of 24 *Acacia mellifera* seeds were planted per bin on 10 December 2006 in the tree only and tree + grass bins and germinated within four days. Seeds of *Eragrostis curvula* grasses were introduced into bins one month after the germination of tree seedlings on 19 January 2007 and allowed to grow until the end of winter (end of July 2007). Soil nutrient content was low being 0.02 % (w/w) N and 0.01 % (w/w) P and 0.74% (w/w) organic matter. No fertilizer was added in the experiment and plots were watered on a weekly basis using an automated sprinkler system.

At the onset of the second growing season (beginning of August 2007), grasses from half the bins (i.e. grass-only as well as tree-grass treatments) were harvested by cutting the above-ground material at 2 cm above ground. Harvesting of grass above-ground material from treatment bins was repeated every two months until the end of the experiment in July 2008. This clipping of above-ground grass material to simulate grazing was performed on 18 of the bins exclusively planted with grass, and 18 of the bins with mixed tree-grass plants, nine having sand-rocky combinations and the other nine containing pure sand. Although grass clipping is not an accurate representation of herbivory by diverse animals (Irving et al. 1995; Tripathi & Shukla 2007) and may preclude mutualistic animal-plant interactions, trampling or selective herbivory (Walker et al. 1989), it does have the benefit of uniformity and repeatability. We also note that *E. curvula* is a highly palatable grass species that is preferred by domestic livestock (Van Oudtshoorn 1999).

Clipped grass from each bin was collected in paper bags, weighed, dried at 65°C for 48 h and re-weighed. Grass biomass from each sequentially harvested bin over the course of the season was added progressively to estimate biomass accumulation. At the end of the experiment in July 2008, after one and half years (two growing seasons) all tree seedlings were harvested (including roots). Final stem heights/root lengths, number and lengths of branches and the basal diameters of stems/roots were documented. Grass not harvested during the course of the experiment served as controls for simulated herbivory and was collected at the end of the experiment. All uncut grass (herbivory controls) was carefully removed (including roots) from bins. The roots of regularly clipped grass were also harvested at the end of the experiment. Excess soil was washed from the roots with water. Final harvested above-ground biomass of grass from bins not subjected to grass clipping was compared to the cumulative biomass of grass from bins subjected to regular harvesting.

Harvested plant material was separated into above- and below-ground components and oven dried at 65 °C for 48 h. While above- and below-ground components of tree seedlings were dried individually, all grasses from a particular bin were combined. In the case of regularly-harvested grass, only the below-ground parts were collected for drying. All oven-dried plant components were weighed. Tree leaves from individual seedlings were separated from twigs and bulked into a single composite sample per bin.

Plant material was milled to a fine powder with a Culatti Type MFC micro-fine pulverizing electrical grinder (Janke and Künkel GmbH, Staufen, Germany) to pass through a 1 mm pore size sieve. Ground plant samples were analysed for total N and total P. Samples were digested with sulphuric acid, hydrogen peroxide and a selenium catalyst using a block digester at 360 °C. Total N in dry plant samples was determined with a LECO FP2000 Nitrogen Auto Analyser (Leco Corporation, St. Joseph, Michigan, USA) using the micro-Dumas Combustion method (AOAC International 2000). Phosphorus was determined using continuous flow analysis with Technicon Autoanalyser II colorimeter (SEAL Analytical, Hampshire, UK) which measures the absorbance of the phosphomolybdovanate complex at a wavelength of 420 nm.

3.4. Statistical Analysis

We measured the competitive responses of trees and grasses to interspecific competition. We did not measure competitive effects *sensu* Goldberg and Fleetwood (1987) and Miller and Werner (1987), which would have required altering the densities of tree seedlings (Goldberg 1996). We did, however, measure the effects of grass competition on tree seedlings by our clipping treatment (plus control). Final harvest plant biomass, tree seedling stem/root diameters for plots subjected to grass clipping were compared to the plant biomass and tree seedling stem/root sizes from plots not subjected to clipping by means of two-way Analysis of Variance (ANOVA) as were the comparisons of similar plant-combinations on different soil types (rocky *versus* sandy). Resultant values were used to evaluate plant performances under inter-specific competition, biomass accumulation by plants on sandy/rocky substrate as well as a comparative study of tree/grass biomass accumulation under conditions of regular grass clipping and when left to grow undisturbed on either soil type. Repeated measures of grass biomass and nutrient concentration were compared in a general linear repeated measure ANOVA model using tree competition as a between subject factor and substrate type as a covariate. Means and pairwise multiple comparisons were tested with Bonferrori *post hoc* tests with adjusted confidence intervals set at α = 0.05 significance level.

3.5. Results

3.5.1. Responses of tree seedling biomass to grass competition

Competition from uncut grass had a significant negative effect on above- (F = 6.89, error d.f. = 17, P = 0.018) and below-ground (F = 11.54, error d.f. = 17, P = 0.010) biomass accumulation by tree seedlings on both rocky and sandy substrates (Fig. 3.1). We found no significant difference between tree-only and frequently-cut grass bins in either above-ground (F = 1.17, error d.f. = 17, P = 0.284) and below-ground (F = 1.14, error d.f. = 17, P = 0.290) biomass of trees, indicating that grass clipping resulted in a situation similar to that of grass absence. Tree seedling overall shoot (10.4 ± 1.3 g) and root (7.9 ± 0.9 g) biomass was reduced by half to 5.9 ± 0.8 and 4.6 ± 0.7 g, respectively, in the presence of uncut grass. Uncut grass also had a significant negative effect on shoot and root diameters as well as the mean number of branches per seedling on both substrates (Table 3.1). While the root:shoot ratios of seedlings on sandy substrate were significantly reduced by uncut grass competition relative to seedlings without grass, this was not the case for root:shoot ratio on rocky substrates (Table 3.1).

3.5.2. Responses of grass biomass to tree seedling competition

The presence of tree seedlings had no significant (F = 3.08, error d.f. =17, P = 0.100) influence on the above-ground biomass of uncut grass on rocky substrates. In contrast, on sandy substrates, uncut grass growing together with tree seedlings yielded significantly (F = 7.20, error d.f = 17, P = 0.020) higher above-ground biomass (172.2 ± 23 g), which was double the yield of grass on their own (85.6 ± 10 g). Thus, in the absence of clipping, the presence of trees on sandy substrates increased grass productivity relative to the absence of tree seedlings (Fig. 3.2). The converse was observed when grass was subjected to repeated clipping where tree seedling competition had a significant deleterious effect (F = 3.1806, error d.f.= 32, p = 0.002) on cumulative above-ground grass biomass (Fig. 3.3). Clipped grass in half of the tree + grass combination bins failed to regrow after the 4th harvest.

Significant differences were found between harvested biomass of grass at different harvest dates with a significant harvest x interspecific competition interaction (Table 3.2). Tree seedlings suppressed biomass accumulation in the repeatedly clipped grass treatment such that grasses competing with trees yielded lower biomass towards the later sampling dates than grass growing on its own on either substrate (Fig. 3.3). No significant differences were found between harvested grass biomasses for any treatments at first harvest (F = 0.63, error d.f. = 30, P = 0.603) or cumulative biomass at subsequent harvests until the last two harvests (Fig. 3.3). For the last two harvests in March 2008 (F = 13.94, error d.f. = 32, $P \leq 0.001$) and May 2008 (F = 3.62, error d.f. = 32, P = 0.023) grass grown without tree seedlings had greater biomass than grass competing with trees, but the cumulative biomass of the grasses was not significantly different (F = 2.17, error d.f. = 32, P = 0.111) (Fig. 3.3). Significant differences (F = 26.29, error d.f. =17, $P \leq 0.001$) were found in below-ground biomass between regularly harvested grass growing on its own (which had greater root biomass) and grass growing with tree seedlings, while no significant differences (F = 0.17, error d.f. = 17, P = 0.686) were evident for uncut grass (Fig. 3.2).

3.5.3. Substrate influence on tree and grass growth

There was no significant influence of substrate type (rocky or sandy) on individual tree seedling mean stem (P = 0.988) or root (P = 0.426) diameter, number of branches (P = 0.981), above- (P = 0.803) or below-ground (P = 0.895) biomass. Substrate type had no significant effect on uncut grass biomass grown without trees either above- (F = 0. 085, error d.f. = 17, P = 0.775) or below-ground (F = 0.014, error d.f. = 17, P = 0.591). Similarly, there was no significant effect on N (F = 0.255, error d.f. = 17, P = 0.621) or P (F = 0.236, error d.f. = 17, P = 0.634) concentrations. The most notable substrate-related difference was the effect of grass competition on tree seedling biomass in which grasses significantly suppressed tree shoot biomass (F = 6.89, error d.f. = 17, P = 0.018) on rocky substrate and suppressed root biomass (F = 11.54, error d.f. = 17, P = 0.003) on sandy substrate (Fig. 3.1). While grass competition significantly suppressed tree seedlings in all size aspects on sandy substrates, it had no significant effect on stem or branch length and root:shoot ratios on rocky substrate (Table 3.1). Substrate type had no significant influence on the biomass and nutrient content of regularly clipped grass (Table 3.2).

3.5.4. Nutrient concentrations

Competition with grass had no significant effect on tree seedling nutrient concentration (Tables 3.3 and 3.4). Tree seedlings on rocky substrate without grass competition did not have significantly different N (F = 1.17, error d.f. = 17, P ≤ 0.293) and P (F = 0.77, error d.f. = 17, P ≤ 0.393) concentrations compared to tree seedlings without grass competition on sandy substrate. Substrate had no significant effect on tree seedling nutrient concentration in all other treatment combinations.

The presence of tree seedlings had significant (range in F = 8.37 - 45.75, range in error d.f. = 24 - 32, $P \leq 0.001$) positive effects on the N concentration of regularly-harvested grass at all harvest dates. However, no significant differences were found between uncut grasses grown with and without tree competition (Table 3.3). Repeated measures ANOVA showed significant differences in N concentration at different harvest dates with a significant harvest date x tree competition interaction (Table 3.2). Regularly-clipped grass growing with tree seedlings showed a consistent increase in N concentration in grass-only treatments while grass grown with trees had declining N at the 3rd and 5th harvests (data not shown).

As with N, the P concentration of frequently-clipped grass was significantly (range in F = 3.46 - 34.91, range in error d.f. = 20 - 32, P = 0.029 - ≤ 0.001) enhanced by the presence of tree seedlings at all except the first harvest (F = 0.44, error d.f. = 29, P = 0.725). There were significant differences in P concentration of grasses competing with tree seedlings (higher P than grass grown on its own) with a significant harvest date x tree competition interaction (Tables 3.2 and 3.4). When considered separately for either soil type, the presence of trees had a significant influence on P concentration of grass on sandy substrates only (Table 3.4). Relatively higher initial mean N:P ratios of 7.1 and 6.9 at the 1st and 2nd harvests respectively, dropped significantly to 3.8 - 4.5 at the 3rd and subsequent harvests (F = 87.10, error d.f. = 65, $P \leq 0.001$) (data not shown).

3.5.5. Responses to simulated herbivory

Comparisons of tree seedlings competing with cut grass on sandy substrate to tree seedlings free of grass competition demonstrated that sustained clipping significantly influenced tree seedling sizes in terms of stem (F = 9.01, error d.f. = 17, P = 0.003) and root diameters (F = 5.93, error d.f. = 17, P = 0.016), mean branch number (F = 5.81,error d.f. = 17, P = 0.017) as well as above-ground biomass (F = 5.36, error d.f. = 17, P = 0.021). The mean number of branches per tree seedling was also significantly different (F = 3.95, error d.f. = 17, P = 0.048) between those competing with harvested grass and those without grass competition. In all but the case of mean branch number, tree seedlings competing with uncut grass on either substrate had significantly smaller sizes than tree seedlings with clipped grass and as indicated earlier, frequent cutting of grass reduced the negative effect of grass competition on tree seedling biomass on rocky substrate (Fig. 3.1). While mean branch length of tree seedlings competing with uncut grass was significantly longer (F = 183.31, error d.f. = 17, $P \leq 0.001$) than tree seedlings growing with clipped grass on rocky substrates, there was no significant difference (F = 0.84, error d.f. = 17, P = 0.362) on sandy substrates.

In comparison to seedlings grown in rocky substrate without grass competition, the seedlings growing with cut grass had a significantly smaller mean number of branches (F = 3.953, error d.f. = 17, P = 0.048), that were significantly shorter (F = 183.313, error d.f. = 17, $P \leq 0.001$). No significant differences were found for other parameters on rocky substrate. On the sandy substrate, similar significant differences were found between tree-only seedlings and those grown with clipped grass as occurred with the comparison of tree seedling competing with clipped grass to tree seedlings competing with uncut grass (see preceding paragraph). Competition from grass subjected to clipping disadvantaged seedlings in all aspects relative to growing without grass on sandy soils. Thus, the frequent clipping of grass did not benefit tree seedlings on the sandy substrate where grass had similar effects on tree seedlings regardless of clipping, but significantly benefited tree seedlings on rocky substrates. Grass clipping had no significant effect on tree seedling N (F = 0.002, error d.f. = 17, P = 0.968; F = 0.507, error d.f. = 17, P = 0.489) and P (F = 0.006, error d.f. = 17, P = 0.939; F = 0.002, error d.f. = 17, P = 0.968) concentration on rocky and sandy soil types, respectively.

Uncut grass growing with trees accumulated significantly more biomass both above- and below-ground than grasses subjected to clipping, with the greatest contrast being found for root biomass (Fig. 3.1). However, in terms of nutrient concentration, harvested grass growing with tree seedlings on both soil types had higher N and P concentrations than uncut grass (Tables 3.3 and 3.4).

3.6. Discussion

Grass competition exerted a significant inhibitory effect on the growth of tree seedlings in all size-related aspects (both above- and below-ground) on both soil types. This result is consistent with the widely-reported capacity of grass competition to suppress woody plants at seedling and sapling life-history stages (Weltzin & McPherson 1997; Jurena & Archer 2003; Cramer et al. 2007; Riginos 2009). Reduction of tree seedling biomass by half due to grass competition could translate to reduced productivity, slow growth and delayed reproductive success of woody plants, creating a bottleneck for succession of trees in savannas (Higgins et al. 2000; Bond 2008).

Competition from uncut grass led to significantly greater tree seedling rooting mass on rocky substrates relative to tree seedlings on sandy substrates. Low water-holding capacity in rocky bins combined with grass competition would have necessitated enhanced below-ground root expansion by tree seedlings to increase resource capture. The physical obstruction presented by the rocks forced roots to grow around the rock barriers, resulting in a larger root mass. Nobel et al. (1992) demonstrated that arid species growing among rocks and boulders had a greater number of lateral roots per length of main root, as well as greater total length of main roots, primary lateral roots and secondary lateral roots. The suppressive effect of grass competition on tree seedlings in rocky bins was eliminated when the competing grass was cut frequently, such that tree seedlings growing with harvested grass were not significantly different in biomass to tree seedlings growing on their own, but had greater biomass than those competing with uncut grass. It is probable that the architecture and overall morphology of grass roots render them prone to restriction by physical obstructions by rocks and other barriers, which in turn reduces grass competitiveness (see also Mbatha & Ward 2010 for differences in cover of grasses growing on rocky and sandy substrates at the Pniel study site from where the *A. mellifera* trees were taken). When this was combined with simulated herbivory of aerial shoots, there was a two-fold suppression of grass vitality and its competitive effect (Fig. 3.2). Sparse grass cover on rocky substrates would mean low fuel load and thus less frequent fires of lower intensity, so that the combined effect of rocky substrate and release from competition by defoliation on trees would be accentuated with fire (Meyer et al. 2005; Bond 2008). However, on sandy substrates grass suppressed tree seedling sizes despite clipping, possibly because root growth was less impeded in sand and the grass could compete more vigorously.

Competition by herbaceous plants such as grasses for soil moisture, nutrients and light is thought to present a barrier to the establishment of woody seedlings (Scholes & Archer 1997; House et al. 2003) and weakening of grass competition by grazing may promote woody seedling growth leading to bush encroachment (van Vegten 1983; van Langevelde et al. 2003; Ward 2005). In our experiment we allowed the tree seedlings to establish for a month before introducing grass competition, but grass nonetheless outgrew the tree seedlings after two months. While the tree seedlings emerged above the grass sward later, shading by uncut grass most likely led to light competition. Our results suggest that bush encroachment is likely to be enhanced by grass herbivory, especially on rocky substrates. A similar result was obtained by Ward & Esler (2010) in a field experiment at the Pniel study site.

Our results demonstrated the suppressive effect of uncut grasses on tree shoot biomass on rocky substrates while its influence on sandy substrates was most significant on the root biomass of tree seedlings. This has major implications for the persistence of the deciduous encroaching *Acacia mellifera* in savannas with rocky soils. Rocks appear to either partially protect tree seedlings from below-ground competition for space and resources or to require greater root investment. The storage of carbon in plants is predominantly below-ground rather than above-ground (Schlesinger 1991; Schutz et al. 2009). Storage of assimilated resources in roots serves to increase plant survival and growth following adverse changes in environmental conditions (Iwasa & Kubo 1997; Schutz et al. 2009). Thus, relatively larger root biomass production by *A. mellifera* in rocky areas may enable the trees to resprout, resume growth and reproduce after fire (Meyer et al. 2005), herbivory or drought dormancy and proliferate into dense thickets. Additionally, larger and deeper rooting in the presence of rocks may allow access to more soil moisture and nutrients.

The presence of tree seedlings significantly enhanced grass biomass accumulation above- and below-ground in the absence of clipping on sandy substrates, but tree seedlings had little effect on the grass on rocky substrates. Contrary to our hypothesis, tree seedling competition did not suppress grass biomass production, but rather seemed to facilitate grass biomass accumulation on sandy substrates when the grass was not subjected to frequent clipping. Low nutrient content in a sandy arid environment may necessitate greater below-ground investment by both grasses and trees. Grasses may be expected to establish greater rooting mass on sand than on rocky areas due to the physical restriction presented by rocky barriers, and thus exert stronger competition for resources. While the roots of tree seedlings may be able to grow around and past such rocky barriers, the roots of grasses are restricted by rocks. Our results showed uncut grasses to have invested more in root production in competition with trees relative to when they grew without interspecific competition. This may in turn induce competing tree seedlings to fix atmospheric N_2 (Cramer et al. 2007) from which the grasses then benefit.

When subjected to repeated clipping, grass growing with tree seedlings had smaller above-ground and root biomass than grass growing free of tree competition. This result is consistent with findings that demonstrated a reduction in total grass biomass production as a result of intense defoliation (Pandey & Singh 1992). Tree seedlings thus had a positive influence on grass biomass output, but this benefit was reversed by the clipping perturbation (see also Ward & Esler 2010). The above-ground biomass showed tree seedlings to significantly reduce above-ground biomass of grazed grass (Fig. 3.3). Grass clipping also suppressed below-ground biomass of grass, particularly when the grass was grown with tree competition on rocky soil types. The uniform clipping achieved in the greenhouse may not be replicated by herbivores in nature because some animals remove all of the above-ground material while others may take the more palatable sections only (Wolfson 1999). Tree-grass dynamics as well as plant-herbivore feedbacks in natural systems may create gradients in plant density and intensity of herbivory (Van Auken 2000).

Frequent defoliation of grass meant that most resources were allocated to shoot reproduction and less to root growth, giving rise to shallower rooting depth of clipped grasses. Frequent clipping of grass may have depleted stored root resources and curtailed their photosynthetic replenishment thus retarding grass vigour (Chapin & Slack 1979; McNaughton & Chapin 1985). Tolsma et al. (2010) showed that while plants show resilience to occasional clipping, more frequent defoliation led to significant depletion of carbohydrate reserves accompanied by high mortality of tillers. The grass may have had to rely on translocation from an otherwise limited root biomass or photosynthate from shoot remnants to replace harvested parts above-ground and sustain growth. Studies from temperate regions have shown that N allocated to shoot growth by forage grasses/legumes during the first days following severe defoliation is primarily relocated from roots rather than taken from the soil (Ourry et al. 1989; Culvenor & Simpson 1991; Louahlia et al. 1999).

Unlike the responses in biomass, the influence of tree seedlings on grass nutrient concentration was only significant when the grass was frequently cropped. Frequent clipping initiated the replacement of old tissues with fresh growth high in nutrient content whereas the bulk of uncut grass biomass had senesced at harvest at the end of the experiment. Relatively higher nutrient concentrations in grazed compared to ungrazed grass (McNaughton 1984, 1985; Frost & Robertson 1987; Mbatha & Ward 2010) may result from enhanced nutrient uptake by defoliated grass (Ruess 1984) and compensatory amplification of photosynthesis (Caldwell et al. 1981; Senock et al. 1991). Although care was taken to mill and analyse green foliage, the interference of a large amount of senescent leaf material in the uncut grass sample could not be entirely avoided. The possibility of nutrient dilution (Warren Wilson 1966; Shaver & Chapin 1980) as a contributing factor to the observed trends is unlikely as the nutrient concentration of grasses continued to increase when grass biomass output increased for the first harvests. The likely cause of elevated nutrient concentration in grass when grown with tree seedlings is that the grasses benefitted from fixed N_2 by the *Acacia* tree seedlings. Low relocation of N from leaves to shoots in *Acacia* tree species (Tolsma et al. 1987), coupled with comparatively higher N content in legumes relative to non-nodulated woody plants (Durr & Rangel 2000) resulted in relatively high N concentrations in the *Acacia* litter fall from which grasses profited (Tolsma et al. 1987). N in fallen tree leaves may have benefitted the grasses in our study, but high N concentration of grasses grown with trees than in grass-only bins was found from the 1st harvest at which stage no tree leaves had been abscised. Transfer of fixed N_2 from the woody seedlings to the grasses probably via nodule and root turnover as well as root exudates (Sierra & Nygren 2006; Sierra et al. 2007) was therefore the most likely contributor to elevated grass N when grown with tree seedlings.

Sustained heavy defoliation of grasses delays and retards root activities for days (Wolfson 1999) and restricts root production to the shallow soil depths (Schuster 1964). Repeated removal of above-ground biomass through herbivory might promote rooting niche separation by constraining below-ground biomass production and root depth penetration by grasses, but the resultant scant remnants of root mass (Hild et al. 2001) may not be sufficient to fully intercept and extract significant moisture. More water from precipitation is likely to infiltrate deeper and benefit the relatively deep-rooted trees when competing grass is continually removed by grazers. This is in agreement with the assertion that heavy grazing pressure that continually removes grass cover allows deeper percolation of soil moisture to subsoil layers exploited by woody plant species (Knoop & Walker 1985; McNaughton et al. 1988; Skarpe 1990) thereby encouraging shrub encroachment (Jeltsch et al. 1997; Graz 2008). When not subjected to regular harvesting, grasses were able to exploit a deeper and larger volume of soil for nutrients and moisture utilizing a greater rooting mass, thus directly competing with the tree seedlings. Access to a larger volume of resource pool may have negated or balanced the reliance on fixed N_2 from tree seedlings, removing differences in nutrient concentration of uncut grass growing with and without tree seedling competition.

3.7. Conclusions

We found that competition with grasses suppressed tree seedling growth and biomass accumulation. This is consistent with our prediction that encroachment by woody plants may result from heavy grazing pressure on the grass component of savanna ecosystems. The outcome of clipping and interspecific competition between trees and grasses combined to severely curtail grass performance. We found that this response was particularly acute on rocky substrates, which matches the empirical findings of Britz and Ward (2007) and Ward and Esler (2010). We suggest that encroachment resulting from heavy grazing occurs most frequently on rocky substrates because the trees and grasses are forced to share a depth niche for water and/or nutrient resources due to direct root competition.

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Table 3.1 The influence of uncut (intact) grass competition on final shoot, root and branch lengths, diameters (mean ± S.E cm) and root:shoot ratios of tree seedlings grown on their own (Trees Only) and in competition with grass on rocky and sandy substrates. Error d.f in all cases was 17. Note that grass competition had a more severe effect on tree seedling diameter and branch length on sandy soil than it did on rocky soils.

Plant Measured Parameter	**Substrate Type**	**Measurement**		**F**	**Significance (P)**
		Tree-Grass	**Trees Only**		
Stem Length	Rocky	50 ± 2	53.2 ± 2.0	1.41	0.236
	Sandy	48 ± 2	56 ± 2	8.093	0.005
Stem Diameter	Rocky	0.51 ± 0.20	0.59 ± 0.10	14.97	<0.001
	Sandy	0.47 ± 0.10	0.59 ± 0.2	31.06	<0.001
Root Diameter	Rocky	0.44 ± 0.02	0.51 ± 0.02	9.40	0.002
	Sandy	0.42 ± 0.01	0.53 ± 0.02	22.45	<0.001
Number of Branches	Rocky	0.92 ± 0.10	2.05 ± 0.10	34.70	<0.001
	Sandy	1.02 ± 0.10	2.06 ± 0.10	45.39	<0.001
Mean Branch Length	Rocky	32 ± 2	32 ± 1	0.004	0.953
	Sandy	29 ± 2	34 ± 1	5.26	0.023
Root:Shoot Ratio	Rocky	0.90 ± 0.03	0.89 ± 0.03	0.07	0.796
	Sandy	0.91 ± 0.03	0.84 ± 0.02	5.23	0.023

Table 3.2 Results of Repeated Measures ANOVA General Linear Model (GLM) for regularly clipped grass. Differences in error d.f. result from failure of harvested grass to regrow at subsequent harvest as well as some grass samples being too small for nutrient analyses on certain harvest dates.

Source of Variation	Wilks' λ	F	Error d.f.	Significance (P)
Biomass at Harvest				
Harvest Date	0.084	58.58	27	<0.001
Harvest Date x Tree Competition	0.178	24.96	27	<0.001
Harvest Date x Substrate Type	0.913	0.52	27	0.762
Cumulative Biomass				
Harvest Date	0.064	79.43	27	< 0.001
Harvest Date x Tree Competition	0.205	20.94	27	<0.001
Harvest Date x Substrate Type	0.936	0.37	27	0.864
Nitrogen Concentration				
Harvest Date	0.032	125.06	21	<0.001
Harvest Date x Competition	0.129	28.48	21	<0.001
Harvest Date x Substrate Type	0.926	0.34	21	0.884
Phosphorus Concentration				
Harvest Date	0.024	129.00	16	<0.001
Harvest Date x Tree Competition	0.221	11.26	16	<0.001
Harvest Date x Substrate Type	0.842	0.59	16	0.701

Table 3.3 Final shoot (grass) or leaf (trees) nitrogen (N) concentration (mean ± S.E mg/g dwt) in grass and tree seedlings grown on their own (=Grass/Trees Only) and in combination on rocky and sandy substrates.

Plant & Herbivory Treatment	Substrate Type	Plant Combination N Tree-Grass	Grass /Trees Only	F	Error d.f.	Significance (P)
Uncut Grass	Rocky	1.01 ± 0.09	0.97 ± 0.03	0.276	17	0.608
	Sandy	0.95 ± 0.05	0.94 ± 0.05	0.016	17	0.900
Cut Grass	Rocky	2.10 ± 0.05	1.74 ± 0.07	13.475	10	0.004
	Sandy	2.21 ± 0.07	1.73 ± 0.06	21.594	14	≤ 0.001
Trees (Uncut Grass)	Rocky	3.71 ± 0.10	3.89 ± 0.11	1.143	17	0.301
	Sandy	3.53 ± 0.32	4.04 ± 0.07	2.712	17	0.118
Trees (Cut Grass)	Rocky	3.88 ± 0.07	3.89 ± 0.11	0.002	17	0.968
	Sandy	3.92 ± 0.08	3.99 ± 0.06	0.507	17	0.489

Table 3.4 Final shoot/leaf phosphorus (P) concentration (mean ± S.E mg/g dwt) in grass and tree seedlings grown on their own (= Grass/Trees Only) and in combination on rocky and sandy substrates.

Plant & Herbivory Treatment	**Substrate Type**	**Plant Combination P**		**F**	**Error d.f.**	**Significance (P)**
		Tree-Grass	**Grass /Trees Only**			
Uncut Grass	Rocky	0.081 ± 0.008	0.069 ± 0.006	1.168	17	0.299
	Sandy	0.092 ± 0.007	0.074 ± 0.009	2.524	17	0.138
Cut Grass	Rocky	0.385 ± 0.041	0.312 ± 0.028	2.173	8	0.179
	Sandy	0.469 ± 0.045	0.313 ± 0.023	17.341	12	0.001
Trees (Uncut Grass)	Rocky	0.175 ± 0.036	0.194 ± 0.031	0. 161	17	0.694
	Sandy	0.157 ± 0.018	0.263 ± 0.076	1.680	17	0.212
Trees (Cut Grass)	Rocky	0.191 ± 0.027	0.194 ± 0.031	0.006	17	0.939
	Sandy	0.208 ± 0.024	0.273 ± 0.084	0.375	17	0.551

Figures

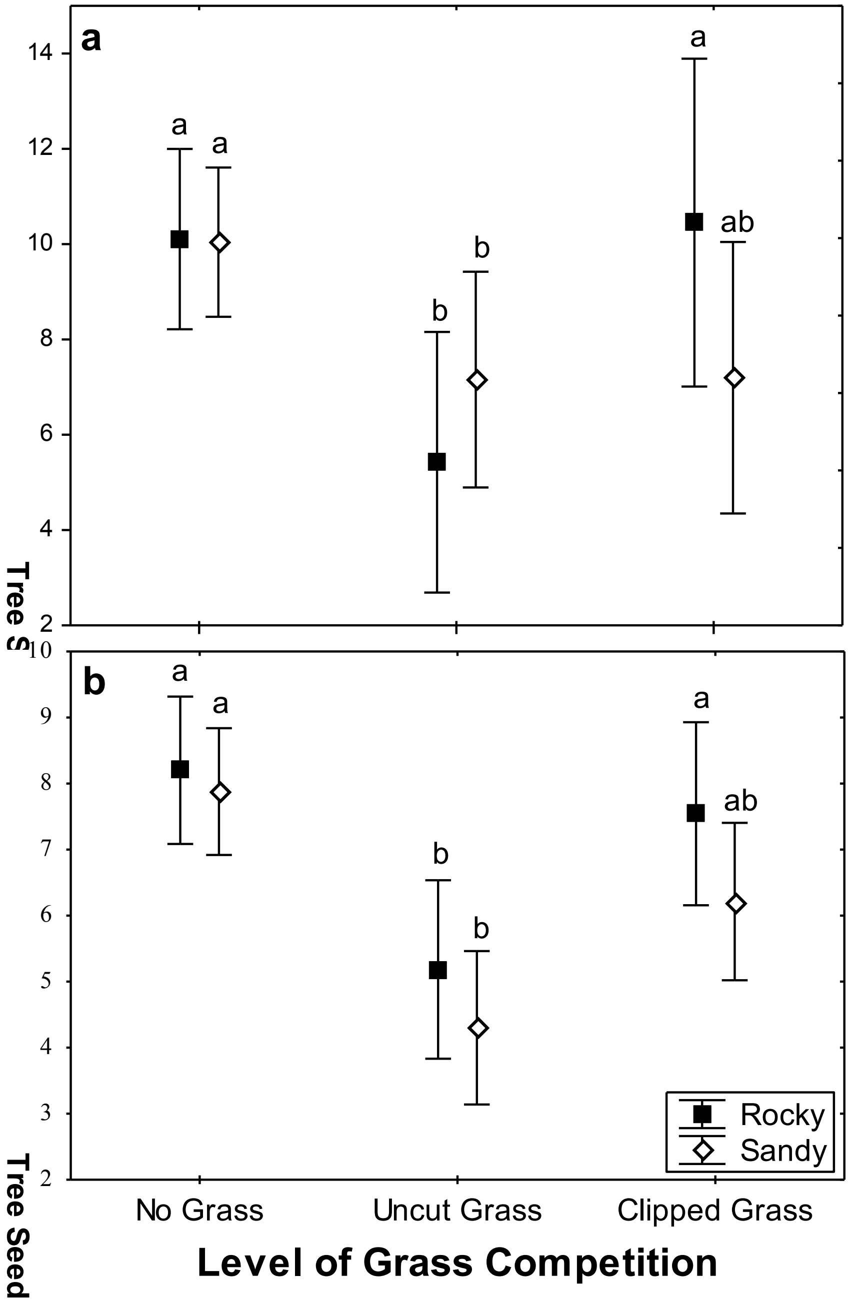

Figure 3.1 Comparisons of (a) above- and (b) below-ground biomass of tree seedlings when grown with and without either uncut or cut grass and on either rocky or sandy substrates. Note that the suppressive effect of grass competition in the absence of clipping significantly suppressed shoot biomass on rocky substrates and root biomass on sandy substrates. Vertical error bars denote 95% confidence intervals.

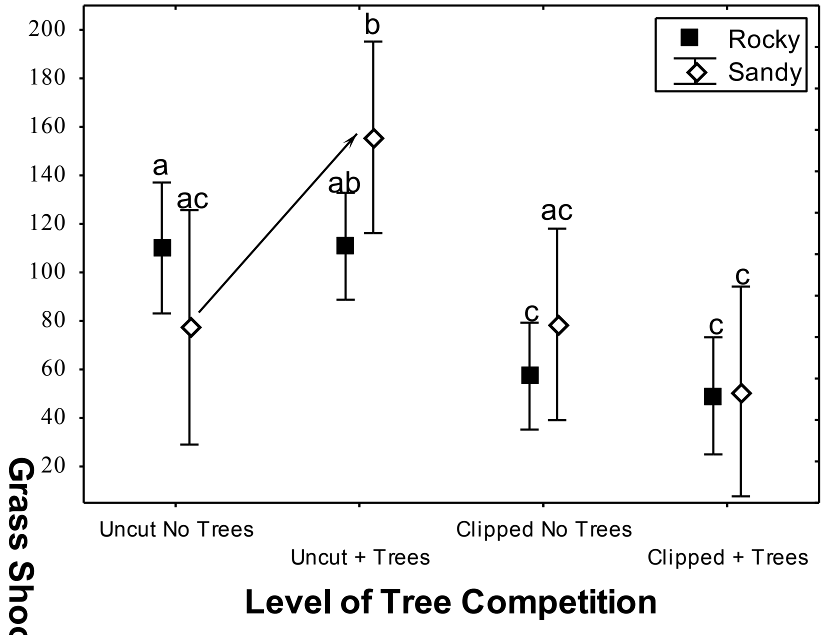

Figure 3.2 Comparisons of cut and uncut grasses with and without tree seedling competition in terms of above-ground biomass on rocky and sandy substrates. Vertical error bars denote 95% confidence intervals. The arrow indicates the significant increase in uncut grass biomass on sandy substrates in the presence of tree seedlings relative to when grass was grown on its own.

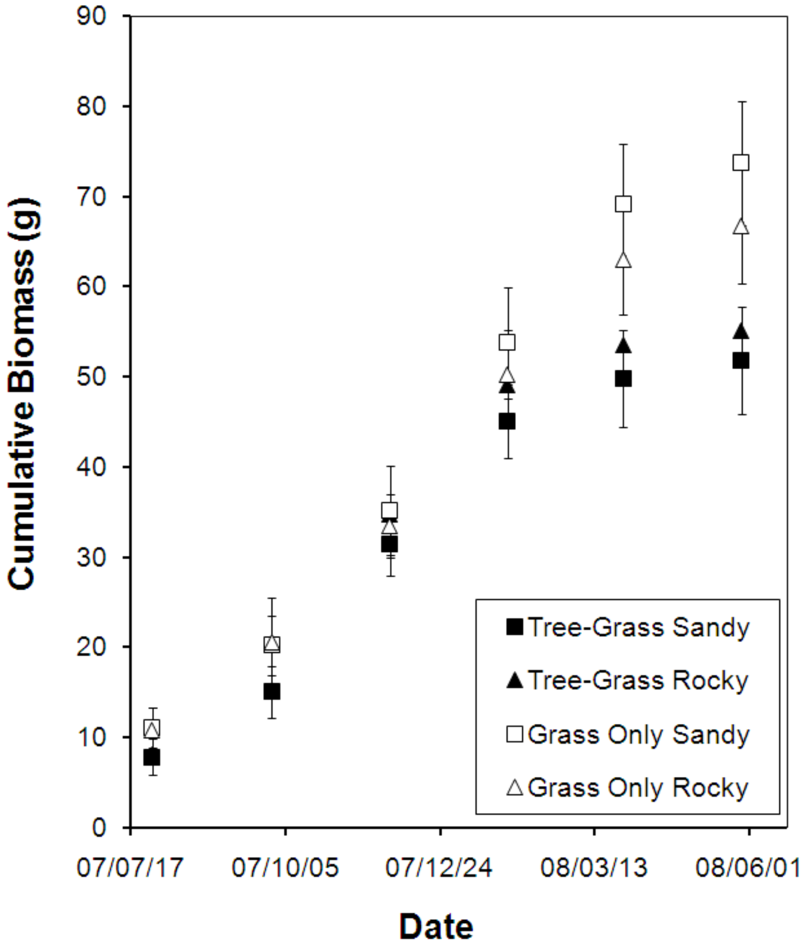

Figure 3.3 Plots of above-ground accumulative biomass (mean ± S.E) of frequently harvested grass on sandy and rocky substrate at different harvest dates as recorded at bimonthly intervals starting with July 2007 (1st harvest) and ending in May 2008.

4. Chapter 4: Overlap in soil water sources of savanna woody seedlings and grasses

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4.1. Abstract

Seasonal availability of water is a key controlling factor in semi-arid savanna vegetation structure, function and interactions. Understanding of woody plant interactions with grasses in savannas has long been underpinned by Walter's two-layered niche differentiation hypothesis that postulates that grasses and trees source water and nutrients from different depths. The Walter hypothesis persists in the literature, despite contrary evidence and a lack of quantitative empirical tests of the theory. We conducted a greenhouse experiment to determine: 1) whether tree seedlings and grasses obtain water from different depths on rocky and sandy soils; 2) whether interspecific competition affected tissue water content of clipped grasses; 3) the influence of repeated grass clipping on soil moisture. Tree seedlings on rocky substrates responded to competition with grass by growing deeper roots compared to when tree seedlings grew without grass competition (21 ± 2 cm), regardless of whether the grass was clipped (80 ± 3 cm) or not (72 ± 3 cm). Grass competition significantly reduced tree seedling rooting depth on sandy substrate (65 ± 2 cm) but not on rocky substrate. Results indicated a three-tier soil moisture depletion pattern, with a top layer (0.15 m) exclusively exploited by grasses, an intermediate zone (0.25-0.35 cm) utilised by both grass and tree seedling roots and deeper subsoil exclusively tapped by tree seedling roots. Our results are consistent with Walter's hypothesis, but we distinguished between three rather than two layers of tree-grass root interactions in acquiring soil moisture.

Keywords: *Acacia mellifera*, inter-specific competition, niche separation, rocky, soil layers, soil moisture, woody plant encroachment, substrate type

4.2. Introduction

Water, disturbance and edaphic factors determine the distribution (Stephenson 1990, Sankaran *et al*. 2008) and productivity (Webb *et al*. 1983, Sala *et al*. 1988) of savannas. Semi-arid savannas characteristically have low total precipitation combined with high variability (Ward 2009). The timing and magnitude of precipitation pulses control soil moisture availability for plants and are thus principal drivers of key ecological processes in semi-arid ecosystems (Noy-Meir 1973, Weltzin *et al*. 2003, Chesson *et al*. 2004). The influence and significance of moisture availability is a function of the amount and spatial distribution of precipitation combined with the influence of underlying soil structure and texture (Vincent & Thomas 1961, Walter 1971, Tinley 1982). Soil structure influences moisture availability to plants as a consequence of differences in pore size, rate of water diffusion and redistribution through the soil profile and evaporation (Hanks & Ashcroft 1980, Campbell 1985, Jury *et al*. 1991). The influence of substrate characteristics on rainwater infiltration, retention, storage and deep percolation (Sperry *et al.* 1998, Hacke *et al.* 2000, Sperry & Hacke 2002) also makes edaphic factors important to plant growth and competition (Noy-Meir 1973).

Many investigations of the role of limited water availability in shaping savanna vegetation have addressed the possibility of competition for soil moisture or its avoidance between grasses and woody plants (e.g. Walter 1971, Walker *et al.* 1981, Walker & Noy-Meir 1982, Knoop & Walker 1985). The precursor to this line of enquiry was Walter's (1939) two-layer hypothesis that there is niche separation between trees and grasses in accessing soil moisture. According to this hypothesis, grasses monopolise rainwater in the upper soil layer while trees have access to deeper soil moisture. Woody species in semi-arid environments generally extend their rooting structures deeper than herbaceous vegetation (Sala *et* *al*. 1989, Scholes & Archer 1997, Weltzin & McPherson 1997), resulting in distinct partitioning of soil moisture sources. In savannas, this promotes tree-grass coexistence.

Intense selective defoliation of grass by herbivores may alter the availability of soil moisture to woody plants by eliminating surface water interception and uptake by grass roots and allowing deeper percolation (Walter 1971, Graz 2008) that increases the availability of soil moisture to trees (McNaughton *et al.* 1988). Removal of grass cover further diminishes the competitive effect of grasses to allow successful recruitment by woody seedlings (Walker & Noy-Meir 1982, Stuart-Hill & Tainton 1989, Jeltsch *et al*. 1997), which often leads to shrub encroachment (Van Auken 2003). Introduction of predominantly selective grazers in the form of cattle has altered tree-grass ratios, often resulting in impenetrable thickets within a few decades (Van Vegten 1983). Bush encroachment has mainly been viewed to have been caused by cattle ranching (Jeltsch *et al.* 1997, Roques *et al.* 2001) which altered the timing and intensity of grass defoliation as well as recovery periods for the grass (Graz 2008). However Wigley *et al*. (2009, 2010) showed higher increases in woody tree cover in a conservation area with predominantly browser game populations than on adjacent cattle ranches. Disruption of natural fire regimes by anthropogenic land management changes (Knapp & Seastedt 1986, Archer *et al*. 2001, Meyer *et al*. 2005) and physical disturbances of the soil (Ward 2009) may also be important. While local-level drivers such as interspecific competition, fire and herbivory as well as land use practice (Ward 2009, Wigley *et al*. 2009) are important factors, shrub encroachment has been shown to be ubiquitous across sites with drastically distinct land tenure systems (Wigley *et al*. 2010). This raises the possibility that global drivers such as elevated CO_2 levels and atmospheric deposition of nitrogen (Wigley *et al*. 2010, Ward 2010) may escalate woody plant encroachment.

Deep storage of soil moisture favouring deep-rooted woody plants (Walter 1971, Walker & Noy-Meir 1982) is not possible in areas with shallow soil profiles underlain by rocks. Nonetheless, proliferation of woody shrub encroachment tends to be more frequent in rocky areas and/or very shallow subsoils relative to deep sandy terrains (Ward 2005, Wiegand *et al.* 2005, Kraaij & Ward 2006). In many cases, tree roots are able to bypass or penetrate between rocks and cracks to exploit deeper sources of soil moisture. Walter's (1939) two-layer hypothesis has often been dismissed as a crude oversimplification that does not account for the complexity of savanna plant interactions (Knoop & Walker 1985, Teague & Smit 1992, Jeltsch *et al*. 1996, Ward 2005). Furthermore, evidence for soil moisture partitioning between trees and grasses is inconsistent (McCarron & Knapp 2001, Stuart-Hill & Tainton 1989, Ehleringer & Dawson 1992). Depth separation of soil water acquisition appears more common in very arid (Wan et al. 1994, Dodd *et al*. 1998, Golluscio *et al*. 1998) to subtropical savanna environments (Brown & Archer 1990, Weltzin & McPherson 1997) and less so in humid regions (Belsky 1994, Le Roux *et al*. 1995).

Generally, soil moisture content is lower beneath grasses than below woody plant communities (Belsky 1994, Li & Wilson 1998). Grasses are able to deplete soil moisture by up to five-fold more than woody vegetation per unit plant biomass (Köchy & Wilson 2000) due to relatively higher root densities (Caldwell *et al*. 1987) and greater length of fine roots per volume of soil within the upper (≤ 0.30 m) soil layers (Jackson *et al.* 1997). However, trees are able to switch from a dependence on deep reserves of water during periods of depleted soil moisture to near-surface sources of moisture following precipitation (White 1985, Sher *et al*. 2010). This utilization of moisture from the upper soil layers by woody plants in arid regions is highly seasonal (Gebauer & Ehleringer 2000). Using oxygen isotopes, Nippert and Knapp (2007) noted collective reliance by both C_4 grasses and C_3 shrubs/forbs on rainwater within the upper soil layers during the wet season with separation of soil moisture uptake depths during dry periods (see also Sher *et al*. 2010 for similar results with C_3 trees). As the upper soils dry towards the end of the wet season, plants may gradually transfer their soil water uptake functions downward to deeper soil layers (Taylor & Klepper 1975, Rambal 1984, Sala *et al*. 1989).

Depletion of moisture by competing plant species would mean that vegetation either has to tolerate low levels of moisture availability or exploit other untapped deeper soil layers (Grime 1994). Thus, tree seedlings growing with grasses could be expected to grow deeper roots away from the grass rooting layers. Direct measurements and empirical evidence for soil moisture withdrawal by savanna grasses and trees at different depths are rare. If grass/tree water uptake differs and the rooting depths are separated, distinct zones of moisture depletion ought to be detectable beneath mixed tree-grass stands. We tested these propositions by growing trees/grasses alone and in combination in 0.6 m deep bins and monitoring changes in the soil moisture profile over time in each treatment. We further tested for the influence of substrate texture and repeated grass clipping on plant soil moisture uptake patterns.

4.3. Methods

4.3.1. Treatment factor combinations

A total of 100 X 95L containers (bins) about 0.45 m top diameter X 0.60 m tall were arranged in a randomized complete block experimental design in the greenhouse. The containers had drainage holes in their bottoms and were filled with an alluvial sandy-gravel aggregate from the Umgeni River near Pietermaritzburg in the KwaZulu-Natal Province, South Africa. Rocks (>64 mm) and boulders (>256 mm) were placed in half (50) of the containers and mixed with sand aggregate in alternating layers. Bins were filled with a column of sand/rock ± 0.5 m deep. Of the total 100 bins used in the experiment 50 were rocky and 50 sandy. Ninety bins, (45 with rocks and 45 with no rocks), were planted with seeds of

Acacia mellifera trees and *Eragrostis curvula* grass (Ermelo variety, McDonald Seeds, Pietermaritzburg, South Africa). Tree seeds were collected from wild plants at Pniel Estates near Barkly West (28^o 34′ S, 24^o 25′ E) in the Northern Cape Province of South Africa in November 2006. Of the 50 bins from each substrate type, 18 were planted solely with grass, 18 with a combination of trees + grass and 9 exclusively planted with trees. The remaining 5 bins served as 'complete' controls devoid of plants to examine unimpeded vertical distribution and loss of water. In addition to substrate type (rocky or sandy) and plant type combination (trees only, grass only, grass + trees or neither) a third treatment factor of simulated selective grazing (grass clipping) was incorporated in the experimental design. A minimum of 16 combinations was thus necessary for a completely crossed experimental design. However, grass clipping was only feasible for bins containing grass. This effectively reduced the number of workable combinations to 12, each replicated 9 times, except for the controls. Containers were arranged in 6 subsets of replicates each containing all of 10 possible plant-type treatments excluding the controls.

4.3.2. Plant cultivation and harvesting

Acacia mellifera seeds were planted in the bins on 12 December 2006 (summer). A total of 24 tree seeds were planted per bin and thinned to 16 trees per bin after 30 d. Grass seeds of *E. curvula* were sown 10 d after seedling thinning on 28 January 2007. Bins were weeded on a regular basis and plants were grown through the winter. At the onset of a second growing season (August 2007), grass in half of the grassy bins was clipped to 0.02 m above soil surface. At each harvest, the clipped grass was weighed for fresh weight, oven dried at 65°C for 48 h and re-weighed. "Water content" (WC) in clipped grass tissues was calculated by subtracting the dry weight from the fresh weight, dividing by fresh weight and multiplying by 100. This was calculated for every second month until the end of the experiment (June

2008) when tree seedlings and grasses were harvested. The soil in bins was carefully removed and plant roots separated from the sand by washing with water. Vertical depths of tree and grass roots were estimated from the overall mean root length per bin.

Each container had a sprinkler delivering water at ± 0.3 L/min for 45 min once a week during the growing season and fortnightly during winter. Five bins per replicate subset were fitted with 0.6 m long 50 mm polycarbonate access tubes with closed bottoms permanently embedded in the soil column. The bins fitted with access tubes were randomly selected to represent half the vegetated treatment combinations per subset. Access tubes were installed in all 10 'complete' controls. A Diviner 2000 (Sentek Pty Ltd, Stepney, Australia) Frequency Domain Reflectometry (FDR) soil moisture probe was used to obtain readings of moisture content with depth in bins at 0.10 m intervals via the access tubes. Factory settings and calibration were used to derive percentage water (content θ%) per depth. FDR readings were collected just before noon (11h00) within a few hours after irrigation and at intervals of 4 d following irrigation once every month between July 2007 and June 2008. At the end of experiment, a 60 cm^3 soil sample was cored from sandy controls at 0.10 m intervals using a hand auger immediately after FDR readings. Soil water content (θm) was determined gravimetrically (Hanks & Ashcroft 1980, Gardner 1986) and multiplied by soil bulk density (1.37 ± 0.04 g/cm^3) to estimate soil volumetric water content (θv). The θv values were regressed with FDR readings to derive an equation for calibrating FDR readings (θ%) to soil moisture volume ($cm^3\ H_2O/cm^3$soil). A positive relationship between FDR readings and θv was obtained ($r^2 = 0.77$, $F = 58.61$, $P \leq 0.001$, error d.f. = 18) giving a best-fit (linear) regression equation of $FDR = 38.05\ \theta v + 4.738$.

Collected soil moisture data for each treatment was combined for 2 months and the mean values used. The non-vegetated controls served to gauge soil moisture regimes due to surface evaporation, diffusion, capillary motion and free drainage of gravitational water in the absence of plants. Subtracting the mean volume of soil water of treatment types from the water volume in controls with matching substrates gave an estimate of the amount of water ‘used’ or redistributed by plants at given depths.

4.3.3. Soil analysis

One sample was taken per sandy control bin and from different depths. An additional sample was obtained from the original soil not used in the experiment to provide an indication of *in situ* soil characteristics. These six soil samples were analysed for particle size distribution, saturated hydraulic conductivity (K_s), pH and electrical conductivity (EC). Particle size distribution in accordance with the United States Department of Agriculture (USDA) classification scheme into sand (2.0 – 0.05 mm), silt (0.05–0.002 mm) and clay (< 0.002 mm) was analysed for air-dried soils with a combination of sieving analysis for the coarse fraction and hydrometer (micro-pipette and sedimentation) procedures for the fine fractions (Gee & Bauder1986). The pH and EC were measured at a soil to solution ratio (paste) of 1:1 for the <2 mm fraction of the soil using hand held conductivity meter and pH electrode (Mclean 1982, Rhoades 1982). Values of K_s were determined using the constant head method over a specific time (Klute 1965, Klute & Dirkson 1986). On average, 17.2% of the soils consisted of gravel (≥ 2 mm) whereas the sand fraction was 53.1% coarse, 25.2% medium and 16.6% fine. The soils had very low silt (3.3%) and clay (1.8%) content with a neutral pH of 7, high K_s (3469 mm h^{-1}) and EC 46.6 (µS/cm) values.

4.3.4. Statistical Analysis

We employed a randomised complete block experimental design and took repeated measurements of soil moisture content at regular time intervals. Hence, a repeated measure multivariate analysis of variance (MANOVA) was used to analyse results using SPSS 15.0 for Windows statistical package (SPSS Inc., Chicago, Illinois, USA), with subsurface depth as a covariate. Between-subject factors (seasons) and the differences among them were tested using general linear full factorial model with Type III Sums of Squares. Comparisons of group means and multiple pair-wise comparisons were evaluated with Bonferroni *post hoc* tests to verify significance of differences between and within treatments. The main effects were compared using confidence intervals set at $\alpha \leq 0.05$ significance level. The soil moisture data after 14 d obtained in May-June 2008 was analysed with one-way Analysis of Variance (ANOVA) with Scheffe *post hoc* tests.

4.4. Results

4.4.1. Rooting depths

The tree seedlings produced one tap root with occasional side roots branching at the bottom. Fine lateral side roots were found all along the length of the tap root. Tree seedlings had greater mean rooting depths than grasses (Fig 4.1). In treatments that contained trees, the tree seedlings growing with unclipped grass produced significantly shorter roots than tree seedlings without grass competition or with clipped grass on both substrates (F = 11.88, error d.f. = 17, P = 0.003). However, in each treatment tree seedlings on rocky substrates had longer roots than on sandy substrates (Fig. 4.1). Nonetheless, the rooting depth of tree seedlings on rocky substrate competing with unclipped grass was not significantly different (F = 1.58, error d.f. = 26, P = 0.226) than the depth of tree seedlings roots on sandy substrate without grass competition or with clipped grass (Fig. 4.1).

The mean rooting depth of unclipped grass on sandy substrate was positively affected by tree seedling competition such that grass competing with trees on sandy substrate had significantly (F = 19.93, error d.f. = 12, P = 0.001) longer roots than grasses in all other treatments. On rocky substrate, the mean rooting depths of unclipped grass without tree competition was not significantly different (F = 4.27, error d.f. = 16, P = 0.055) from unclipped grass growing with tree competition (Fig. 4.1). Clipped grass on its own or in competition with tree seedlings had shallower roots than unclipped grass in either case on both substrates, but tree competition had more negative effect on the rooting depth of clipped grass (Fig. 4.1). Substrate type had no significant influence on average grass rooting depths with the exception of unclipped grass competing with trees (Fig. 4.1).

4.4.2. Moisture niche separation

There was a significant interaction between soil moisture content (θv) and depth eight days post irrigation (Table 4.1), indicating differences between the effects of various treatments at different soil depths. Unclipped grass-only treatments invariably took up more moisture at 0.15 m depth than all treatments and significantly more ($P \leq 0.001$) than the tree-only treatments (Fig. 4.2). The tree + grass combination (unclipped grass) took up significantly more moisture at 0.25 m depth than tree-only treatments ($P \leq 0.001$) but not significantly more than grass-only (unclipped) treatment (Fig. 4.2). Thus, trees and grasses used comparable θv on their own or in combination at 0.25 m soil depth (Fig. 4.2). At the relatively deeper soil layers of 0.35 and 0.45 m, trees on their own or in combination with grasses took up more moisture than all treatments. Unclipped grass had a significant negative effect on soil θv at 0.35 m depth, but there was no significant influence of grass at the lowermost 0.45 m depth (Fig. 4.2).

With regard to quantitative differences in θv from 'complete' controls (± volumetric water use by plants), all tree-treatments on sandy substrates 'consumed' nearly identical and the largest θv (~180 cm^3 H_2O/ cm^3 soil) from 0.45 m (Fig. 4.2). The unclipped grass without tree seedling competition treatment at 0.25 m on sandy substrates resulted in the second largest θv difference which was not significantly different from the θv difference of unclipped tree + grass at 0.25 m or grass-only at 0.15 m depth on sandy substrate (Fig. 4.2). Grasses on their own had a significantly larger effect on soil moisture at 0.15 m compared to grass growing among trees on both substrates (Fig. 4.2). Trees-only used the least θv at 0.15 m and the smallest difference in θv at 0.15 m depth was found in the rocky tree-only treatment. Clipped grass-only treatment utilised the least θv at all depths beneath 0.15 m on either substrate and was wetter than the controls (negative θv difference) at 0.45 m depth on rocky substrate (Fig. 4.2).

There was no significant interaction between substrate type (rocky or sandy) and θv at different depths (Table 4.1), but the quantitative difference in θv from controls showed significant differences between rocky and sandy substrates (Fig. 4.2). In all cases, plants on sandy substrates used more moisture than on rocky substrates (given the larger soil volume in sandy bins). Trees took up more θv from 0.45 m on sandy substrate but on the rocky substrate they took more water at 0.35 m. The unclipped grass treatment showed a significantly larger difference in θv at 0.25 m depth from rocky bins than was found at 0.15 m, but there was no significant difference at the two depths on sandy substrate (Fig. 4.2). Unlike in other treatments at other depths, θv deficit on rocky substrates relative to sandy ones for the grass treatments at 0.35 m did not differ significantly (Fig. 4.2).

4.4.3. Influence of grass clipping on soil water content

Grass clipping had a significant influence on soil moisture distribution with depth (Wilks' λ = 0.622, d.f. = 177, $P \leq 0.001$). Regularly clipped grass took up significantly less θv than unclipped grass on its own or in combination with trees (Fig. 4.2). However, the influence of regular grass clipping on θv depletion was only significant at lower depths of 0.25 m (grass-only swards) and 0.35 m (rocky tree + grass combination). Regular clipping of grass did not significantly affect the grass' ability to deplete soil moisture in the topsoil at 0.15 m. The largest θv difference between grass-only clipping treatment and unclipped grass on either substrate was at 0.25 m depth. Unclipped grass on sandy substrates showed double the moisture depletion compared to clipped grass at 0.25 m depth (Fig. 4.2).

On sandy substrate, grass clipping had no significant effect on the moisture 'uptake' of the tree + grass treatment at any depth relative to tree-only treatments (Fig. 4.2). On the rocky substrate, the tree + grass combination with clipped grass had comparable θv difference to the tree-only treatment at all depths < 0.15 m and significantly smaller than the tree + grass treatment without clipping (Fig. 4.2). Thus, grass clipping altered soil moisture availability to woody seedlings more on rocky substrates than on sandy ones. There was a significant moisture x plant-combination x clipping interaction (Wilks' λ = 0.908, d.f. = 177, $P = 0.004$).

4.4.4. Water content of clipped grass

There was a significant effect by harvest date on the "water content" (WC) of clipped grass tissue (Wilks' λ = 0.052, d.f. = 18 $P < 0.001$) but no significant harvest date x tree seedling competition (Wilks' λ = 0.815, d.f. = 18, $P = 0.554$) or harvest date x substrate (Wilks' λ = 0.948, d.f. = 18, P =

0.960) interactions. Tree seedling competition had a significant effect on grass WC on certain dates (Table 4.2). However, clipped grass growing with tree seedlings had significantly higher WC than grass growing on its own on these harvest dates (Table 4.4). Substrate did not have a significant influence on grass WC at any harvest date nor was there an interaction between tree seedling competition and substrate (Table 4.2). The first harvest (Harvest 1) was taken at the end of the dry season when the bulk of the grasses were dead and dry, thus physiologically inactive or dormant, hence the very low water content values (Table 4.2).

4.5. Discussion

There was distinct separation of soil moisture depletion zones by different tree and grass combinations in this study. Consistent with predictions by Walter (1939, 1971), grasses used up more moisture than tree seedlings at very shallow depths (< 0.15 m) while trees used the most water at greater depths. These depletion zones overlapped at intermediate depths (0.25-0.35 m). Gravimetric water content (WC) in a soil is not only indicative of soil moisture availability to plants, but is also a function of soil water potential (Rundel & Jarrell 1989), but WC changes provide an estimate of the water usage by roots at each depth. Given that the same soils were used in the experiment, soil properties could be discounted in evaluating changes in volumetric soil moisture content (θv). The θv in the 'complete' controls (unvegetated) after > 4 d could be taken to represent the drained upper limit after which no free drainage takes place (Ratliff *et al.* 1983). The differences between trees-only and unclipped grass-only treatments from the controls were equivalent at 0.25-0.35 m on both substrates, indicating that trees and unclipped grass effectively shared this depth layer as a source of moisture. On sandy soils, grasses had larger θv differences than trees at 0.25 m but the reverse was observed at 0.35 m, showing a diminished capacity for moisture uptake by grass with depth as the absorptive capacity of tree roots increased.

Higher water content in regularly clipped grass tissue growing with tree seedlings compared to grass on its own could indicate that grasses benefited from hydraulic redistribution by the trees (Caldwell & Richards 1988, Burgess *et al*. 1998). Hydraulic lift, the upward redistribution of water, entails the nocturnal release of moisture taken up from deep moist soil layers by shallow tree roots to the drier surface soils (Richards & Caldwell 1987, Schwenke & Wagner 1992, Dawson 1993). Possible rewetting of shallow soil layers in our study was more likely to cause a reduction in the magnitude of θv depletion by roots until the rate of transpiration by trees surpassed that of moisture uptake from deep soil layers with re-opening of stomata at dawn (Richards & Caldwell 1987, Dawson 1993). Since our measurements were taken just before noon when stomatal conductance was likely at a maximum, the contribution of hydraulic lift to soil θv would probably have been negligible. Differences in θv between controls and vegetated bins at the time of measurement would mainly have resulted from transpirational water uptake. Moreover, differences in θv between trees + grass and tree-only treatments imply more water uptake by grass than the amount of soil moisture depletion by tree seedlings, including hydraulically-redistributed moisture through 'water parasitism' (Caldwell & Richards 1989, Liste & White 2008, Hawkins *et al*. 2009). That tree + grass treatments where the grass was clipped had higher water content than unclipped tree + grass treatments at 0.25-0.35 was indicative of both trees and grasses taking up water from these depths. On the rocky substrate, unclipped grass had a competitive advantage over trees, indicated by larger volumes of water uptake from the topmost layer (0.15 m) down to 0.35 m, indicative of 'niche-sharing' by trees and grasses. When subjected to clipping however, the grass-only treatment took up much less moisture than tree-only treatment, retaining more water than the controls at

0.45 m on rocky substrate. Grass clipping thus reduced the grass ability to compete for soil moisture and allowed more water to reach to deeper soil layers.

Grass clipping had a significant effect on soil moisture storage at intermediate to deeper soil layers. Our results confirm field studies showing that repeated removal of grass (simulating heavy grazing) allows more soil moisture to percolate to deeper soil layers exploited by woody plant species (see also Knoop & Walker 1985, McNaughton *et al.* 1988, Skarpe 1990). The magnitude of differences between moisture 'depletion' by clipped and unclipped grasses was smaller at 0.15 m depth and greatest at 0.25 m. In addition to a larger evapotranspirational surface for unclipped grass relative to clipped grass, there was also a negative effect on grass rooting depth in the grass clipping treatments at 0.25 m depth. In contrast, unclipped grass had roots present at that depth. That the effect of grass clipping was not immediately evident in the first months after commencement of grass clipping may suggest that there were roots present at 0.25 m which then died back due to repeated aboveground defoliation. Frequent defoliation eventually depletes carbohydrate reserves and leads to tiller mortality (Schutz *et al*. 2009, Wigley *et al*. 2009, Tolsma *et al*. 2010) and possibly also root death. However, the grasses seemed to have maintained their roots and competitive capacity in the upper 0.15 m despite repeated clipping and could exploit and benefit from hydraulically-lifted water.

The presence of a third intermediate soil depth layer equally exploited by both trees and grasses may partially account for the mixed results on root distribution and moisture uptake reported from other field studies (e.g. Knoop & Walker 1985, Mordelet *et al.* 1997, Weltzin & McPherson 1997, Hipondoka *et al.* 2003). Layering of plant roots may vary greatly depending on environmental and genotypic attributes. Dimorphic rooting systems consisting of superficial lateral roots and deep penetrating tap roots exploiting both shallow and deep soil layers are common in savanna woody species (Seghieri 1995, Smit 1999). Stable isotope studies suggest that woody plant species are able to redirect carbon allocation towards root production in response to moisture availability (Kolb *et al.* 1997, Dawson & Ehleringer 1998, Snyder & Williams 2003), indicating adjustments and plasticity of root activity as well as allocations at depth (Sher *et al*. 2010) or shifts in functional rooting zones. It is unlikely that plants would shut off the uptake of moisture by roots in one rooting zone and exclusively utilise another if they maintain root presence at all depths. Patterns of moisture uptake by plant roots may simply mirror the spectrum of relative resource availability at various depths (Fitter 1994, Lynch 1995, Snyder & Williams 2007, Sher *et al*. 2010) and the plant's ability to exploit the spatiotemporal heterogeneity of resource distribution in the soil (Robinson 1996). Reported switching by trees/shrubs from a reliance on infiltrated rainwater at shallow depth to exploitation of deeper sources (Gebauer & Ehleringer 2000, Nippert & Knapp 2007, Sher *et al*. 2010) may rather reflect shifts in the proportions of moisture sourced from various depths, rather than occupation of distinctly separate niches.

Dual competing exploitation of moisture by concurrently abundant tree and grass roots from the same soil depth (Knoop & Walker 1985, Sala *et al*. 1989, Belsky 1994) has been reported before to occur within the shallow 0.2-0.3 m depth layer (Lawson *et al*. 1968, Seghieri 1995). These were the depth layers in our greenhouse experiment at which tree seedlings and grasses utilised comparable θv, indicating joint exploitation of this depths. When shallow subsurface layers are more saturated than the deeper soil layers, following rainfall or during the wet season (Wan & Sosebee 1990, Seghieri 1995), tree/shrub roots in the shallower 'common rooting zone' would most likely obtain proportionally more water than roots penetrating deeper and *vice versa*. This is consistent with findings by Nippert and Knapp's (2007) of increased potential for subsurface competition for soil moisture between C_4 grasses and C_3 shrubs when water is abundant and its reduction in times of limited moisture availability. With progressive drying of the shallow soil layers with onset of the dry season, the contribution of resource-depleted layers (Robinson 1994) to a plant's water budget may approach zero, resulting in a 'switch' towards exclusive reliance on deeper soil layers for moisture (Taylor & Klepper 1975, Rambal 1984, Sala *et al*. 1989). This leads to a strong seasonality of water uptake from the shallow soil layers by woody plants of arid zones (Gebauer & Ehleringer 2000). In our study, a distinct 'deeper' layer was limited by the depths of dustbin and regular watering, but was nonetheless discernable on especially sandy substrates. Indiscrete deeper soil layer in rocky bins may have resulted from an absence of sufficient tree roots at the bottom. All plants (including trees) in bins containing rocks had similar or less θv at 0.45 m than at 0.35 m (Fig. 4.2), which may point to possible initial retardation of root elongation on rocky substrates.

Grass roots are restricted to a depth that has variably been reported to lie between 0.2-0.3 m (Lawson *et al.* 1968, Mordelet *et al*. 1997, Hipondoka *et al*. 2003). Grasses are thus solely reliant on moisture in the limited topsoil volume, derived from rainfall infiltration, seepage of overland flow or hydraulic redistribution by roots of woody species (Schulze *et al*. 1998, Burgess *et al*. 2001, Brooks *et al*. 2006). The range of active rooting depth measured for *A. mellifera* (based on field data from the site where our tree seeds were sourced) was 0.15-0.5 m (Meyer *et al*. 2007). In the present study, we found that grasses monopolise moisture uptake at ≤0.15 m. The rooting zone shared between grasses and seedlings in our study is likely to have been between >0.15 and ≤0.4 m, but this will vary for mature trees and under field conditions.

The main edaphic factors influencing transpiration and thus moisture uptake from soil depths are the vertical distribution of roots and actual soil moisture supply (Minhas *et al.* 1974, Feddes *et al.* 1976, Molz 1981). The θv differences did differ significantly for rocky and sandy substrates at all depths. This was due to rocky controls holding less moisture (low water holding capacity) than their sandy counterparts. Plants growing on the sandy substrate thus had access to greater volumes of moisture than those on the rocky substrate. A large θv difference found in all vegetated treatments except clipped grass after irrigation reflected the antecedent moisture content patterns before watering. Apparent fluctuations in θv differences by the clipped grass-only treatment on rocky substrate in particular may have been an artefact of the (unvegetated) controls drying out faster than vegetated rocky bins due to relatively larger evaporative losses due to an absence of shading by plants. This implied that the presence of plants significantly minimised moisture loss and improved the water retention in rocky bins, not only as result of reduced surface evaporation but possibly also due to remobilisation of water to the surface soils and reducing drainage. Grasses have indeed been shown to hydraulically lift soil moisture (Espelata *et al.* 2004). Both the top and lower soil layers easily lost water to surface evaporation and probable free drainage. Retention of moisture in the near-surface soil layers is generally of extremely short duration (Richards 1986). In essence, clipped grass may have enhanced moisture infiltration due to minimal interception of irrigation water, improved the water-holding capacity of soils relative to bare soils as a result of the presence of roots, reduced surface evaporation by shading and minimal transpired water, thus losing less moisture than the controls.

Our results showed higher moisture content in grass treatments than the controls. Roots of grasses in particular may have enhanced water infiltration and improved the soil's moisture retention capacity, thus increasing the wetting of soils at depths through grass hydraulic redistribution (Epelata *et al*. 2004) relative to controls. Organic compounds produced by plants and degraded root biomass may act as surfactants at the water-air interface in the soil (DeBano 2000, Read *et al.* 2003). This then increases the affinity of the soil around roots (rhizosphere) to water, leading to improved wetting of the soil matrix. Elevated soil moisture content aids the percolation and retention of water to deeper soil layers where the roots of woody plants may be favoured (Graz 2008). Grasses produce dense roots that may augment the hydraulic continuity of pores in the soil (Archer *et al.* 2002, Hallett *et al.* 2003) as shown by a positive correlation between root volumes in the soil and saturated hydraulic conductivity (Archer *et al.* 2002).

The belowground competitive ability of grasses for soil moisture beneath 0.15 m was reduced by growing with tree seedlings, even in the absence of clipping when grass competition was expected to be stronger. With clipping, grasses growing with trees were affected more severely than grass on its own. Such disadvantageous influence on grass is consistent with assertions that herbivory weakens grass competitive capacity to the benefit of woody seedlings (Walker & Noy-Meir 1982, Stuart-Hill & Tainton 1989, Jeltsch *et al*. 1997). Restricted root extension due to rock barriers necessitates sharing of shallow niches by trees and grasses (Kambatuku *et al*. in press). Thus, when one component is disproportionally disadvantaged by herbivory the other may predominate and monopolise the 'shared niche'. This is among the reasons advanced for widespread woody encroachment onto extensively grazed rangelands (Archer 1989, Skarpe 1990, Ringrose *et al*. 1996, Ward 2009). On sand, trees are able to extend roots beyond grass rooting zones without hindrance, permitting competition avoidance and coexistence via niche differentiation. Ward & Esler (2010) found that *A. mellifera* saplings growing on sand at the Pniel study site were larger than those growing on rocks. West *et al*. (2004) found elevated production of deep fine roots with increasing profile depth and sand content across varying soil types dominated by *Pinus palustris* trees and *Aristida stricta* grasses in a savanna ecosystem the southeastern USA, South Carolina. These authors did not attempt to identify the roots of species but rather estimated the roots lengths (using number as a proxy) through minirhizotron imagery. Field investigations comparing the sources of moisture uptake by two co-occurring shrubs, *A. mellifera* and *Tarchonanthus camphoratus* using water isotopes showed these shrubs to partition moisture uptake with depth on relatively deeper sandy areas while sourcing the same resource on rocky outcrops (Reinsch *et al.* in prep). It can therefore be expected that the outcome of heavy grazing will be more profound on shallow rocky terrains where the removal of grass competition may allow annexation of the only available soil layer by tree/shrub roots. This may partially explain the rapid increase and proliferation of encroaching plants on rocky/shallow soils relative to areas with deep sandy horizons (Wiegand *et al*. 2005, Britz & Ward 2006, Ward and Esler 2010).

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Table 4.1 Results of Repeated Measures Anova General Linear Model (GLM) for soil moisture content (θv) at 8 d intervals over time as function of depth, substrate type (rocky/sandy), plant combination (trees-only, grass-only, tree + grass, no plants) and the regular clipping of grass. Error degree of freedom (d.f.) was 177.

Source of Variation	**Wilks' λ**	**F**	**Significance (P)**
Period of Measurement	0.797	9.01	<0.001
Period x Soil Depth	0.516	33.18	<0.001
Period x Substrate Type	0.969	1.13	0.346
Period x Plant Combination	0.905	3.70	0.003
Period x Grass Clipping	0.622	21.49	<0.001
Period x Substrate x Plant Combination	0.954	1.72	0.132
Period x Substrate x Grass Clipping	0.929	2.70	0.022
Period x Plant Combination x Grass Clipping	0.908	3.58	0.004

Table 4.2 Two-way ANOVA comparisons of predicted mean ± S. E of water content (WC) in regularly clipped grass tissue at different harvest dates with tree seedlings competition and substrate type (sandy/rocky) as factors. Differences in error d.f. were due to failure of grass to regrow after clipping and for this reason an unbalanced design was used for harvests 5 and 6.

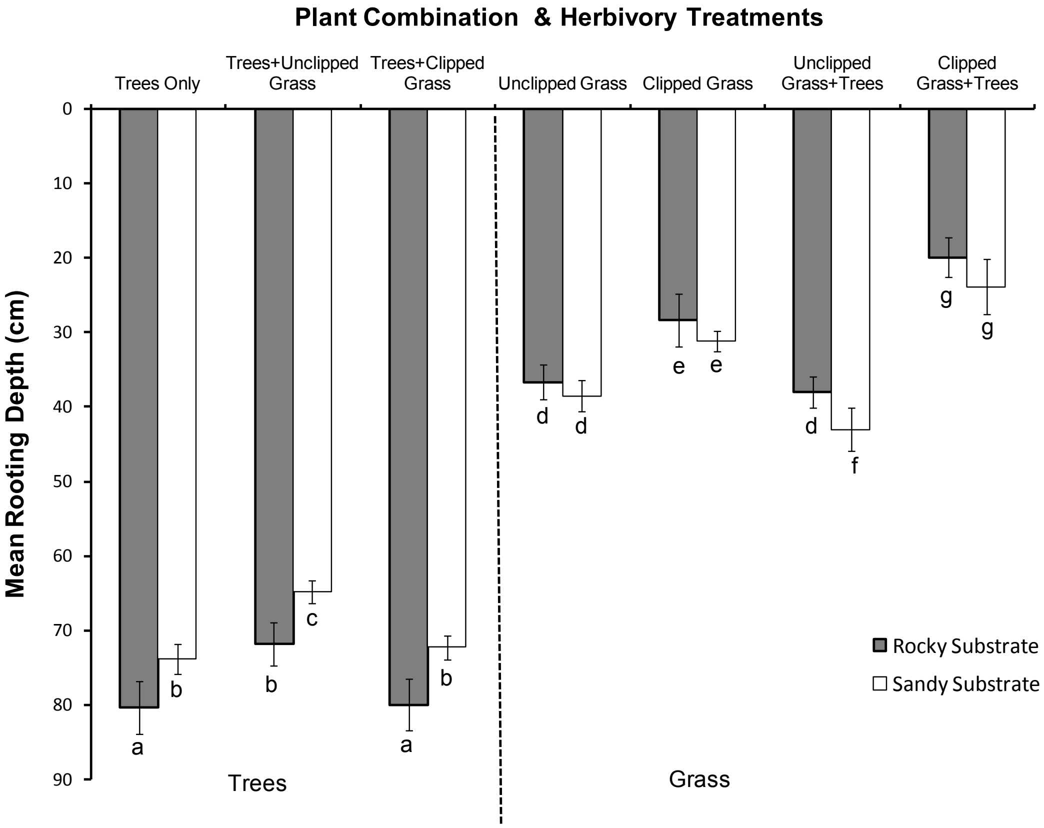
Harvest & Date	**Factors & Interactions**	**Water Content (%)**		**F**	**Error d.f.**	**Significance (P)**
		No Trees /Sandy	**Trees/Rocky**			
Harvest 1 2007/07/28	Tree Competition	7.17 ± 4.25	13.380 ± 4.25	12.35	35	0.001
	Substrate Type	10.21 ± 4.22	9.58 ± 4.22	0.24	35	0.630
	Trees x Substrate	5.71 ± 5.99	14.49 ± 5.99	0.33	35	0.571
Harvest 2 2007/09/28	Tree Competition	49.55 ± 1.48	54.28 ± 1.48	8.67	35	0.006
	Substrate Type	51.42 ± 1.47	52.15 ± 1.47	0.06	35	0.807
	Trees x Substrate	49.19 ± 2.08	55.45 ± 2.08	0.37	35	0.555
Harvest 3 2007/11/28	Tree Competition	51.47 ± 1.05	54.62. ± 1.05	2.67	35	0.112
	Substrate Type	53.27 ± 1.05	52.64 ± 1.05	0.02	35	0.898
	Trees x Substrate	51.46 ± 1.48	52.64 ± 1.48	0.42	35	0.522
Harvest 4 2008/01/28	Tree Competition	53.22 ± 1.75	56.08. ± 1.75	0.06	33	0.807
	Substrate Type	54.86 ± 1.74	54.28. ± 1.74	2.41	33	0.131
	Trees x Substrate	52.39± 2.46	56.39 ± 2.46	0.85	33	0.364
Harvest 5 2008/03/28	Tree Competition	50.90 ± 3.62	53.86 ± 3.62	11.56	25	0.002
	Substrate Type	55.33 ± 3.62	49.08. ± 3.62	2.41	25	0.107
	Trees x Substrate	49.26 ± 5.15	48.85 ± 5.15	0.08	25	0.780
Harvest 6 2008/05/28	Tree Competition	62.06 ± 2.75	70.25.86 ± 2.75	12.02	23	0.007
	Substrate Type	64.64 ± 2.75	65.83± 2.75	2.12	23	0.158

Figure Captions

Fig. 4.1 The mean ± S.E for final root depths of tree seedlings grown with grass competition and grass grown on their own and in combination with tree seedlings as affected by grass clipping.

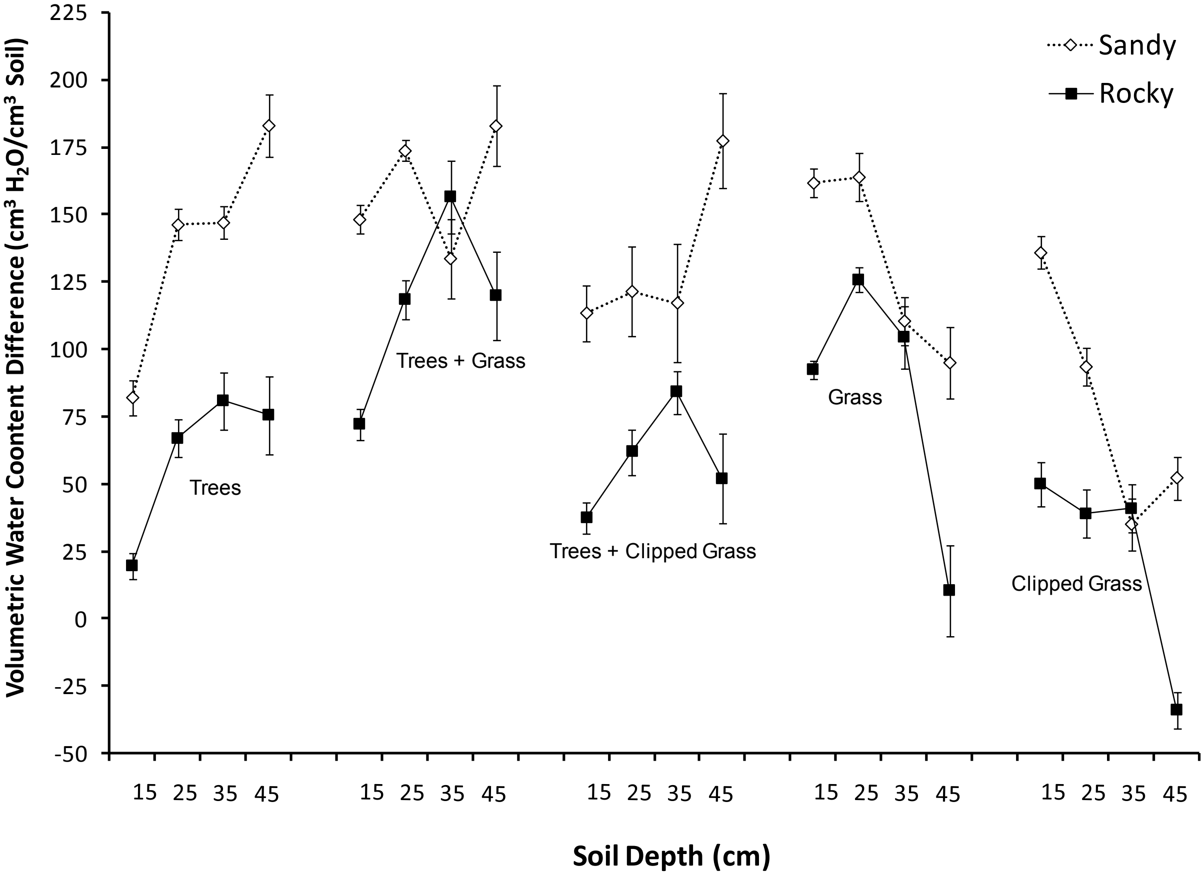
Fig. 4.2 The differences in mean ± S. E volumetric soil water content (θv) between the 'complete' controls and vegetated treatments (cm^3 H_2O/cm^3 soil) on sandy and rocky substrates for all weekly readings (treatment means) between September 2007 and February 2008.

Fig. 4.1



Plant Combination & Herbivory Treatments
Trees Only
Trees+Unclipped Grass
Trees+Clipped Grass
Unclipped Grass
Clipped Grass
Unclipped Grass+Trees
Clipped Grass+Trees
Mean Rooting Depth (cm)
0
10
20
30
40
50
60
70
80
90
a
b
b
c
a
b
d
d
e
e
d
f
g
g
Rocky Substrate
Sandy Substrate
Trees
Grass

Fig. 4.2



Sandy
Rocky
Volumetric Water Coontent Difference (cm³ H₂O/cm³ Soil)
225
200
175
150
125
100
75
50
25
0
-25
-50
Trees
Trees + Grass
Trees + Clipped Grass
Grass
Clipped Grass
15 25 35 45
15 25 35 45
15 25 35 45
15 25 35 45
15 25 35 45
Soil Depth (cm)

5. Chapter 5: Nitrogen fertilisation reduces grass-induced N_2 fixation by tree seedlings of semi-arid savannas

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5.1. Abstract

Coexistence of trees and grasses in nutrient-poor arid savannas may result in competition for soil nitrogen (N). While grasses may be more effective than woody plants in acquiring N from the soil, some leguminous woody species may rely on fixing atmospheric N_2. This greenhouse study determined the contribution of N_2 fixation to the N-budget of *Acacia mellifera* seedlings under several levels of N addition and competition from grass. We also determined the influence of N addition and grass competition on growth, nutrient concentration, biomass accumulation and ^{15}N natural abundance of trees. Tree seedlings were four times taller and twenty times heavier in the absence of grass. The leaf $\delta^{15}N$ values were lower with grass (-0.25 ± 0.2‰, n = 19) than without grasses (5.2 ± 0.1‰, n = 64). On the basis of these $\delta^{15}N$ data, the contribution of biological N_2 fixation to the N pool when tree seedlings grew with grass decreased with increasing level of N fertiliser. *A. mellifera* was more reliant on N_2 fixation in the presence of grasses, but this did not result in higher biomass accumulation or tissue N concentration relative to seedlings grown without grass competition. Tree seedlings competing with grass were significantly more negative $\delta^{13}C$ (-29.5 ± 0.6‰) than those of seedlings without grass competition (-28.8 ± 0.5‰). Induction of inefficient water use and BNF by grass may have resulted from competition for nutrients. N_2 fixation may enable the tree seedlings to compensate for soil N deficiency and survive competition with grass at a critical and vulnerable developmental stage of germination and establishment.

Keywords: *Acacia mellifera*, carbon isotope discrimination, $\delta^{15}N$ values, nitrogen fixation, tree-grass coexistence, WUE

5.2. Introduction

Over half of southern Africa is savanna (Rutherford 1997) in which leguminous woody species feature prominently (Midgley and Bond 2001). Savanna ecosystems are dominated by a variably spaced woody tree/shrub component and continuous herbaceous cover, mostly comprised of grasses (Frost *et al.* 1986). Conceptual models of savanna tree-grass coexistence assign prominence to competitive plant interactions or disruptive environmental perturbations on germination, survival and maturation of trees (House *et al.* 2003; Sankaran *et al*. 2004; Ward 2005). Co-occurrence of trees and grasses in semi-arid savannas may be maintained by inter-specific exploitation of distinct sub-soil niches in acquisition of limiting resources (Walter 1971; Walker and Noy-Meir 1982), opening of patches via intraspecific competitive exclusion (Wiegand *et al*. 2005, 2006) and perturbations such as fire (Higgins *et al*. 2000; Midgley and Bond 2001), low rainfall with recurrent droughts (Harrington 1991; Scholes and Archer 1997) and disturbance (Jeltsch *et al*. 1996). Although soil nutrients and moisture are the main resources for which competition occurs in savannas (Stott 1991; House *et al.* 2003; Sankaran *et al*. 2004), attention has mostly focused on water (Walter 1971; Walker and Noy-Meir 1982; Knoop and Walker 1985; Wiegand *et al*. 2006). Despite this, direct interactions between savanna trees and grasses may mainly be driven by nutrient uptake (Scholes and Hall 1996; House *et al.* 2003). Plant-available soil nitrogen (N) is the nutrient most commonly limiting productive output in terrestrial ecosystems under natural conditions (Miller and Cramer, 2005) and, along with water, arid savanna ecosystems are particularly susceptible to N limitation (Skarpe and Bergström 1986; Solbrig 1996; Breman and De Wit 1983; Kraaij and Ward 2006).

Coexistence of trees and grasses could lead to intense competition for limited soil mineral N (Smith and Goodman 1986; Cramer *et al*. 2007). Grass competition suppresses tree seedling survival

(Kraaij and Ward 2006; Ward and Esler 2010), growth (Knoop and Walker 1985; Cramer *et al*. 2007) and maturation (Van Auken 2000), representing a demographic obstacle to seedling recruitment in savannas. The most critical developmental period for trees is germination and seedling establishment (Grubb 1977; Jeltsch *et al.* 1998; Higgins *et al.* 2000; Wiegand *et al*. 2005).Woody seedlings germinate and set root in the uppermost soil layers presumed to be the domain of grass roots (Walter 1939; Higgins *et al*. 2000), thus directly competing for belowground resources (Ward 2005). Rooting depths and distribution of adult trees overlap with those of grasses (Johns 1984; Knoop and Walker 1985; Belsky 1994; Seghieri 1995), although verification of distinct rooting niches remains equivocal (Scholes and Archer 1997; Sankaran *et al*. 2004; Wiegand *et al*. 2005; Meyer *et al*. 2007).

Woody legumes often occur at high densities in African savannas, despite their relatively low species richness (Cramer *et al.* 2007). Increasing encroachment of leguminous *Acacia* trees into nutrient-poor arid African savannas (Walter 1964) is also common and often results in the exclusion of grasses (O'Connor 1995; Hudak and Wessman 2001). As the majority of acacias nodulate (Allen and Allen 1981; DeFaria *et al.* 1989; Scholes and Walker 1993; Sprent 1995; Scholes *et al*. 2002) it is postulated that their ability to symbiotically transform atmospheric dinitrogen (N_2) into plant-usable reduced N enables them to dominate in these savannas.

Competition with grass limits available soil N (Barea *et al.*1989) and triggers reliance by legumes on N_2 fixation (Zanetti *et al*.1996; Loiseau *et al*. 2001). Bond *et al.* (2001) noted a selective ability to form nodules subject to grass competition in *Acacia karroo* and *A. nilotica*. Grass competition results in lower $\delta^{15}N$ natural abundances in acacias, indicating enhanced N_2-fixation through inter-specific competition (Cramer *et al*. 2007). Differences in the influence of N supply on responses of *Acacia mellifera* compared to grasses (Kraaij and Ward 2006) could be ascribed to reliance by grasses on soil N and N_2 fixation by *A. mellifera.* However, extensive excavations of mature *A. mellifera* roots in the field failed to uncover nodules (Cramer and Ward; pers. comm.), casting doubt over whether N_2 fixation occurs in adults of this species. Nonetheless, *A. mellifera* exhibited the highest dependency on N_2-fixation of all legumes studied by Schulze *et al.* (1991) in Namibia, indicating that the reliance on N_2 fixation is dependent on the environmental context.

N_2 fixation is conventionally considered to be energetically costly (Vitousek and Howarth 1991; Sprent 1985) so that plants only rely on it when its benefits outweigh its costs (Sprent 1985; Crews 1999), such as under conditions of very low soil N and/or strong grass competition for available N (Cramer *et al.* 2007). The differences in the energy cost of N_2 fixation relative to non-fixation of N_2 may not be as acute as was once thought (Pate *et al.* 1979, Lambers *et al.* 2008). This has been used to explain the lack of suppression of growth by reliance on N_2 fixation in African acacias (Cramer *et al.* 2010). High levels of plant-available N are nonetheless known to inhibit formation of nodules (Streeter 1988; Huss-Danell 1997) and decreasing N_2 fixation by legumes (Fried and Broeshart 1975; Unkovich *et al.* 2000). Depletion of soil N by grasses may encourage N_2 fixation in legumes by reducing the pool of available N (Carlsson 2005). Symbiotic biological nitrogen fixation (BNF) by woody legumes may be a facultative response to grass competition for limited soil N. This could facilitate increased densities of trees in sparsely wooded savannas or bush encroachment.

Widespread bush encroachment (Archer 1989) results from a perturbation of tree-grass coexistence, with direct implications for rangeland and pasture management (Skarpe 1992; Ward 2005). *Acacia mellifera* is a common encroaching species on rangelands in Botswana, Namibia and northern parts of South Africa (Skarpe 1991; Molele *et al.* 2002; De Klerk 2004; Kraaij and Ward 2006). We tested the hypothesis that the capacity for N_2 fixation of woody leguminous plants confers a competitive advantage with respect to grass. We further hypothesised that the degree of reliance of the woody legume *A. mellifera* on N_2 fixation for N would decrease with increasing soil N availability. We thus predicted increased BNF in response to both limited soil N and grass competition. To test this, biomass accumulation and ^{15}N isotope abundance was assessed in pot-grown *A. mellifera* plants growing with and without grass competition with varying concentrations of fertilizer N.

5.3. Methods

5.3.1. Plant cultivation

An alluvial sand aggregate from the Umgeni River near Pietermaritzburg in the KwaZulu-Natal Province of South Africa was placed in 0.3 m diameter X 0.28 m tall pots. Umgeni sand aggregate is mostly very coarse material with particle distribution sizes ranging from pebbles and gravel aggregates to fine silts with low nutrient content. Pots were filled with 16 kg of Umgeni sand to which 32 g of Omnia Nutriology “Superphosphate 10.5” (N-P-K-Ca-S, 0 -10.5 -0-17 -10.2) fertiliser (Omnia Fertilizer Ltd., Bryanston, Johannesburg, South Africa) was added uniformly as a source of P and other nutrients. “Azolon 39 N” (N-P-K, 39-0-0), a controlled slow-release methylene urea fertiliser (Aglukon Spezialdünger GmbH Co, Düesseldorf, Germany) was used as a source of N. Of the N content of Azolon 39 N fertiliser, 3.5% was in the form of carbamide nitrogen (urea) with ureaformaldehyde N making up 36%. The pots were supplied with 0, 0.1, 2.1, 4.1 and 8.2 g of “Azolon 39 N” per pot in 5 treatment groups comprising 30 pots each, the untreated group with no N addition serving as a control treatment. Fertilizers were thoroughly mixed with dry sand in the pots and topped off with 0.04 m of fertiliser-free Umgeni grit aggregate.

Grass seeds of *Eragrostis curvula* (Ermelo variety, McDonald Seeds, Pietermaritzburg, South Africa) were planted in the 0.04 m untreated soil layer in half of the pots within a treatment group on 26

November 2005 and covered with an additional 0.04 m layer of unfertilized Umgeni grit. Grasses were allowed to establish for two weeks following emergence. *A. mellifera* seeds collected from 20 individual trees at Pniel Estates near Barkly West (28° 34′ S, 24° 25′ E) in the Northern Cape Province of South Africa in November 2004 were planted directly in all the pots at 0.04 m depth within the untreated soil layer on 11 December 2005. A total of 12 seeds were planted per pot. Germination of seeds was over 90% for grasses and below 30% for trees. No thinning of emerging seedlings was undertaken and the final number of seedlings per pot was a random outcome of germination and survival rates, allowing for the evaluation of the effects of intraspecific and interspecific competition. All pots were provided with an equal amount of water for 30 min every 3 d throughout the experiment. The treatment groups in the greenhouse as well as the pots within a treatment group were moved regularly to homogenise the greenhouse environment.

5.3.2. Plant growth and harvesting

After six weeks (February 2006), the aboveground heights of all tree seedlings were measured weekly (initial 110 d), and later fortnightly to allow calculation of relative height increases. All seedlings were harvested 142 d after emergence (in May 2006), having grown for about 5 months. Sand was washed from seedling roots and the number of nodules on roots counted. Tree seedlings were separated into above- and belowground components, weighed to determine fresh weight, oven dried at 65 ºC for 36 h and re-weighed. A total of 24 tree seedlings were randomly selected among pots with and without grass, but not more than three individual trees were chosen from a single pot. Selected seedlings were stripped of all leaves which were milled to a fine powder to pass through a 1 mm mesh using a Culatti Type MFC micro-fine pulverizing electrical grinder (Janke and Künkel GmbH, Staufen, Germany) fitted with 1 mm pore size sieve. Where there was insufficient leaf material due to small plant size, leaves from more than one seedling in the same pot were bulked into a single sample.

5.3.3. Soil and fertiliser analysis

Six samples of the original Umgeni grit were collected and analysed for N and P content. The Umgeni grit N concentration was 0.012 ± 0.004 % (w/w) and soil total P was 11.7 ± 4.1 mg/g with soil pH being 6.97 ± 0.12. Despite this low N concentration, the soil had a large amount of organic matter (0.59 ± 0.04%) due to the presence of undecomposed plant material. Six pots, three with grass and trees and three with trees only, were randomly chosen subsequent to harvesting and the soil sampled (> 0.05 m depth) for analysis of N concentration and isotopic composition. Three subsamples of Azolon 39 fertiliser were also collected for analysis. Soil and fertilizer samples were dried at 80 ºC for 48 h and sieved through a 1 mm mesh prior to analysis.

5.3.4. Mass spectrometry

Leaf and soil samples were milled to pass through a 0.5 mm mesh using a Wiley mill with 2.2 mg of leaf and 40 mg soil sub-samples weighed into tin capsules (Electrical Microanalysis Ltd., Okehampton, UK) for combustion in a Thermo Flash EA 1112 series elemental analyser (Thermo Electron Corporation, Milan, Italy). Resultant gas emissions were fed into the Delta Plus XP isotope ratio mass spectrometer (Thermo Electron Corporation). Isotopic ratios for plant materials were calibrated through comparisons to an international standard and the results expressed in parts per thousand (‰) as $\delta^{15}N$ (Evans 2001) values relative to the natural abundance signature of N in atmospheric air and $\delta^{13}C$ (Ehleringer and Rundel 1989) relative to Vienna Pee-Dee Belemnite (VPDB) for carbon (C).

In most plant species, the rate of net photosynthesis per amount of transpired water (*A/E*), or water use efficiency (WUE) is positively correlated to $\delta^{13}C$ (Farquhar *et al.* 1982; Knight *et al.* 1994). The $\delta^{13}C$ of tree seedlings at harvest was used as an indicator of integrated WUE during the experiment. To account for the fertilizer N isotope ratio and the differential N fertilizer application levels, the fertiliser $\delta^{15}N$ was subtracted from the foliar $\delta^{15}N$ of grass and tree seedlings to derive plant $\delta^{15}N$ signatures. The $\delta^{15}N$ value was used to calculate the extent that BNF contributed to plant N by using the $\delta^{15}N$ value of seedlings growing without grasses. The mean $\delta^{15}N$ of tree seedlings without grass competition at the same N fertilizer application level (treatment) which were not nodulated was used as a reference value ($\delta^{15}N_{ref}$) to calculate the %Ndfa by co-occurring seedlings ($\delta^{15}N_{fix}$) within the same treatment group using the equation of Shearer and Kohl (1986) with a B-value (the ^{15}N of N fixed from the atmosphere obtained from legumes that obtain all their N through BNF) of -2‰:

$$\%\mathrm{Ndfa} = 100 \times \left(\frac{\delta^{15}\mathrm{N}_{\mathrm{ref}} - \delta^{15}\mathrm{N}_{\mathrm{fix}}}{\delta^{15}\mathrm{N}_{\mathrm{ref}} - B} \right)$$

The mean B-values reported for most woody species ranges between -3‰ and +1‰ (Shearer and Kohl 1986; Boddey et al 2000) with 0‰ and -2‰ being the midrange values (Roggy *et al.* 1999; Gehring and Vlek 2004). To estimate the degree to which the fertilizer (Azolon 39 N) at different levels of application (Foliar $\delta\ ^{15}N_{fertilizer}$) supplemented the N budget of the plants, the equation below, was used for non-nodulated tree seedlings (Foliar $\delta\ ^{15}N_{control}$) grown without grass, with the isotopic signature of the soil ($\delta\ ^{15}N_{soil}$) less that of the fertilizer ($\delta\ ^{15}N_{Azolon\ 39}$) as denominator (Cramer *et al.* 2010).

$$\text{Azolon 39 N Contribution (\%)} = \frac{\text{Foliar } \delta^{15}\text{ N}_{\text{control}} - \text{Foliar } \delta^{15}\text{ N}_{\text{fertilizer}}}{\delta^{15}\text{ N}_{\text{soil}} - \delta^{15}\text{ N}_{\text{Azolon 39}}}$$

5.3.5. Statistical analysis

Data were analysed with SPSS 15.0 for Windows statistical package (SPSS Inc., Chicago, Illinois, USA). One-way Analysis of Variance (ANOVA) including Scheffe *post hoc* tests was employed in validating the significance of differences between treatments within and between groups. Regression analysis and curve estimation were performed to test the influence of intraspecific competition between *A. mellifera* seedlings and relationships between growth parameters relative to each other as well as to BNF and N concentration in leaves using the SPSS 15.0 statistical programme. Shoot development measurements at regular intervals were analysed with a repeated measures MANOVA general linear model.

5.4. Results

5.4.1. Soil and fertilizer

The only significant difference in post-harvest soil N concentration with and without grass was at 0 g/pot (control) and the highest N level (Table 5.1), which resulted in overall significant differences between the soils. Little change in soil N indicated that the N applied was used up by the plants. Soil δ^{15}N after harvest was positive (4.2 ± 0.1 ‰ with grass and 4.4± 0.1 ‰ without grass) and did not vary significantly (F = 0.76, P = 0.556, error d.f. = 54) with differences in N fertiliser levels (Fig. 5.3a). There was no significant ($P > 0.05$) difference in soil $\delta^{15}N$ between the soils with and without grass. The level of N fertiliser did not have a significant effect on the $\delta^{13}C$ of soil with tree + grass (F= 1.92, P = 0.139, error d.f. = 25) or tree seedlings only (F= 1.14, P =0.363, error d.f. =25). However, soil with grass grown in it was less depleted in $\delta^{13}C$ isotope (-15.8 ± 0.4‰) than soil with tree seedlings only growing in it (-20.7 ± 0.1‰) (F= 147.65, P = 0.001, error d.f. = 57) probably due to the presence of C_4 grass roots. Mass spectrometry analysis of the Azolon 39 fertiliser showed its N concentration to be 40.9 % (w/w), with a $\delta^{15}N$ ratio of -0.1‰.

5.4.2. Tree seedling development

Significant shoot elongation occurred over five months in *A. mellifera* seedlings grown without grasses (Wilks' $\lambda = 25.87$, d.f. = 235, $P \leq 0.001$), but there was no significant fertiliser level x shoot length interaction (Wilks' $\lambda = 0.80$, d.f. = 892.95, $P \leq 0.059$). However, fertilisation levels had a significant effect (F = 30.89, error d.f. = 4, $P \leq 0.001$) on shoot elongation. In the absence of grass, the slowest shoot elongation occurred at the lowest N addition (Fig. 5.1a). The presence of grass strongly retarded the development of tree seedlings, resulting in little shoot elongation by trees growing among grasses (data not shown).

Grasses significantly affected final tree shoot lengths across N treatments, with shorter shoots in the presence of grass compared to shoots in the absence of grass competition (Fig. 5.1b). In the absence of grass, tree seedling shoot lengths varied as a function of N treatment, with N fertiliser significantly influencing eventual shoot lengths (F = 12.05, $P < 0.001$, error d.f = 81). At the low N addition levels, tree seedlings' shoot lengths did not differ significantly, but shoot lengths at the highest level of N significantly differed from shoot lengths at the low N treatments.

Tree seedlings grown without grass had significantly higher above- and belowground final biomass than tree seedlings grown with grass at all N levels (Fig. 5.2a). Grass consistently depressed the biomass accumulation of trees at all N levels. In the absence of grass, N fertilizer increased total biomass accumulation of trees ($F = 7.90$, $P < 0.001$, error d.f. = 81) by increasing both shoot ($F = 10.32$, $P < 0.001$, error d.f. = 81) and root ($F = 4.05$, $P = 0.003$, error d.f. = 81) biomass. However, root biomass was only significantly different between the control (0 g/pot) and maximum treatment. Increases in aboveground biomass occurred at the higher N levels, but there was no significant difference between the low N treatments.

Tree seedlings with grasses yielded lower (smaller) root:shoot ratios relative to when they were grown without grasses ($F = 53.45$, $P < 0.001$, error d.f. = 73, Fig. 5.2b). Root:shoot ratio was significantly negatively affected by the level of N treatment overall ($F = 10.35$, $P < 0.001$, error d.f. = 81) and in trees growing without grass competition. For grass-exposed trees, fertiliser addition did not have a significant effect ($F = 2.20$, $P = 0.078$, error d.f. = 69) on root:shoot ratio. Among control seedlings without grass, the mean root:shoot ratio was 0.56 ± 0.03, indicating that over half the biomass was produced belowground.

5.4.3. $\delta^{15}N$ Natural abundance and BNF

Grass competition caused significant differences ($F = 179.19$, $P < 0.001$, error d.f. = 73) in the ^{15}N natural abundance of *A. mellifera* seedlings (Fig. 5.3a). The $\delta^{15}N$ values in tree seedlings exposed to grass competition were predominantly close to zero (-0.3 ± 0.2‰), indicative of high N_2 fixation rates. The tree seedlings without grass competition had positive $\delta^{15}N$ values (5.4 ± 0.2‰), indicating low/no

N_2 fixation. In competition with grass, the mean $\delta^{15}N$ values of tree seedlings were significantly (F = 223.62, P = 0.001, error d.f. = 79) lower than those of tree seedlings grown free of grass competition.

The effect of added N on $\delta^{15}N$ was significant in trees grown with grass (F = 6.95, P < 0.001, error d.f. = 33) and without grass (F = 24.87, P < 0.001, error d.f. = 81). A weak pattern of increasing $\delta^{15}N$ with incremental N was apparent in tree seedlings, starting at different N levels with grass than without grass (Fig. 5.3a). There was a significant (F = 5.32, P = 0.002, error d.f. = 39) difference between the $\delta^{15}N$ of grass grown at different N levels but, *post hoc* tests showed this difference resulted from comparison of the $\delta^{15}N$ of grass at the lowest N addition level (0.1 g/pot) to that of grass at other levels of N addition.

N fertiliser addition significantly (F = 7.57, P = 0.002, error d.f. = 14) affected the estimated mean percent N derived from the atmosphere (%Ndfa) by tree seedlings exposed to grass competition. Ndfa was highest at the low N fertiliser application level of 2.1 g, after which it declined with increasing N level. There was no significant difference between estimated Ndfa at higher N application levels (Fig. 5.3b). The fertiliser was estimated to have contributed a mean of 12, 23, 10 and 63% to the N budget of tree seedlings without grass competition at the different incremental N treatment levels.

The foliar N concentration per dry weight (%N) was not significantly different between tree seedlings with and without grass (F = 0.01, P = 0.911, error d.f. = 146). Significant differences in tissue %N were induced by different N addition rates among tree seedlings not competing with grass (F = 24.82, P < 0.001, error d.f. = 146) but not in tree seedlings competing with grasses (F = 0.53, P = 0.717, error d.f. = 17). Leaf tissue %N at low N levels was higher in trees grown with grasses than those in monoculture but this was reversed at high N levels (Fig. 5.3c). A linear increase in %N with increasing N fertiliser was evident in tree seedlings without grass competition as well as in grasses.

5.4.4. $\delta^{13}C$ Natural abundance and WUE

Grass competition significantly (F = 55.61, P = 0.001, error d.f. = 81) influenced the $\delta^{13}C$ of tree seedlings, such that trees competing with grass had isotopically more depleted C (-29.5 ± 0.6‰), than tree seedlings growing without grass (-28.8 ± 0.5‰). Tree seedlings competing with grass showed opposite trends to those without grass with incremental N level (except for the highest N level, Fig. 5.4). Thus, tree seedlings without grass competition had higher water use efficiency (WUE) than tree seedlings competing with grass. The level of N fertiliser addition had a significant effect (F = 3.17, P = 0.047, error d.f. = 14) on the $\delta^{13}C$ of tree seedlings competing with grasses but no significant effect on tree seedlings without grass competition (F = 1.66, P = 0.172, error d.f. = 59).

5.4.5. Correlations among measured parameters

Biomass accumulation above-ground showed a weak but significant negative correlation with tree density in the pots (data not shown), whereas there was no significant relationship between the foliar %N concentration of trees and tree seedling density overall (Table 5.2). Tree seedling numbers per pot had a significant negative effect (r = -0.55, F = 86.16, P < 0.001) on tree seedling shoot biomass, but no significant influence on biomass (F = 0.22, P = 0.150) in the presence of grasses. An overall strong positive correlation was found between $\delta^{15}N$ and both above- and below-ground biomass. For trees with grasses, $\delta^{15}N$ was not significantly correlated with shoot biomass (F = 0.55, P = 0.786) whereas it increased with higher biomass in trees growing free of competition (r = 0.37, F = 21.58, P < 0.001), indicating decreasing reliance on BNF with increasing size in the absence of grass competition.

5.5. Discussion

The ^{15}N results indicated grass competition to have induced N_2 fixation in tree seedlings in our study. Predominantly low (near zero) $\delta^{15}N$ in trees competing with grasses relative to higher positive values when grown without grasses probably indicates biological N_2 fixation in *A. mellifera* to be inducible by grass competition. This has previously been shown by Cramer *et al*. (2007) for other *Acacia* species in field experiments. It is discrimination against ^{15}N in favour of ^{14}N during N_2 fixation (Shearer and Kohl 1986) that gives rise to negative $\delta^{15}N$ in legumes. An increased reliance on N_2 fixation by tree seedlings competing with grass did however not lead to greater biomass accumulation or foliar N concentration relative to tree seedlings growing on their own. Tree seedlings competing with grass were more reliant on biological nitrogen fixation (BNF), yet they had significantly lower biomass and did not have significantly higher foliar N concentration than tree seedlings growing without grass competition. There was no significant correlation between ^{15}N and biomass or %N. Contrary to our hypothesis, an increased reliance on BNF in response to grass competition did not confer a competitive advantage to *A. mellifera* seedlings over the grasses which grew vigorously and suppressed the trees. Grass competition severely retarded the development and final sizes attained by seedlings relative to absence of grass. This indicated that grasses competitively suppressed tree seedling growth and biomass accumulation in spite of BNF. However BNF may have enabled the tree seedlings to survive the strong competitive effect of grass.

BNF dependence by *A. mellifera* was coupled to the level of N fertilizer application in our study. Our results show that N fertilization can suppress the BNF induced by grass competition. Tree seedlings relied progressively less on BNF input with increasing applied N. This is consistent with our second hypothesis and findings of others establishing a trend of general increase in $\delta^{15}N$ with incremental N availability (Unkovich *et al.* 2000). Addition of N fertiliser also inhibits N_2 fixation by crop legumes with an apparent substitution of BNF by soil N (Doughton *et al.* 1995; Herridge *et al.* 1995; Peoples *et al.* 1995; McNeil *et al.* 1996). High plant-available N inhibited BNF by reducing nodular biomass (Hellsten and Huss-Danell 2000) and also retards the rate at which N_2 is fixed (Svenning and Macduff 1996). Livestock urine which is high in N resulted in a 50% drop in BNF-derived N when added to plots of mixed ryegrass and white clover (Menneer *et al.* 2003). Combined, these trends of increased reliance on BNF in the presence of grasses and progressively reduced BNF reliance with incremental N supplementation suggest the facultative nature of N_2 fixation in *A. mellifera*. This result may indicate functional plasticity of BNF activity in response to limited access to N brought about by naturally low levels of N or competition with grasses. Grasses are strong competitors for N, with increased legume BNF reliance in their presence compared to legumes grown on their own (Zanetti *et al.* 1996; Loiseau *et al.* 2001).

N fertilizer addition was of little benefit to tree seedlings competing with grasses in terms of shoot elongation, biomass accumulation and %N concentration, implying that competition between trees and grass may have been for other resources in addition to N. Belowground resources for which grasses possibly outcompeted tree seedlings may include other nutrients such as P (Cramer *et al.* 2010) and soil moisture. Higher N addition may stimulate the growth of grasses and intensify the negative competitive effect of grass on tree seedlings (Cohn *et al*. 1989; Debain *et al*. 2005). Without grass competition, trees produced shoots almost four times as heavy as those growing with grasses, demonstrating a strong suppressive effect of grasses on seedling biomass accumulation, consistent with previous results for other *Acacia* species (Cramer *et al*. 2007, 2010). The root:shoot ratio of tree seedlings competing with grass was unexpectedly lower than that of tree seedlings without grass competition. Plants generally invest in root development when competing for nutrients and this was evident in the *decreasing* root:shoot ratio with increasing N levels in this study. However the suppressive effect of belowground grass competition may have been more severe than the effect on tree seedling shoots resulting in lower root:shoot ratios. A strong belowground effect by grass competition on tree seedling roots may partially explain the relative lack of response to N levels by tree seedlings competing with grasses.

The negative influence of intraspecific competition (as indicated by the number of tree seedlings per pot) on tree seedling biomass was significant, but it was weaker than the positive correlation between $\delta^{15}N$ and tree seedling biomass (Table 5.2). An increasingly positive $\delta^{15}N$ signature with increasing tree seedling biomass was indicative of decreasing N_2 fixation with incremental tree sizes. This may imply that trees rely less on BNF as they grow bigger. The majority of acacias nodulate (DeFaria *et al*. 1989) and their N_2 fixation is taken for granted, yet estimates of their dependence on BNF for N varies considerably (Shearer *et al*. 1983; Högberg 1986; Handley *et al.* 1994; Aranibar *et al*. 2004). Varied results in the literature may indicate the extent of N_2 fixation by *Acacia* species to be contingent on specific ecological controls (such as degree of grass competition) varying in time and space. This study confirms N supply, tree size and grass competition to be among the key factors affecting BNF in *A. mellifera*. Contrastingly, the mean $\delta^{15}N$ value of 5.2‰ in grass-free tree seedlings for this study denotes soil-sourced N. This is supported by the similarity of soil $\delta^{15}N$ to that of tree seedlings without grass competition (Fig 5.3a). Thus, when not faced with grass competition, *A. mellifera* seedlings primarily derive N from the soil rather than through BNF. Estimation of BNF input in natural ecosystems is generally complex, due to heterogeneous soil and foliar $\delta^{15}N$ values often obscuring the distinction between N_2-fixing and non-N_2-fixing plants (Shearer *et al*. 1983; Hansen and Pate 1987; Pate *et al.* 1993; Handley *et al*. 1994; Boddey *et al*. 2000) with overlaps recorded in values (Högberg 1986). Seasonal/annual changes in N assimilation by long-lived perennials (Evans 1989, 2001; Ladha *et al.* 1993; Peoples *et al.* 1996), intraspecific differences in growth, mycorrhizal infection and nodulation may lead to discrepancies in BNF estimates in *Acacia* species. The indication of high N_2 fixation in *A. mellifera* indicated by Schulze *et al*. (1991) was solely based on $\delta^{15}N$ and no indication of the species nodulation capacity was indicated.

There was a significant difference in the mean $\delta^{13}C$ of tree seedlings grown with and without grass competition. More depleted $\delta^{13}C$ isotopic signature in tree seedlings competing with grass indicated that they had their stomata open for longer (Farquhar *et al.*1982; Knight *et al*. 1994). The implication is that competition with grasses resulted in tree seedlings having difficulty in obtaining nutrients and increasing their uptake of moisture to facilitate more nutrient acquisition. This is supported by the fact that with grass competition, the $\delta^{13}C$ of tree seedlings was affected by N addition whereas this was not the case in the absence of grass competition. Similar results are evident from previous work on *Acacia*-grass interactions (Cramer *et al.* 2007, 2010), although these authors did not comment on the possible mechanism. Cramer *et al*. (2009) demonstrated the important role played by nutrient availability in regulating the flow of water in plants, indicating that an important component of nutrients acquisition associated with nutrient mass-flow driven by the flux of water to the root powered by transpiration (mass-flow). Regular watering every three days ensured sufficient water supply to all pots. Thus, competition for water was not likely. Rather, tree seedlings used more water when competing with grass, presumably to increase the mass-flow of nutrients to the roots.

The ability of leguminous woody plants to fix N_2 has been advanced as being among the driving forces behind bush encroachment in savannas (Scholes and Walker 1993; Sprent 1995; Scholes *et al*. 2002, Wiegand *et el*. 2005). Our results show that trees are not able to outcompete grasses solely through a reliance on BNF and that N_2 fixation may thus not confer a direct competitive advantage to woody legumes over grasses. However grass-induced BNF may enable woody leguminous trees to survive grass competition at the critical and vulnerable stage of their establishment and development until they reach sizes where their above- and belowground structures put them on a competitive footing with grasses. Thus while N_2 fixation may not immediately lead to bush encroachment, it may prevent establishing tree seedlings from being competitively excluded from savannas by a competitive grass component. BNF may therefore allow woody leguminous seedlings to persist in savannas despite strong grass competition and take advantage of factors that weaken the suppressive effect of grass such as selective grazing pressures and droughts.

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Table 5.1 One-way ANOVA comparisons of post-harvest N concentration (mean ± S. E) in soils grown with and tree seedlings in competition with grass and without grass competition at different levels of N fertiliser addition.

Fertiliser Level (g/pot)	Grass Competition		F	Error d.f.	Significance P
	With Grass (N%)	No grass (N%)			
0.0	0.025 ± 0.001	0.018 ± 0.001	17.21	10	**0.002**
0.103	0.026 ± 0.001	0.019 ± 0.001	2.45	9	0.152
2.05	0.024± 0.001	0.021± 0.001	3.08	10	0.110
4.10	0.024 ± 0.001	0.020 ± 0.001	4.16	10	0.069
8.20	0.029 ± 0.001	0.022± 0.001	24.95	10	**0.001**

Table 5.2 Correlation analysis of plant performance parameters evaluated against *Acacia mellifera* seedling densities per pot, above- and belowground biomass, "N concentration" (N %) and $\delta^{15}N$ (‰) values in tree seedlings.

Independent Variable	Mean	Dependent Variable	Mean	r	F	P
Seedling Number per pot (density)	5.79 ±0.25	Shoot Biomass	3.51±0.15	-0.21	7.33	0.008
		Root Biomass (g)	1.83±0.07	-0.18	5.37	0.022
		Root: Shoot Ratio	0.53±0.01	0.12	2.56	0.070
		$\delta^{15}N$ (‰)	4.62±0.18	-0.11	1.99	0.160
		N%	2.83±0.83	-0.34	21.66	0.001
Aboveground Biomass (g)	3.51±0.15	Root Biomass (g)	1.83±0.07	0.90	709.64	0.001
		N%	2.83±0.83	0.14	3.43	0.066
Belowground Biomass (g)	1.83±0.07	N%	2.83±0.83	0.01	0.02	0.897
$\delta^{15}N$ (‰)	4.62±0.18	Shoot Biomass (g)	3.51±0.15	0.67	133.11	0.001
		Root Biomass (g)	1.83±0.07	0.62	106.12	0.001
		Root: Shoot Ratio	0.53±0.01	-0.09	1.36	0.245
		N%	2.83±0.83	0.15	3.64	0.058

Figure Captions

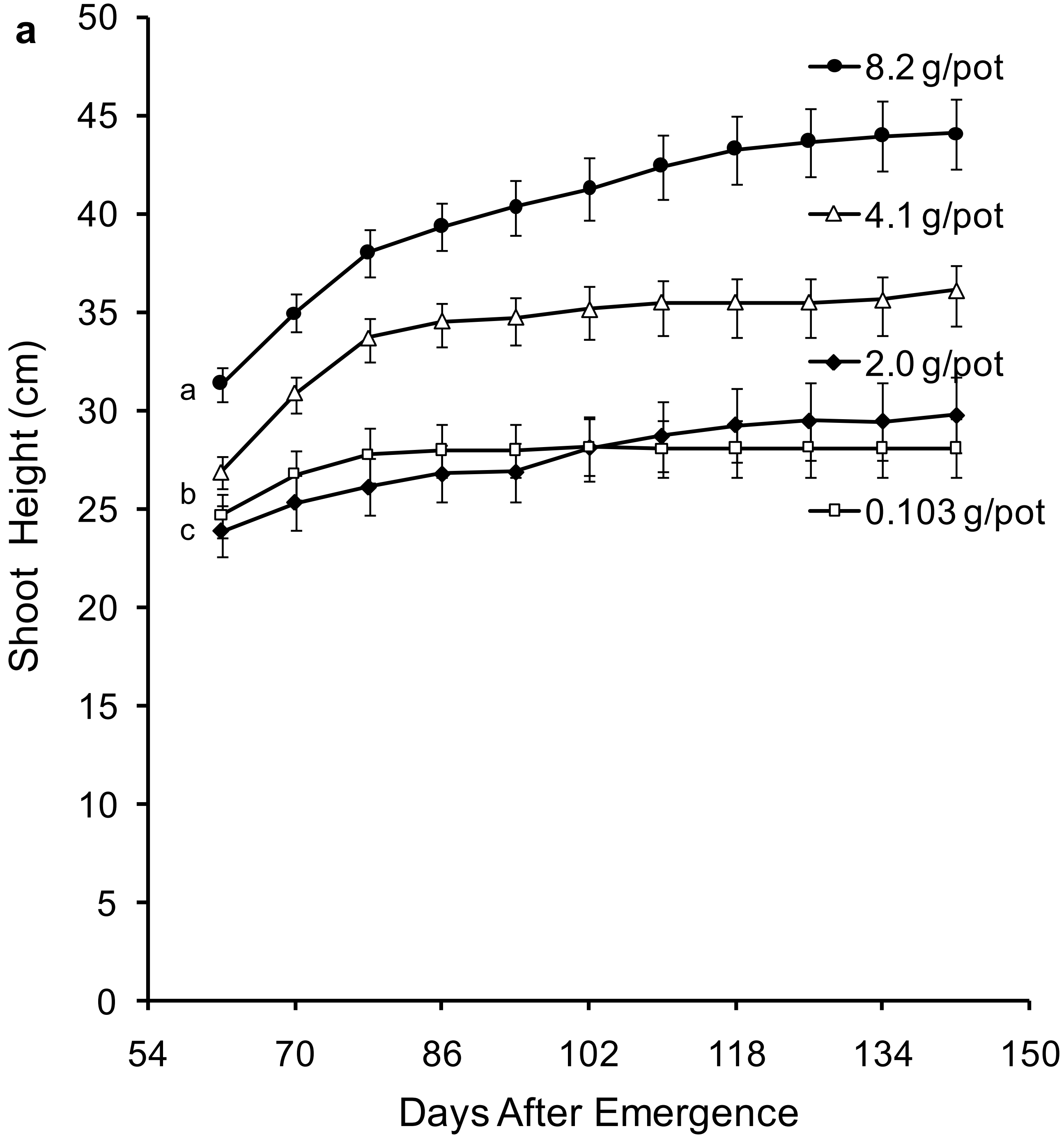
Fig. 5.1 Tree seedling (a) shoot elongation (mean ± SE) in absence of grass competition over time following emergence at different application levels of N fertilizer per pot and (b) final shoot lengths at harvest with and without grass competition. Shoot development over time at zero (control) and 2.1 g/pot were similar and not significantly different from the trend at 0.1 g/pot.

Fig. 5.2 The (a) aboveground (shoot) and belowground (root) biomass accumulation (mean ± SE) with grass competition and without grass and (b) tree seedling root:shoot ratio in competition with grass and without grass competition at different application levels of N fertilizer per pot.

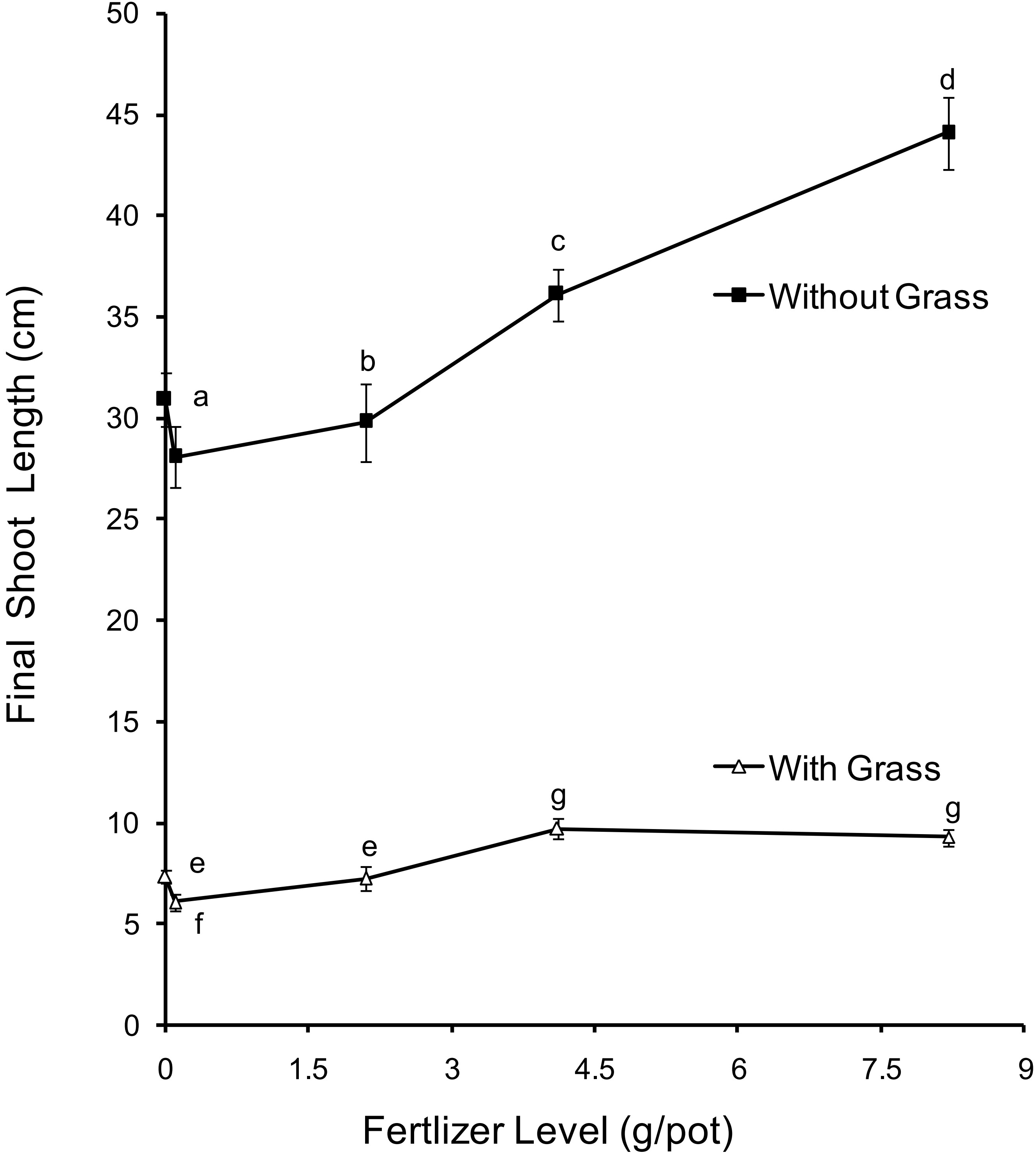
Fig. 5.3 The (a) ^{15}N isotopic composition (mean ± SE) of grass and tree seedlings with/without grass competition, grasses, soils and fertiliser and (b) % contribution of N by fertiliser to tree seedlings without grasses as well as estimated percentage nitrogen derived from the atmosphere (%Ndfa) by tree seedlings competing with grasses at different application levels of N fertilizer per pot and (c) tissue N concentration (%) in tree seedlings and grasses at different N fertilizer application levels.

Fig. 5.4 The $\delta^{13}C$ data (mean ± SE) of grass and tree seedlings without and with grass competition at different application levels of N fertilizer per pot.

Fig. 5.1

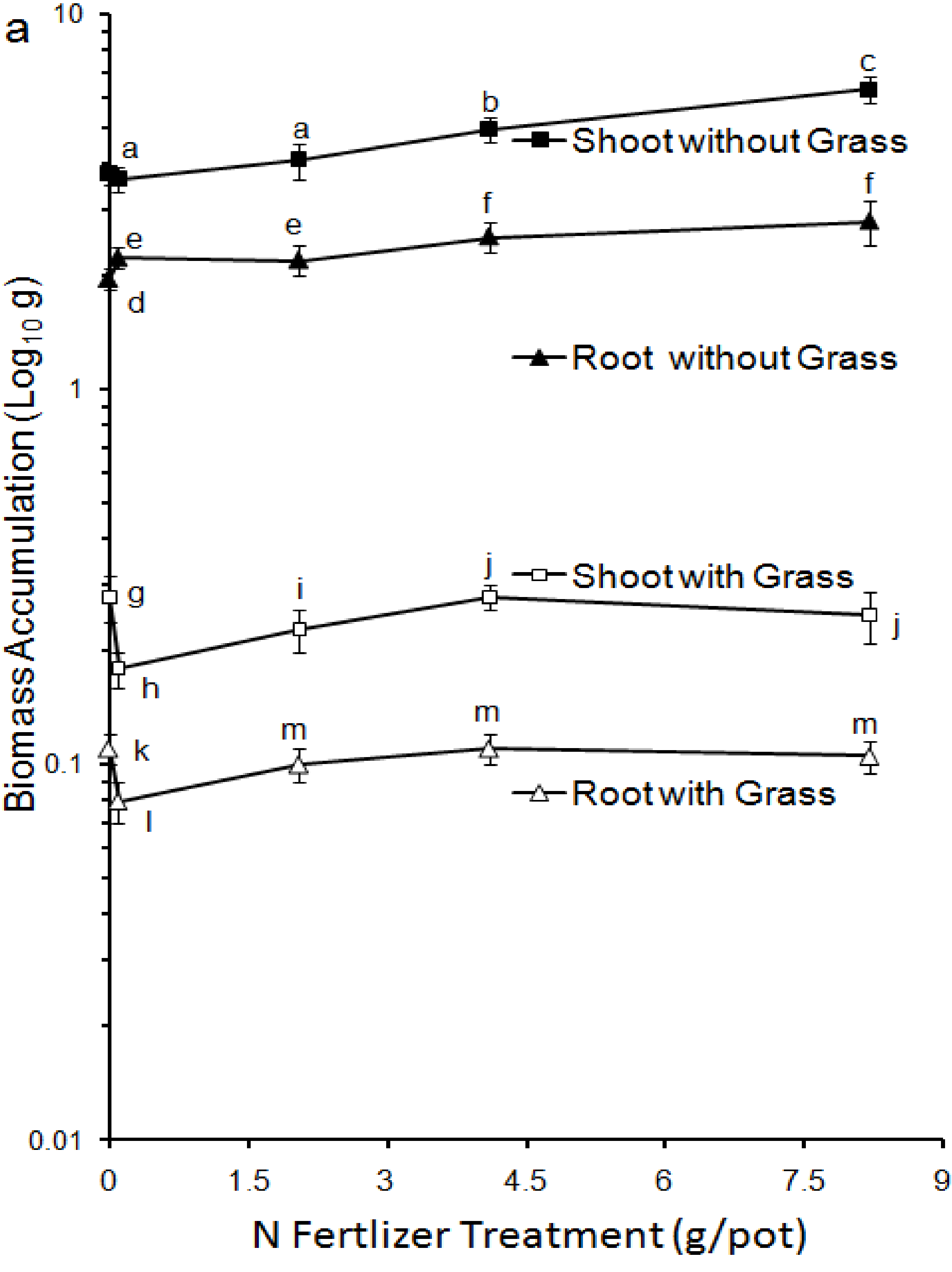

a
Shoot Height (cm)
50
45
40
35
30
25
20
15
10
5
0
a
b
c
8.2 g/pot
4.1 g/pot
2.0 g/pot
0.103 g/pot
54
70
86
102
118
134
150
Days After Emergence

b



Final Shoot Length (cm)
50
45
40
35
30
25
20
15
10
5
0
0
1.5
3
4.5
6
7.5
9
Fertlizer Level (g/pot)
Without Grass
With Grass
a
b
c
d
e
f
e
g
g

Fig. 5.2


a
10
1
0.1
0.01
Biomass Accumulation (Log₁₀ g)
0
1.5
3
4.5
6
7.5
9
N Fertlizer Treatment (g/pot)
Shoot without Grass
Root without Grass
Shoot with Grass
Root with Grass
a
a
b
c
e
d
e
f
f
g
h
i
j
j
k
l
m
m
m

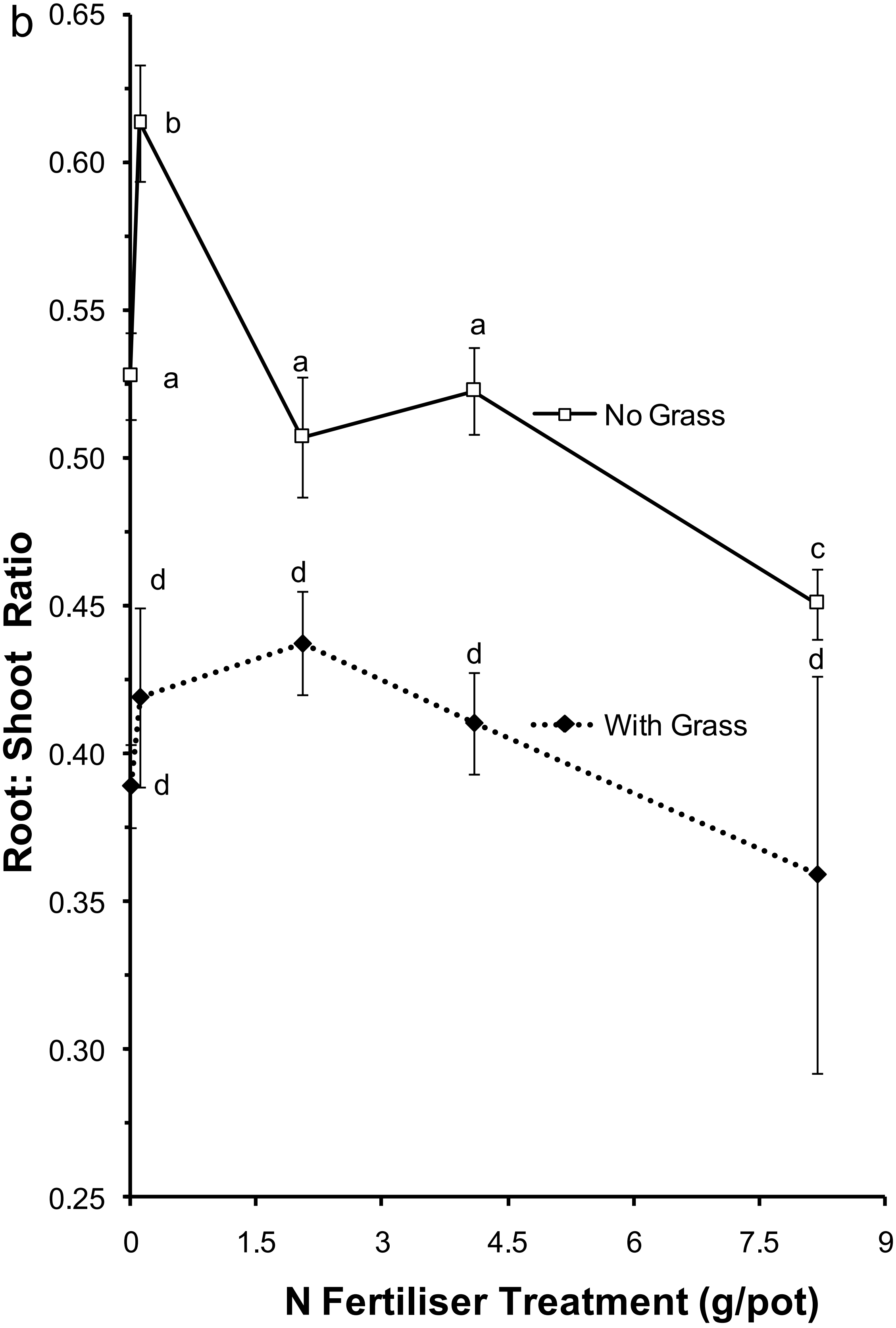
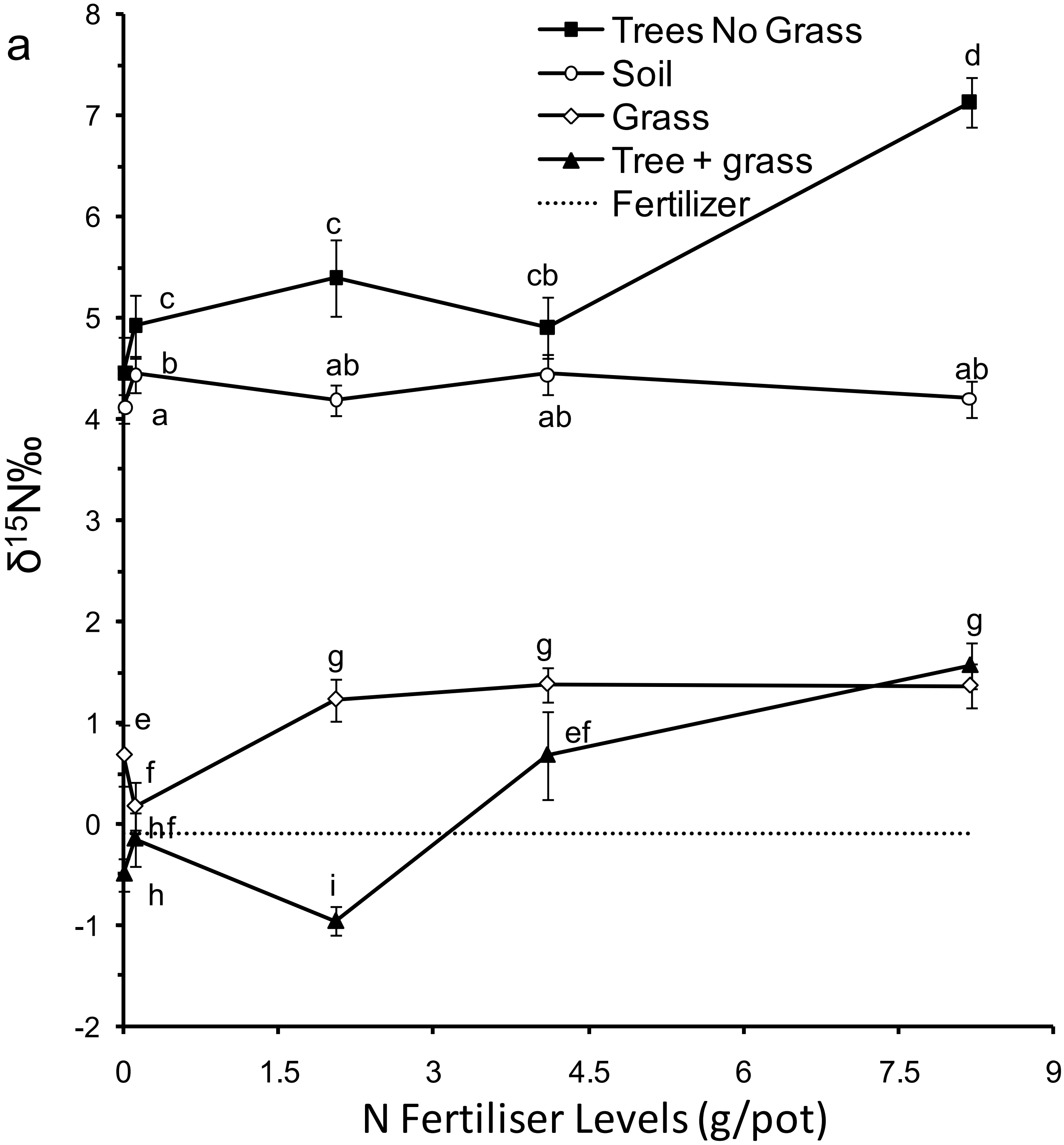
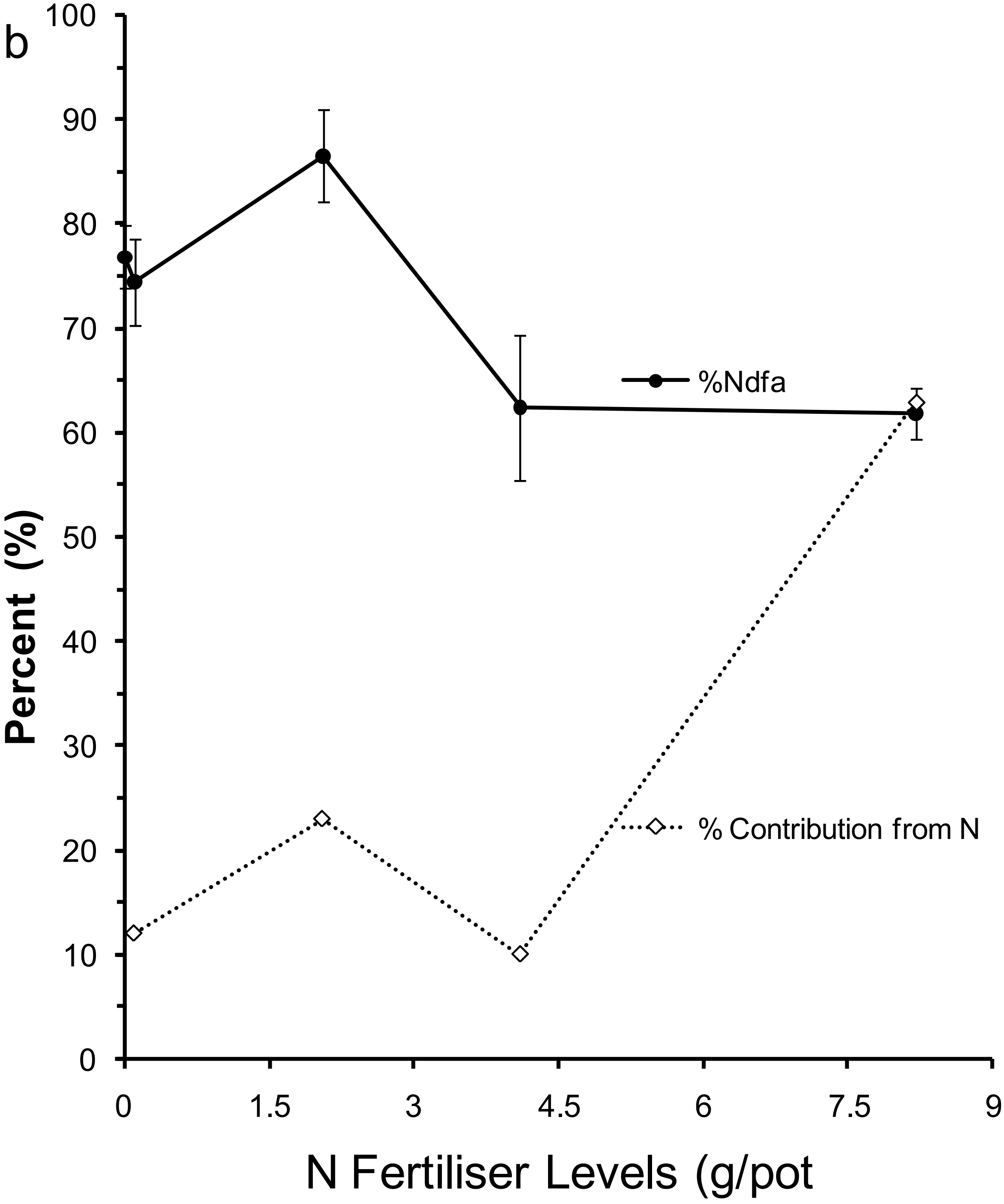

b
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
Root: Shoot Ratio
0
1.5
3
4.5
6
7.5
9
N Fertiliser Treatment (g/pot)
No Grass
With Grass
a
b
a
a
c
d
d
d
d
d

Fig. 5.3



a
Trees No Grass
Soil
Grass
Tree + grass
Fertilizer
δ15N‰
N Fertiliser Levels (g/pot)
8
7
6
5
4
3
2
1
0
-1
-2
0
1.5
3
4.5
6
7.5
9
d
c
c
cb
b
ab
ab
a
ab
g
g
g
e
ef
f
hf
h
i


b
100
90
80
70
60
50
40
30
20
10
0
Percent (%)
0
1.5
3
4.5
6
7.5
9
N Fertiliser Levels (g/pot
%Ndfa
% Contribution from N

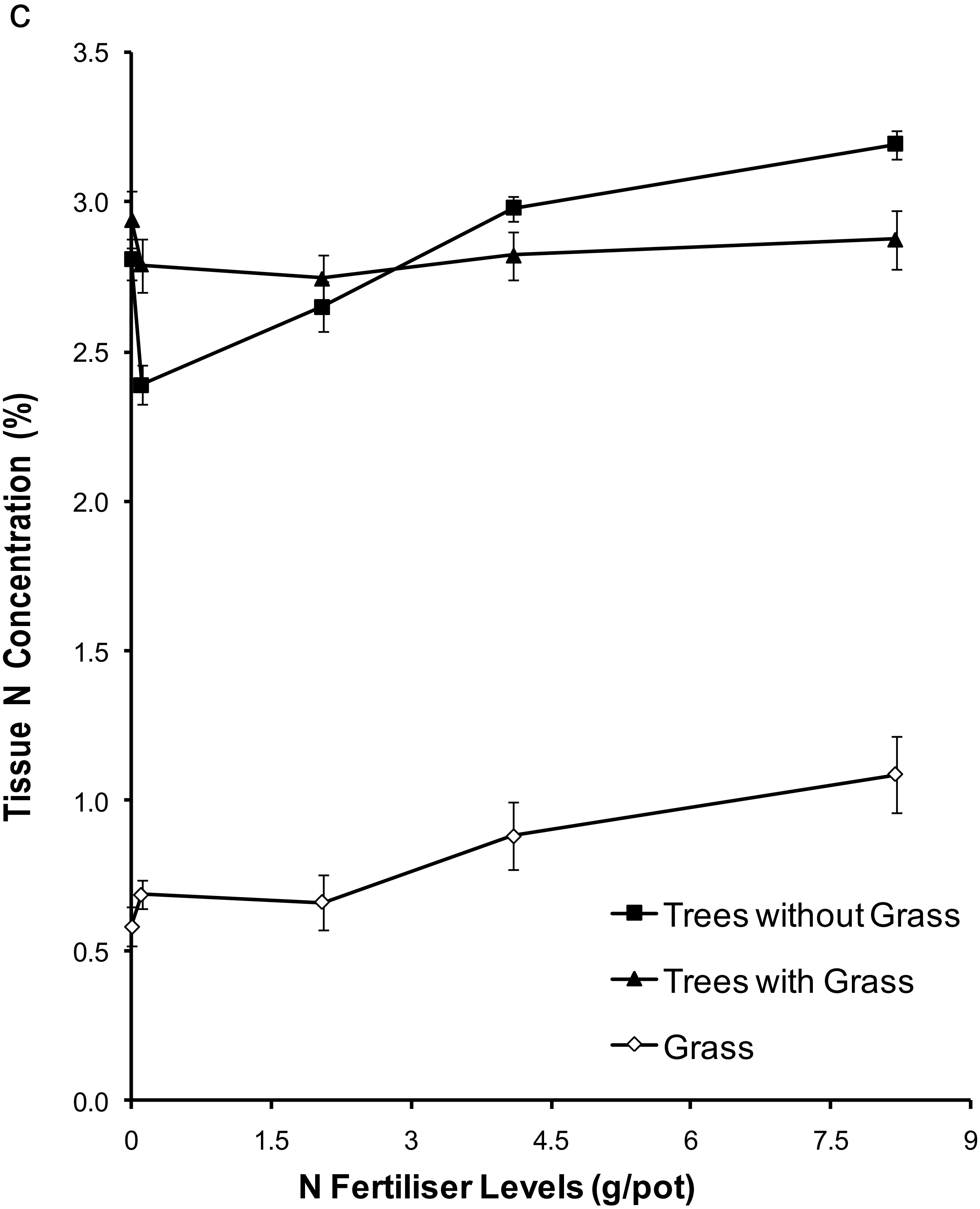
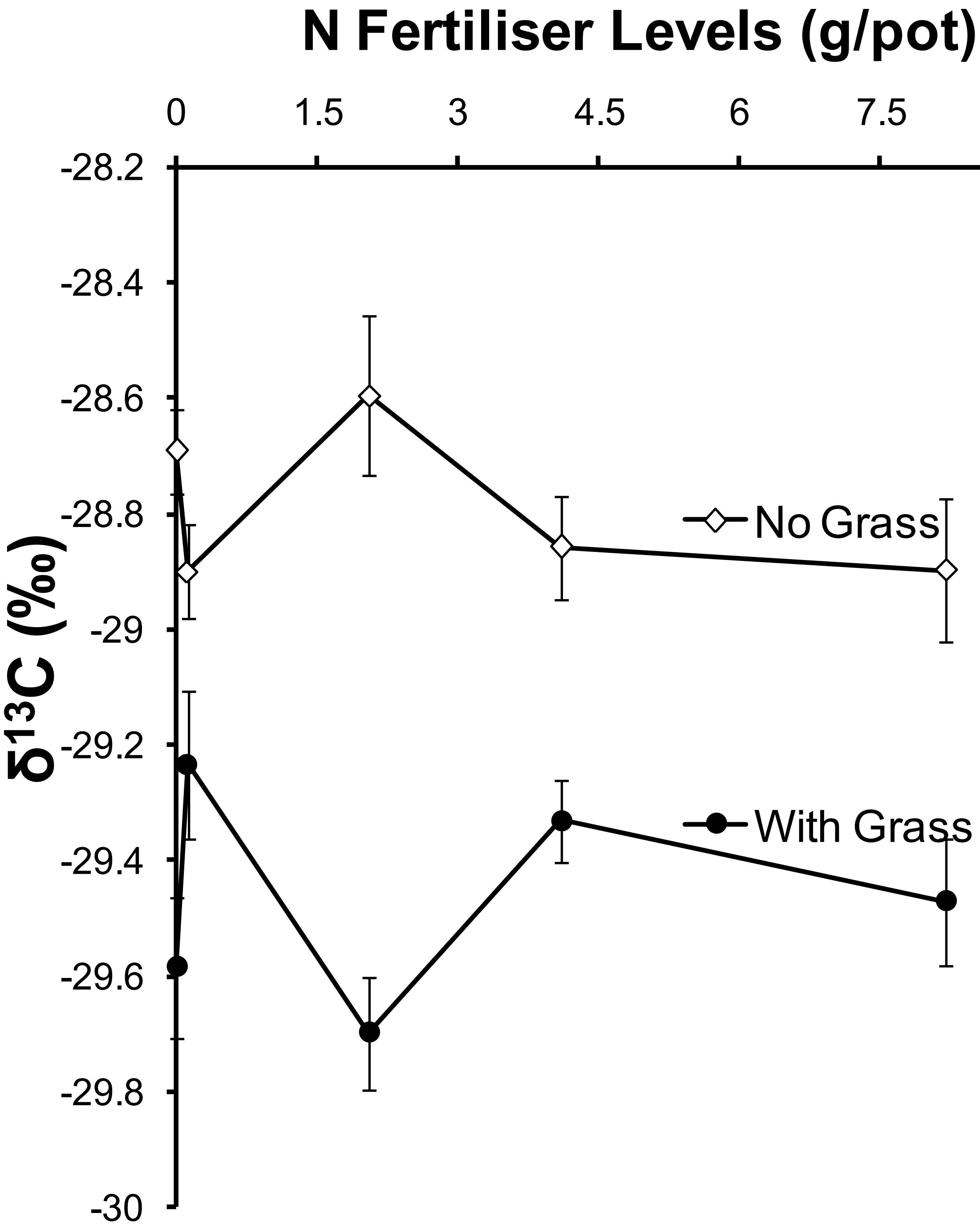

C
Tissue N Concentration (%)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1.5
3
4.5
6
7.5
9
N Fertiliser Levels (g/pot)
Trees without Grass
Trees with Grass
Grass

Fig. 5.4


N Fertiliser Levels (g/pot)
0
1.5
3
4.5
6
7.5
9
-28.2
-28.4
-28.6
-28.8
-29
-29.2
-29.4
-29.6
-29.8
-30
δ13C (‰)
No Grass
With Grass

6. Chapter 6: Constraints of resource availability and substrate type direct inter- and intraspecific competition in savannas: a synthesis

6.1. Relevance of the sudy to the understanding of arid savanna environments

Ecological models accounting for the continual tree-grass coexistence in savannas can be grouped into two broad categories in which competitive plant interactions or environmental perturbations (fire, droughts, herbivory) regulate and inhibit tree germination, survival and maturation (Sankaran *et el*. 2004, Ward 2005). These models are seen to address the *functioning* and the *disturbances* of the system respectively (Graz 2008). These paradigms may not necessarily be mutually exclusive because the impact of disturbances could be superimposed on competition-mediated pre-disturbance vulnerability of plants to disruptions. Within the two main groups of *competition* and *disturbance* models, four subdivisions of ecological models on savanna dynamics pertaining to grass-tree coexistence or interactions can be discerned (House *et al.* 2003). These rationalizations of how trees and grasses coexist encompass:

i. *niche separation,* whereby the woody and herbaceous components of savannas inhabit and exploit discrete spatial and temporal niches for resources (Walter 1971, Harrington *et al.* 1984, Sala *et al*. 1997).

ii. *balanced competition* between trees and grasses where intraspecific competition between either life form is stronger than interspecific competition leading to self-thinning at a certain density by the superior competitor (grasses), thus opening up niches for the inferior competitor (trees) to exploit (Wiegand *et al*. 2006).

iii. *competitive exclusion* where the competitively robust life form monopolises access to resources to the exclusion of the weaker competitor which is then eliminated from the system. Complete dominance/elimination of either competitor is prevented by regular disturbances primarily acting on the competitively superior component (House *et el.* 2003).

iv. *multiple stable states* which allow for the existence of opposing tree-to-grass ratios at any site at different times. Alterations in the availability of resources transform tree-grass interactions and lead to a shift in the ratio of woody and herbaceous plants from one stable state to another (Walker & Noy-Meir 1982, Archer 1989, Skarpe 2000).

Sankaran *et al*. (2005) noted that there is an increase in tree density with increasing mean annual precipitation up to approximately 650 mm of annual rainfall. Thereafter, there is no direct relationship between tree density and rainfall. Thus, it appears that there is a fundamental difference between arid/semi-arid savannas (<650 mm annual rainfall) and mesic/humid savannas. In mesic savannas, Bond & Midgely (2000) contend that the spread of grasses in formerly forested biomes long before anthropogenic influences has been facilitated by fires and it is fires that continue to prevent the woody component of savannas from growing to their climatically-determined maximal potential cover or density. Fire, among other factors plays a key role in maintaining the relative ratio of trees and grasses in savannas (Higgins *et al*. 2000).

In contrast, in arid or semi-arid systems (here, I use the term “arid” for the sake of brevity) different factors are important, the overriding factor being rainfall. In general, fires are considered less important in arid savanna systems because fuel loads are seen as being insufficient for frequent and intense fires (Meyer *et al*. 2005, 2007). Hereafter, I focus on this fundamental difference and emphasize the importance of my research to the understanding of arid savanna function. The rate of change in arid ecosystems is characteristically very slow, requiring long periods for conversion to another state to be completed (Sankaran 2005). The relative proportions of trees to grass in savanna would therefore be expected to be relatively stable and maintained for long periods of time. In fact the existing models which denote savannas to be an outcome of a suite of interacting ecological controls producing a stable balanced system that persist despite environmental variations (Walker *et al*. 1981, Walker & Noy-Meir 1982, Belsky 1990, Menaut *et al*. 1990) or an unstable system regularly disturbed and prevented from stabilising by fires, droughts and herbivores (Skarpe 1992, Scholes & Walker 1993, Scholes & Archer 1997, Jeltsch *et al.* 2000, Higgins *et al*. 2000, Wiegand *et al.* 2005) or anthropogenic interventions (Scholes & Archer 1997, Jeltsch *et al*. 1998, Jeltsch *et al.* 2000, van Langevelde *et al.* 2003) do not predict rapid changes. As systems that depend on and are driven by the demographics of slow-growing trees, it may take a number of centuries for any real pattern to emerge (Menaut *et al*. 1990). However, one of the most rapid alterations of the physiognomy of arid savannas manifests itself in a process known as bush encroachment. Bush encroachment encompasses an increased density of woody plants in previously sparsely wooded savannas accompanied by a marked decline in the grass component, which may occur within a few decades (Archer 1996, Scholes & Archer 1997). For example, the shrub/tree component is reported to have grown three times its original total biomass within 25 years in eastern Botswana (van Vegten 1983). Rapid escalation in dominance by woody plants at the expense of the herbaceous cover is indicative of the responsiveness of savanna tree-grass ratios to alteration of environmental disturbances (Scholes & Archer 1997, Sankaran *et al*. 2005, 2008).

Populations of encroaching woody species do not exhibit gradual growth in numbers of individual trees, but rather show mass recruitments in certain favourable seasons (Story 1952, Donaldson 1969). Kraaij and Ward (2006) demonstrated that above-average precipitation sustained over a number of consecutive seasons was a prerequisite for successful recruitment of encroaching *A. mellifera*. Patch dynamic models (Wiegand *et el.* 2006) predict simultaneously recruited cohorts of individual trees to exert competitive effects on each other such that the stronger competitors eliminate the weaker ones which reopens encroached patches to grass cover. My results show that there is indeed a strong suppressive effect by grasses on establishing tree seedlings (Chapters 3-5), which necessitates a reliance on N_2 fixation by tree seedlings. This further suggest the probability of niche separation on sandy substrates and its potential absence on rocky or shallow-layered savanna soil makes substrate type an important determinant of the outcome of tree-grass interactions and how they are affected by grazing pressures. Finally I propose that the small sizes attainable by shrubs on rocky areas precludes intense intraspecific competition that would otherwise cause density-dependent mortality and re-open encroached patches for grass invasion. Specifically, my findings show that it is possible to amalgamate Walter's two-layer model with Wiegand *et al*. (2006) patch dynamics model, and that differences between these models and those of Jeltsch *et al*. (2000) and Higgins *et al*. (2000) that emphasize the roles of disturbance are minor. The results indicated that fundamentally, the two-layer model of Walter (1939) is correct except for a few details such as the overlap in water use at intermediate depths (Chapter 4). This same point has been made by Knoop and Walker (1985). However, there is a need for patch dynamic models (with rainfall as a driving force) of Wiegand *et al.* (2006) to scale up from the small (patch) scale to the large (landscape) scale, where Walter (1939) indicates that an equilibrium can occur (Walter (1939) was not specific on the spatial scale of the equilibrium). Importantly, my study stresses the role of water (Chapter 4) and nutrients (especially nitrogen, Chapter 5) as the key mechanisms behind these models. Clearly, Jeltsch *et al*. (1998) has indicated the importance of seedling 'safe sites' as important, and the facilitation of tree/shrub recruitment by nurse plants (Archer *et al.* 1988, Vetaas 1992) support the assertions of 'safe sites' as important factors in controlling recruitment of trees to savannas. However, these were not part of this thesis. Higgins *et al*. (2000) is also partly correct in his statement that disturbances control savanna structure, although he emphasized that fire was the most important factor. Fuel loads in arid savannas are however usually insufficient to justify this as a major factor (see also Ward 2005). Herein I did not explicitly consider disturbances (other than herbivory) as a factor controlling recruitment but focused on the understanding of post-recruitment processes.

Enhanced growth, fecundity, survival and fitness of plants in dry inland ecosystems due to neighbourhood associations are widely reported (Maestre *et al.* 2001, Callaway *et al.* 2002, Brooker *et al.* 2007). Where drought stress during the summer constraints seedling recruitment, as is the case in semi-arid grassland environments (Harrington 1991), buffering against excess radiation and heat by shrubs (Valiente-Banuet & Ezcurra 1991, Gómez-Aparicio *et al.* 2008) increases moisture and nutrient availability to the benefit of seedling survival and initial growth (Callaway 1995, Gómez-Aaparicio *et al.* 2004, Pugnaire *et al.* 2004). The outcome of shrubs acting as nurse plants for establishing seedlings and saplings of other shrubs/ tree species (Gómez-Aaparicio *et al.* 2004, 2008) is often an aggregated spatial configuration of vegetation (Callaway & Pugnaire 1999, Maestre 2002) with extensive overlap of individual plant canopies (Lamont *et al.* 1984) as in bush encroached areas. Facilitative effects are often more pronounced with intensity of abiotic stress (Callaway & Walker 1997, Holmgren *et al.* 2000, Callaway *et al.* 2002) and may be stronger in dry hot seasons in contrast to seasons or years with relatively wet and mild climatic conditions (Greenlee & Callaway 1996, Ibañez & Schupp 2001). However, facilitative linkages in space are rather commonly found between seedlings of one species finding refuge beneath adults of a different species, the 'nurse plant syndrome' (Niering *et al.* 1963, Turner *et al.* 1966, 1969, Steenberg & Lowe 1969, 1977) and rarely apply to conspecific interactions among legumes of the savanna. In savanna ecosystems nurse plant facilitated recruitment of trees and shrubs have been linked to the conversion of grasslands to woodlands (Archer *et al.* 1988, Vetaas 1992), thus, bush encroachment.

Gómez-Aparicio *et al.* (2008) found the facilitative effect of shrubs on saplings to be species-specific, benefiting *Acer opalus* and *Quercus ilex* while having little effect on the *Pinus* species. In the sandy areas of Pniel, *Acacia erioloba* appeared to be the main benefactor nurse plant supporting the greatest number and diversity of species such as *Ziziphus mucronata, Grewia bicolour, Grewia flava, Grewia flavescens* and even *Acacia mellifera* (pers. obs.). Schleicher *et al.* (2011) indicated that substrate type may be important in determing interspecific facilitative association between *Tarchonanthus camphoratus* by *A. mellifera* shrubs. In rocky terrains, *T. camphoratus* saplings were positively associated with *A. mellifera* wheras dispersal via seeds from conspecific adults was the apparent process related to saplings distribution on sandy areas (Schleicher *et al.* 2011). The absence of conspecific positive associations may possibly be ascribed to canopy shading and shade intolerance of especially leguminous seedlings as well as direct competition for nutrients and water by adult plants (Lloret *et al.* 2005). This is in keeping with the seed-seedling conflict proposal that holds that the ideal environment for a seedling to grow may not be the best locality for a sapling to mature (Schupp 1995), but beneficial effects of nurse shrubs were also reported for shade-intolerant species (Castro *et al.* 2002). Nonetheless positive associations among near neighbour adult shrubs during periods of high abiotic stress analogous to the effect on seedlings and saplings can be expected. Recent studies emphasise concurrence of positive and negative interactions between neighbouring vegetation alternating in intensity and outcome throughout the plants' life histories (Tielbörger & Kadmon 2000, Brooker *et al.*2007, Nuñez *et al.* 2009).

In my field measurements of plant water stress and canopy dieback at Pniel I reported an apparent shift from direct competition to beneficial associations of near neighbourhood among *Acacia mellifera* shrubs (Chapter 2). A change from positive to negative outcomes between close neighbours in arid environments is mainly regulated by moisture availability (Fowler 1986) such that the same locality can show contrasting neighbourhood effects in different seasons subject to patterns of precipitation (Callaway & Pugnaire 1999, Tielbörger & Kadmon 2000, Pugnaire & Luque 2001). This is in line with the seasonally variable effects of neighbourhood associations I found in the field at Pniel (Chapter 2). What is perhaps more relevant to my field experiment and findings, and by extension *Acacia mellifera* and other arid savanna *Acacias*, is the conclusions by Gómez-Aparicio *et al.* (2008) that protection presented by nurse plants to saplings against abiotic stress (summer drought and winter frost) was of greater significance than protection against herbivory damage. The results I presented in Chapter 2 indicated that during periods of heighted summer drought and extreme temperatures, individuals without neighbours were more likely to suffer whole plant mortality than shrubs growing among neighbours. This is in contrast to the findings of Moustakas *et al.* (2008) who found dead *Acacia erioloba* trees nearer to neighbours than living trees (but see section 6.3). Moreover, my findings that *A. mellifera* shrubs without neighbours, which were invariably small-sized, experienced more water stress during hot dry summers compared to shrubs with neighbours is supported by results of Nuñez *et al.* (2009). These authors found variations in water stress levels of solitary *Austrocedrus chilensis* trees compared to trees under shrub canopies. Smaller trees were more stressed when growing in the open and the opposite was found for medium sized trees while larger trees showed no differences, possibly owing to differences in rooting depths (Nuñes *et al.* 2009). All my field experimental plants were of comparable sizes and did not grow beneath the canopies of shrubs, but rather in close proximity to other shrubs (Chatper 2).

Reported suppressive competitive effects of grasses on woody plants at different life history stages (Weltzin & McPherson 1997, Jurena & Archer 2003, Cramer *et al.* 2007, Riginos 2009, Ward & Esler 2010) may present a critical recruitment bottleneck for trees in savannas (Higgins *et al.* 2000, Bond 2008). The sparse distribution of woody plants in arid savannas is thought to result from obstacles to tree seedling germination, recruitment, establishment and maturation (Higgins *et al.* 2000, Ward 2005, Wiegand *et al*. 2006). Demographic barriers imposed by frequent fires, insufficient magnitudes and frequencies of precipitation as well as grass competition on tree establishment may prevent savanna woody plants from growing at high densities (Higgins *et al.* 2000, Wiegand *et al*. 2006). However, the frequencies and intensities of droughts, rainfall and fires are highly variable in time and space as to create special temporal openings for the successful recruitment of tree seedlings or the maturation of saplings into adults (Higgins *et al*. 2000). Such rare opportunities for woody plant recruitment in arid savannas may be presented by the absence of intense fires coinciding with the occurrence of above-average rainfall of long duration (Kraaij & Ward 2006). Such synchrony of adequate soil moisture conditions is extremely rare in occurrence so as to give rise to a high rate of variance in tree seedling recruitment rates (Harrington 1991).

In arid savannas, seed germination and seedling establishment stages are considered the prime recruitment obstacles for woody savanna vegetation (Jeltsch *et al.* 1998, Higgins *et al.* 2000, Wiegand *et al*. 2005, 2006, Moustakas *et al*. 2006, Meyer *et al*. 2007) controlled by low rainfall and frequent droughts (Harrington 1991, Midgley & Bond 2001). Once savanna woody plants are established, their mortality rates diminish significantly while the lifespan of an adult tree is very long, spanning several decades. This makes early seedling survival and recruitment the most critical stage in the life history of woody plants (Grubb 1977, Harper 1977, McPherson 1997, Scholes & Archer 1997). The ability of seedlings to compete with grasses following successful germination is therefore of critical value to the persistence of woody plants in savannas which are, by definition, grass-dominated systems. A capacity for fixation of N_2 at particularly seedling stage among leguminous species may contribute to their relative abundance in southern African savannas (Cramer *et al*. 2007, 2010). The ability to fix N_2 in leguminous woody plants is thought to increase the probability of bush encroachment in savannas (Scholes &Walker 1993, Scholes *et al*. 2002, Wiegand *et el.* 2005).

By and large, the seeds of many savanna trees are subjected to high incidences of predation, rapid decay or loss of viability (Tybirk *et al*. 1993) and fungal attacks such that savanna woody plants do not build up viable long-term seed banks in the soil (Miller & Coe 1993). Unless the seeds germinate in a relatively short period of time, they may possibly never add to the recruitment of trees. This may be particularly true for *A. mellifera* (Joubert 2008). To this end, the storage of reproductive potential in adult trees of different generations is of critical importance for the long-term persistence of trees in savannas (Higgins *et al.* 2000). It may be in this respect that biological nitrogen fixation (BNF) assumes greater value in the persistence of woody leguminous species in savanna ecosystems. The fixation of atmospheric N_2 induced by grass competition (Chapter 5) may help establishing woody seedlings that germinate within a grass sward to survive to later life history stages and persist in savannas (Cramer *et al*. 2007) rather than confer a competitive advantage to the trees over the grasses. In spite of induced BNF, the results obtained in this work showed that the tree seedlings competing with grasses remained stunted relative to seedlings without grass competition at all N addition levels, indicating that grasses continued to suppress tree seedling growth and biomass in spite of BNF or N addition (Chapter 5). This may partly be ascribed to the fact that N limitation is but one nutrient among many limiting savanna ecosystems and that particularly P may become the most limiting nutrient once N limitation is overcome (Cramer *et al*. 2010).

6.2. Walter's two-layer model

An interaction between the behaviour of a species with environmental variability is necessary to promote species coexistence (Turelli & Gillespie 1980, Chesson & Huntly 1988). Walter (1971) invoked niche differentiation through layered rooting zones in which the shallow grass roots are separated from the deeply penetrating roots of woody plants, thus exploiting segregated soil depths for resources, principally moisture, to explain the coexistence of trees and grasses in savannas. With a complete grass cover intercepting and trapping most of the infiltrating water from small rainfall amounts of arid environments, insufficient moisture percolates to the deep rooting zones of woody vegetation to sustain their establishment and persistence in savannas. This would lead to and maintain sparse savanna tree densities and expansive grass cover. An ecological model by Walker and Noy-Meir (1982) supported the possibility of stable grass-tree coexistence through differentiated rooting niches.

Evidence for vertically divided root distribution and soil moisture uptake have been presented (Knoop & Walker 1985, Weltzin & McPherson 1997, this study), but a number of studies (Johns 1984, Richards & Caldwell 1987, Belsky 1990, 1994, Seghieri 1995, Mordelet *et al*. 1997, Scholes & Archer 1997) have produced results that do not explicitly support rooting depth differentiation as an universal explanation for tree-grass coexistence. Studies have rather shown overlapping rooting zones in shallow soil layers by trees and grasses (Scholes & Walker 1993, Belsky 1994, Wiegand *et al*. 2005) or an ability among trees and shrubs to switch from a dependence on deeper moisture sources to recent rainwater in the top layers during the wet season (Nippert & Knapp 2007, Sher *et al*. 2010). Nonetheless the two-layer hypothesis has had the biggest influence on savanna ecological research (Graz 2008), although it has probably been too successful for its own good (i.e. researchers have assumed that it is correct and have looked no further). It is only recently that non-equilibrial models such as those of

Higgins et al. (2000), Jeltsch et al. (2000) and Wiegand et al. (2006) have been invoked because of the imperfections of Walter's (1939) model.

Grasses are known to grow roots as deep as 1.5 m or deeper while the dimorphic nature of tree rooting patterns (Seghieri 1995) means that woody plants have rooting depth in relatively shallow as well as very deep horizons of the soil profile. Inevitably tree and grass roots are bound to overlap at some depth in the soil profile (Nippert & Knapp 2007, Sher *et al*. 2010). While finding evidence of different water use patterns between trees and grasses in the top and subsoils, Knoop and Walker (1985) reported a wide range of overlap between tree and grass roots of southern African savannas. However these results have either been cited as contradictory (Seghieri 1995, Mordelet et al. 1997) or supporting evidence for Walter's (1939) two layer hypothesis of depth niche separation (Sala et al. 1989). Instead of being entirely segregated or overlapping, the distribution of grass and tree roots in the soil encompasses both aspects of rooting depth niche differentiation and overlap, with grasses dominating the shallower upper soil layers and trees dominating grater depth zones with intermediate layers being shared by overlapping roots (Chapter 4). A three-tiered layering of tree and grass roots is more in keeping with field results of root distribution (e.g. Knoop & Walker 1985, Mordelet *et al.* 1997, Weltzin & McPherson 1997, Hipondoka *et al.* 2003, this study) as well as isotope studies of moisture uptake by plants (Dawson & Ehleringer 1998, Snyder & Williams 2003, this study). The selective repeated removal of grass by grazers may then benefit trees by weakening grass competition for resources (Walker & Noy-Meir 1982, Stuart-Hill & Tainton 1989, Jeltsch *et al*. 1997, this study). Rather than freeing up resources from the shallow surface layer that would otherwise be captured by roots of the grass layer, selective grazing of grass may benefit trees by primarily reducing direct competition from a shared rooting zone (i.e. consistent with Walter's (1939) hypothesis).

Niche separation is less likely on rocky substrates or shallow layered soil types while it is more probable on deep sandy terrains (Chapter 4, Wiegand *et al*. 2005). On rocky substrates or shallow soil profiles, grass and tree roots are likely to share most of the available soil horizon due either physical restriction or unavailability of space, precluding possible niche differentiation. The removal of grasses through selective grazing pressure is therefore likely to lead to the exclusive monopoly of the available soil layer by co-occurring trees and lead to more rapid proliferation of woody plants on rocky or shallow soils (Britz & Ward 2006, Ward & Esler 2010). This, in addition to limited soil volume between rocks with an expectedly low water holding capacity and a small nutrient pool would necessitate extensive investment in rooting mass by trees (Nobel et al. 1992). A sparse grass cover on rocky substrates will not only translate into reduced interspecific competition for the trees (Higgins et al. 2000, Bond 2008, Nano & Clarke 2010, Ward & Esler 2010). Combined with an extensive belowground investment by trees, reduced or absent browsing (Staver *et al*. 2009), less frequent, low intensity fires are less likely to eliminate the woody plants on rocky substrates, leading to woody plant encroachment (Higgins *et al*. 2000). When the grass cover is removed by grazing, the benefit to trees may be increased.

6.3. Tree-on-tree competition

Growth by individual trees is controlled by composite interconnected factors among which competition and microsite heterogeneity may operate spatially and lead to interdependence among neighbouring trees (Reed & Burkhart 1985, Matern 1986, Schoonderwoerd & Mohren 1987, Liu & Burkhart 1994, Magnussen 1994). By regulating the availability of and access to resources of neighbouring plants (Keddy 1989), competitive interactions can result in growth and sizes being negatively affected by neighbourhood proximity. The most vital local environmental factors shaping the dynamics of species in non-equilibrium arid environments are related to aspects of soil nutrients and moisture (Silvertown & Lovett Doust 1993, Tainton *et al.* 1996). At small spatial scales, the availability of a niche for germination and establishment as well as plant-to-plant competition determine tree densities (Jeltsch *et al.* 1998). Plant communities are largely structured by interspecific competition for resources such as soil moisture, mineral nutrients and light (Schoener 1983, Tilman 1990, Wilson & Tilman 1991) as well as herbivory.

In this study, the negative ecophysiological effects of near-neighbour competition such as partial canopy dieback may have helped maintain the shrubs facing intraspecific competition within the threshold of sizes that can be supported by the local resource pools and prevented the death of individuals (Chapter 2). Van Vegten (1981) hypothesized about the presence of a unique genotype as an explanation for invariably undersized shrubs far below the species maximum height in encroached areas. Skarpe (1990) however proposed competition for water in space and time among closely aggregated shrubs as an alternative explanation. Similar to the relative competitive effects of neighbours upon the growth in height of Douglas fir trees (*Pseudotsuga menziesii*) as a function of distance (Wagner & Radosevich 1998), the distance of neighbours would negatively affect the size of shrubs. Rather than the competitive elimination of weaker neighbours by stronger competitors predicted by the Wiegand *et al.* (2006) patch dynamic models, near neighbour competition among small individuals in resource-poor environments may help maintain a dense aggregation of woody plants due to low availability of nutrients and moisture.

Competitive exclusion of weaker competitors proposed in the Wiegand *et al.* (2006) patch-dynamic models are more likely on deeper sandy substrates where trees are able to attain large resource-demanding sizes. Phillips & MacMahon (1981) as well as Skarpe (1991) showed that tree densities change from dense aggregation to random and from random to regular distribution as plant sizes increased. Ward and Esler (2010) showed that saplings of *A. mellifera* were larger on sandy than on rocky substrates. Deep sandy soils of arid environments such as in the Kalahari sands may be characterized by evenly spaced *A. erioloba* parkland type of vegetation (Skarpe 1991, Jeltsch et al. 1996, 1999). Trees reach large sizes in terms of both height and canopy cover. Large tree sizes with resource needs that cannot be met from the available nutrient and moisture pools cannot be sustained and are therefore not feasible in rocky areas with shallow soil profiles. The size of the belowground resource reservoir available to an individual tree is in turn a function of the extent, both vertical and horizontal, of its rooting structures. This is in itself contingent on the availability of physical space (no physical barriers presented by rock boulders or impenetrable soil horizons), mineral nutrients (water, nitrogen, phosphorus and microelements) and an exploitable niche (absence of a comparatively superior competitor in the surroundings). To this end, there may be a finite size attainable by any individual tree within a given landscape and a set of prevailing physical, nutrient and biotic conditions (Chapter 2). The implication is that trees may be similar sizes not necessarily because they are similar in age (cohorts), but rather because they have reached the maximum size attainable within a given environment. Conditions that favour seed germination may not necessarily favour the growth of a seedling into a sapling or the sapling into an adult tree. This would mean that germinated seedlings may be able to catch up with saplings that are unable to escape into mature trees due to some environmental constraint, in the manner that frequent fires create ‘gullivers’ as indicated by Bond & van Wilgen (1996). A rare event that favours the growth of seedlings into saplings or saplings into adults would then release all seedlings/saplings in concert into saplings/adults in spite of differences in their ages.

Britz and Ward (2007) found sandy soils to be relatively resistant to bush encroachment. More often than not, species that occur as shrubs in encroached shallow-layered soils or rocky areas grow into tall trees when occurring on deep sand (see also Ward & Esler 2010). In Pniel Estates this is particularly true for *Acacia tortilis* and *A. mellifera*. Noticeably different sizes observable for the same species of trees growing on different substrates such as rocky outcrops and sandy plains within a short distance from each other (pers. obs.) and within the same climatic region may attest to the limitation imposed by substrate on final tree size classes. Exceptional events such as above-average rainfall or prolonged droughts may push individuals above or below the average size threshold, but the 'normal' size will be restored through shoot or total dieback (Davis et al. 2002) of tree parts that cannot viably be sustained by regular environmental conditions. Alternatively, trees or branches lost due to exceedingly dry conditions may regrow as rainfall patterns return to the long-term mean. This scenario may be applicable to nutrient fluxes and any other pulsed event with direct bearing on tree sizes.

Similar-sized trees which are putatively taken to belong to cohorts may indeed span diverse generations of individuals arrested at a given life history stage by a set of environmental constraints. Pulsed favourable events, principally above-average rainfall events sustained for a longer period than is the norm, may then act to release such differently aged trees into the next life history stage (Seymour 2008). Wiegand *et al.* (2005) highlighted the fact that recruitment events do not necessarily have to result from *en masse* germination or establishment. The recruitment of seedlings in a select set of patches in one recruitment event can be supplemented by an unrelated recruitment event that favours slightly different conditions in an adjacent patch (Wiegand *et al*. 2006).

6.4. *Acacia mellifera* life history traits compared to other savanna species

Acacia mellifera subspecies *detinens* is a widely occurring species in Africa being native to over 15 African countries. It is a very common encroaching shrub on the majority of savanna rangelands adjoining the Kalahari (van Vegten 1981, 1983, Tolsma et al. 1987, Joubert 2008) generally showing a preference for rocky hillsides and shallow soils. *Acacia mellifera* occurs in varied sizes ranging from less than 1 m high shrubs up to 7 m high trees depending on the environment. It is generally multi-stemmed with several branches mostly branching off the base, giving an impression of clustered trees rather than a single individual plant. The species is starkly deciduous and appears dead during the dry winter periods. *A. mellifera* effectively reproduces and disperses via seeds. It flowers in spring between September and October with white honey scented cream yellowish white semi-globoid or elongated blooms. The flowering and fruit sets of the species all occur at the end of the dry season (Forst *et al*. 1986). The transition from flower to pod and the shedding of pods/seeds in *A. mellifera* is remarkably swift. The seeds ripen in the second week of November. This is in contrast to *Acacia tortilis,* found to co-occur with *A. mellifera* in the field study site which produces flowers and pods towards the end of the rainy season. Another contrast between *A. mellifera* and co-occurring *A. tortilis* in the study site was that the latter was depended on rainfall to break dormancy while the phenology of the former appeared less dependent on rainfall (pers. obs.). Seedpod yield show high annual variations and the output per individual tree is highly erratic (de Klerk 2004) and strongly affected by precipitation in the preceding wet season (Joubert et al 2008). Some individuals may skip seedpod production altogether in some years (pers. obs.).

The violent rainstorms that characterise the onset of the rainy season appear to be the main agent of *A. mellifera* seed dispersal as many trees are pruned of ripe pods and seeds get scattered beneath and around the canopy perimeter following a heavy storm (Donaldson 1969, pers. obs.). This is in agreement with the clustering of young *A. mellifera* seedlings or saplings spanning more than two generations around one or a few larger nucleus mother plants with contagious distribution patterns at the onset of encroachment (Donaldson 1969). Animal dispersal of *A. mellifera* seeds is extremely rare (Donaldson

1969) if not absent altogether. The probable explanation for this hinges on the unattractiveness of the yellow-white pods as fodder at a time when every other plant is green.

The seeds of the study species do not remain viable for too long. In the course of this work, seeds collected in 2004 and kept in the laboratory registered a lowly 30% germination rate two years later in 2006. However, seeds collected in 2007 and planted within a week showed a high (more than 90%) germination rate (data not presented). The seeds of *A. mellifera* may not survive long enough to be transmitted to subsequent wet season. Seed viability is compromised by shrivelling, insect attack (Donaldson 1969) and fungal infections and contamination. The implication is that should seeds fail to germinate and establish within the season of their production they are not likely to add to the recruitment of individuals of the species.

Physiological studies investigating water stress, water use efficiency, N uptake and carbon allocation of tree species as they relate to tree-grass coexistence or the persistence of woody plant species in savannas are not uncommon (Medina & Francico 1994, Cramer et al. 2007). In African savannas, many studies on tree recruitment have focused on *Acacia karroo* (O'Connor 1995, Wigley et al. 2009) in relatively mesic environments. Only two species, *Acacia reficiens* (Wiegand et al. 2005) and *A. mellifera* in Namibia (Joubert et al. 2008) have been studied from arid savanna zones. However, these authors concentrated on spatial patterns while the focus of my study was on understanding the mechanisms behind coexistence of *A. mellifera* and grasses. Other savanna species that have been studied extensively in this context are from mesic areas where fire is an important disturbance factor (Trollope 1982, Higgins et al. 2000, van Langevelde et al. 2003). It is generally agreed that there is insufficient fuel load in arid savannas for fires regimes to exert significant influence on woody plant recruitment and survival. Competition for water and nutrients (Walter 1954, Fowler 1986, Skarpe 1990) may thus assume greater importance in coexistence of grasses and woody plants in arid savanna environments.

The species *A. mellifera* mostly accounted for a comparatively higher woody vegetation density and cover in over-grazed areas relative to less disturbed vegetation in Botswana (Skarpe 1990). Sandy terrains exposed to a prolonged dry spell before the onset of rains may develop substantial water deficits, which may not be satisfied by small widely spaced rain showers typical of the arid savannas. Given the fine texture of aeolian surface deposits of the Kalahari, the sandy substrate for most of arid savannas south of the Sahel, retention capacity would expectedly be high. It might thus take exceptional and frequent rainfall to initiate percolation to deeper layers. In this way, most of precipitation frequently falling in arid areas on sandy areas will be retained in the upper soil surface where it is either evaporated or used up by near surface roots (Skarpe 1990). In comparison to other arid savanna woody plants, *A. mellifera* has relatively very shallow lateral roots (van Vegten 1981, Tolsma et al. 1987). This attribute predisposes the species to enhanced access to soil nutrients inclusive of *Rhizobium* fixed N_2 (Tolsma et al. 1987) as well as rainwater readily available to woody plants following overgrazing and suppression of grass (Skarpe 1990). Kraaij and Ward (2006) demonstrated that above average precipitation sustained over a number of consecutive seasons was a paramount prerequisite for successful recruitment of encroaching *A. mellifera*. However, once established, the species' shallow lateral rooting structure would enable it to take advantage of even the smallest rainfall amount more than other woody species if the grass layer is absent.

6.5. Towards ecophysiological understanding of bush encroachment

Results from the current study have indicated negative effects of near neighbours on physiological attributes, growth and partial canopy dieback of shrubs in a bush encroached arid savanna (Chapter 2). Shrubs growing among neighbours experienced more water stress than those without neighbours in most seasons. This may have resulted in suboptimal leaf physiology leading to more pronounced partial dieback of branches and twigs with negative effects on the overall canopy expansion (Chapter 2). Resultantly plants growing in close proximity with neighbours did not grow as much as plants without neighbours. This is in agreement with inference by Skarpe (1990) that invariably small plant sizes far below the maximal potential size of a species when growing in bush encroached areas result from competition for moisture among near neighbours. However, this competition may not be limited to water. The ^{15}N values of field experimental plants showed individuals competing with neighbours to be more reliant on biological fixation of N_2 than plants growing without neighbours (Chapter 2). The implication is that like grasses (Cramer et al. 2007, Chapter 5) belowground competition from conspecifics brings about N_2 fixation in encroaching legumes. This showed that competition for nutrients was also intense among close neighbouring plants, making it one of the limiting factors on shrub sizes. Physical barriers presented by rocks, boulders and shallow soils may further curb shrub sizes leading stunted plants in an encroached area.

Small statures of encroaching plants resulting from competition for water and nutrients as well physical limitations on rooting depths may be advantageous to the long-term survival of individuals as it helps maintain shrubs within a size-threshold that can be supported by their environment. With the removal of competitors, solitary plants may grow beyond what the local resource pool can support when rainfall is above average. Such plants may then become predisposed to whole-plant demise (rather than branch dieback) when the resources recede during unfavourable seasons, leading to whole plant mortalities (Chapter 2). Such unfavourable abiotic condition in arid environments may arise from extended rainfall deficit that exhaust soil moisture (Fensham & Fairfax 2007) leading to xylem cavitation (Rice et al. 2004). Thus despite competitive effects of neighbours, dense aggregation as in bush encroachment may simultaneously facilitate persistence of individuals, albeit at smaller sizes below the maximum growth potential.

Grass competition significantly affected tree seedling biomass (Chapters 3 and 5) and this appears to be related to its effect on nutrient (Chapter 5) and water uptake (Chapters 4 and 5). Tree seedlings competing with grasses showed near zero to negative ^{15}N isotopic composition to the more positive ^{15}N isotopic values in tree seedlings without grass competition (Chapter 5). This indicated that N_2 fixation by encroaching *A. mellifera* is inducible by grass competition as was reported for other *Acacia* trees (Cramer et al. 2007). Yet increased reliance on fixed N_2 from the atmosphere did not lead to improved growth, N concentration or biomass in tree seedlings competing with grass relative to trees seedling not competing with grass and apparently reliant on soil N (Chapter 5). Equally adult control shrubs showing greater reliance on N_2 fixation in the field did not have high foliar N concentration or improved growth. Reliance on N_2 fixation does not seem to add to better plant physiology and may only serve to make up for what is lost to inter- or intraspecific competition.

More water stress resulting from neighbourhood competition among mature shrubs did not significantly affect the shrubs' water use efficiency (WUE) as shown by similar $\delta^{13}C$ valves in control and target shrubs (Chapter 2). However in competition with grasses, the WUE of woody *A. mellifera* seedlings was negatively affected relative to when the seedlings grew without grass competition (Chapter 5). Taken together these contrasting effects of grass and conspecifics on the WUE of encroaching trees may suggest minimal influence of neighbouring shrubs on each other's WUE and detrimental consequence of grass competition. Thus trees are able to coexist in close proximity, i.e. form dense thickets with little effect on each other's WUE, while grass competition lead to inefficiency of water use. This may partly explain why grass competition in arid savannas presents a greater recruitment bottleneck to woody savanna plants than competitive exclusion by conspecifics, and why encroaching woody plants are able to grow in aggregated dense thickets.

6.6. Future directions

Future research on grass-induced BNF should examine the effect of different grass species on the N_2 fixation capacity of different woody legumes as well as the effect of varied grass densities, height and herbivory (Payne 2008). Persistence of competitive effects on invading plants is of temporary duration as it dissipates when invasive plants outgrow the inhibitory influence of the surrounding vegetation (Callaway *et al*. 1991, Miriti 2006). The time it takes the invader species to grow above the shading effect of adjacent plants and/or put down sufficient rooting structures to overcome belowground competitive pressure, depends on the structure of the pre-existing vegetation (Berkowitz *et al*. 1995). Slower growth rates that delay the escape of seedlings from the consequences of competition increases the cumulative probability of seedling demise by compounding annual rates of mortality (Hill *et al*. 1994). Grass-induced BNF in legumes is a competitive response to competition by grass for nutrients and possibly water and may therefore vary with varying intensity of grass competition or inversely with the competitiveness of the woody plant. As trees grow in size and put down deeper roots, their ability to compete with grasses will increase. It would thus be of interest to track the changes in BNF capacity with a plant's maturity over time and establish at what stage if any, BNF is discarded.

The rhizobial associations necessary for N_2 fixation have conventionally been considered costly to the legumes (Sprent 1985; Vitousek & Howarth 1991, Crews 1999). However, Cramer et al. (2010) argued that the fact that the growth of N_2 fixers is not compromised by the energy expended on BNF relative to non-fixation of N_2, energy cost may not be as acute as was once thought (Pate *et al*. 1979, Lambers *et al*. 2008). It is not clear if the same argument applies to carbon costs.

Although it is common practice in BNF studies to count and weigh the nodular biomass (Cramer et al. 2007, 2010), there is little information on the relationship between nodular number, sizes and biomass as to how much N_2 is fixed. A comparative study of nodular number, sizes and biomass and its correlation to the amount of N_2 that get fixed will be informative on whether there is a relationship between nodular load, and thus the cost of BNF and the amount of N_2 fixed by plants.

If indeed N_2 fixation was necessary for woody plants to survive grass competition, this does not explain the persistence of non-leguminous plants in grass dominated savannas. It is probable that arbuscular mycorrhizal (AM) fungi which are known to transport N to host plants (Leigh *et al*. 2008) are involved. This warrants further investigation as AM fungi have the capacity to obtain ^{15}N in the form of NO_3-N or NH_4-N and convert it to arginine before transporting it to structure where it the amino acids broken down prior to uptake by host plant (Govindarajulu *et al*. 2005, Jin *et al*. 2005).

Investigations of soil moisture uptake with deeper containers will be necessary to clearly delineate the layering of the shared soil moisture niches and exclusive zones for trees and grasses (Chapter 4). What will be more informative is to have a field component to the study that does not only look at the depth of shared and exclusive niches between a single species of woody plant and grass, but compares multiple species (such as competition between *Acacia mellifera* and *Tarchonanthus camphoratus* - e.g. Schleicher *et al.* 2011). It has been shown that the competitive effect of grasses on woody *Acacia* species varies among species of grasses (Payne 2008), although all grasses tested had negative effects on *Acacia* sizes. Moreover, different species of grasses have different rooting depths and therefore the rooting depth niche that will overlap (and be shared) with the roots of woody species will vary from species to species of grasses. It would further be necessary to allow a longer drying period between watering intervals for soil moisture depletion zones to be clearly distinguished. Since tree seedlings establish and set root in the shallow soil layers, they would for some time be sharing the same rooting zones as grasses. Niche separation cannot possibly develop during tree seedling establishment stage when tree roots grow through the same soil volumes as the grass roots. In fact the initial soil moisture profiles at the start (Chapter 4) did not show distinct zones of soil moisture depletion. In order to determine the life history stage at which tree seedling rooting depths grow beyond the ‘terminal rooting depth of grasses’, it will be necessary to follow up on root depth development through sequential harvesting.

Shrubs act as nurse plants for the establishment of other woody vegetation in arid environments (Archer et al. 1988, Vetaas 1992) and the results in this study suggest that some facilitative effects could extend to adult neighbouring trees (Chapter 2). Moreover, facilitative effects by plants are postulated to increase with intensity of environmental stress (Callaway & Walker 1997, Holmgren et al. 2000, Callaway et al. 2002). Findings by Nuñez et al. (2009) indicated that the association between a benefactor nurse plant and establishing seedling/sapling may switch from being beneficial to becoming negative as the sapling attains comparable stature and rooting depths as the nurse plant and competes directly for resources. Nursed plants could eventually reach large sizes exploiting greater resource pools as to be free of benefactor influence (Nuñez et al. 2009). There is thus a need, especially in arid environments to investigate the facilitative/competitive effects of neighbourhood proximity, developmental life history stage and sizes of closely associated plants across an aridity gradient.

Future research should examine neighbour removal on deeper soils where trees are able to attain greater sizes and compare these to adjacent rocky terrains preferably within the same climatic zone. This should indicate if indeed the intraspecific competition leading to self-thinning is greater on soils where plants can grow larger and thus exploit greater zones of resource capture.

6.7. References

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