Investigating the benefits an early green-up strategy can provide for two semi-arid savanna trees

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A thesis submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg in fulfilment of the requirements for the degree of Doctor of Philosophy

Johannesburg, May 2017

DECLARATION

I declare that this thesis is my own unaided work. It is being submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other university.

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ABSTRACT

Plant phenology, the study of the timing of biological events such as leaf flush, has been identified as a key tool in monitoring and understanding the impacts that changing climates may have on the world. Unfortunately there is a noticeable lack of phenological research in Africa's savannas in comparison to other global ecosystems, such as temperate forests. Savannas are known for their complexity in structure and composition and the phenology of their flora is no exception to this. Owing to the highly seasonal climates in this system, plants have had to evolve a range of phenological strategies to cope with the limited window of suitable growth conditions during the periodic wet season in the austral summer. One of these phenological strategies is that of early-greening; where deciduous trees will produce new flush prior to the onset of seasonal rainfall when the environmental conditions are at their driest. There is likely no other ecosystem in the world where the early-greening phenomenon occurs, however, it has been recorded in savannas from Africa, Australia, Asia and South America. The global prevalence of this strategy suggests that early-greening trees must gain some benefit from utilising this seemingly risky leaf flushing strategy.

The main aim of this study was to investigate two of the hypothesised benefits of an early-greening strategy for semi-arid savanna trees. The first hypothesis suggested that early-greeners are extending their growing season beyond that of grasses and late-greeners – whose leaf flush is limited by the availability of water through the onset of seasonal rainfall. The second hypothesis is that early-greeners produce new leaves ahead of the emergence of invertebrate herbivores with the onset of the first rains, therefore decreasing the risk of damage to the vulnerable tissues and allowing the defence mechanisms within/upon the leaf to develop. This study took place in the Nylsvley Nature Reserve (NNR) over three consecutive austral growing seasons (August 2012 to April 2015) and was conducted on the two dominant deciduous broad-leaved trees: *Burkea africana* and *Terminalia sericea*. *Burkea africana* is known to flush prior to the onset of seasonal rainfall and was considered the early-greening species in this study. *Terminalia sericea* has been recorded as flushing leaves prior to the onset of rainfall, but is more often a facultative-greener – rapidly producing new leaves with the onset of seasonal rainfall.

This study experienced high seasonal variability between years with early-greening only occurring during the final (2014-2015) season. Nevertheless, I was able to assess the weekly green-up phenology of both species during the first three months of green-up and found that the green-up of the trees was largely disconnected from the main system driver – water – while grass green-up was closely linked to the onset of rainfall. This complements the theory

of temporal niche separation; however, when assessing the functionality of the new leaves produced, carbon gain only occurred after the first two weeks post bud-break. In a system such as the NNR where rainfall onset variability is high, trees will only gain the advantage of an extended growing season if the onset of rainfall is more than two weeks after the start of flush. Using historical rainfall records (1980-2014), I estimated that 46% of years could have potentially experienced early-greening with rainfall commencing after the 15th October – the earliest date of green-up prior to rainfall onset in the NNR during this study.

One of the benefits tested relating to early-greening in this study was that early-greeners avoid invertebrate herbivore damage on vulnerable new leaves. This study provided evidence for the use of a phenological defence strategy to cope with invertebrate herbivory pressure. Leaves which emerged before the rains had lower rates of herbivore damage than those which emerged after. Moreover, it was demonstrated that the constant turnover of leaves with high photosynthetic rates (*T. sericea*) is a reasonable mechanism for dealing with high leaf herbivory, and can result in equivalent end of season leaf area (and carbon gain) to species which invest in defence and have slower turnover rates (*B. africana*).

Using the ground-based phenological measures in conjunction with remotely sensed NDVI imagery, the frequency of early-greening across seven comparable broad-leaved woodland sites in southern Africa from 2002 to 2014 was quantified. Of the environmental variables considered, the predictability of early-season rain (rather than total rainfall amount) was best correlated with early-greening. In savannas where rainfall onset and annual amounts were highly variable (such as the NNR), early-greening was less frequent (20% of the years) while in savannas closer to the equator where rainfall amounts were consistently >900 mm per annum and the onset dates began within a two week window each season, early-greening occurred in 80-90% of all years. The decrease in the proportion of early-greening events in the NNR from the 1980s to the past decade could be driven by the changing rainfall regimes over South Africa – with a predicted decrease in the number of precipitation events, but an increase in the storm intensity and rainfall amounts in each of these events. Fewer precipitation events may increase the risks associated with the early-greening strategy and this may be driving the NNR trees to use this strategy less frequently.

This study has highlighted the need for a long-term phenological monitoring network within southern Africa's savannas and has illustrated how early-greening species can benefit over other flora when environmental conditions are suitable for them to commence early leaf flush. This thesis has shown that early-greening broad-leaved savannas trees in South African savannas are more likely to avoid invertebrate herbivory than extend their growing seasons.

DEDICATION

I dedicate this thesis to my Grandpa, William Sharp Whitecross Snr.

Thank you for always believing in me.

RIP: 15 January 1927 – 16 May 2014

ACKNOWLEDGEMENTS

I received project funding and a bursary top-up from the Centre for Tree Health Biotechnology (CTHB) and running expenses funding from the Council for Scientific and Industrial Research (CSIR) which enabled me to conduct this fieldwork intensive project. I also received a scarce skills MSc grant from the National Research Foundation (NRF) during the first two years of this study (2012-2013) and an innovation PhD grant during the third and fourth years of the study (2014-2015), and these bursaries were topped up by the CTHB. I would like to thank and acknowledge all of these funding bodies for their financial support in making this study possible.

Thank you to my supervisors, Prof Sally Archibald and Prof Ed Witkowski for their continuous support, guidance and encouragement throughout this project.

I do not have enough words to express the enormous thank you that needs to go to my better half Caroline Howes for not only keeping me sane during long periods alone in the field with Skype calls and messages, but for also spending countless months helping me with field and lab work. I cannot begin to express how much having you in my life has meant and how your endless support and love has kept me going throughout this process.

Huge thanks must also go to my parents, Robert and Helenor Whitecross for not only their emotional support during my seemingly endless years of studying, but for countless hours spent assisting me with clearing my study site, isotope collection and preparation, scaffolding construction and irrigation installation. Thanks also to my brother, William, for always encouraging me to be better than my best. Thank you to my many field assistants for their contributions to making my mammoth fieldwork task doable - Wesley Hattingh, Ryan Thomas, Isabelle Inder, Giuseppe Venturi, Kirstin Olsen, Marcelle Johnson, Vidosava Jakovljevic and Nadine Cronk. I must also thank my special and loyal research companion, Roxy – your furry companionship made those long, lonely days in the field bearable and the good days great. Ruby – your furry cuddles made the tough days tolerable, thank you for your company on those long runs to clear my head!

Thank you to Natasha Du Plessis and the numerous staff (especially Johannes and Willie) at Nylsvley Nature Reserve for their support and assistance during the project. Thanks to Limpopo Parks and Tourism Board for granting me access to the Nylsvley Nature Reserve.

Thank you to Brian Frank for allowing me to stay on his beautiful farm, Sandfields and Forests. The tranquillity and peaceful environment allowed for a very productive space in which to write my thesis and analyse my data.

Thank you to Gert Le Roux from Mainline Irrigation for his assistance in designing and supplying the drip irrigation system for my watering experiment – twice – and for acknowledging my student budget constraints.

Thank you to Barney Kgope for taking time to dissect my LICOR data and for offering fantastic advice in taking the project forward.

Thanks to Ricardo Holdo for the use of his long term soil moisture probe data from Nylsvley Nature Reserve.

Thank you to Working on Fire for their donation of a "bakkie-sakkie" water tank for use in my irrigation experiment.

Thanks to Nthambaleni Masia, Mary Scholes, Terri Bird, Stephen Woodburn, Grant Hall, Sven Kaehler, Francesca Parrini, Dave Mycock, Deanne Drake, Penny Mograbi and Jolene Fisher for their advice over the course of this project. Thanks to Caroline Howes, Marijke Els, Marcelle Johnson, Felicia Maluleke and Isaac Mudau for their assistance with lab work.

Thanks to Caroline Surmon (Ouma) for encouraging me from day one to be curious about nature and engage with it every day.

Lastly, thank you to William Sharp Whitecross Snr. (Grandpa) for believing in me and supporting me right from the start through my wild and crazy ways and letting me be the green sheep in our family. I still miss you and our great conversations every day.

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LIST OF ABBREVIATIONS

AICc − Akaike information criterion with a correction for finite samples

ANCOVA – analysis of covariance

ANOVA – analysis of variance

AVHRR − Advanced Very High Resolution Radiometer

BA – *Burkea africana*

CVs – coefficients of variation

DGVMs – dynamic global vegetation models

DPM − disk pasture meter

EVI − enhanced vegetation index

GUSD – green-up start date

IRGA − infra-red gas analyser

LMA – leaf mass area

MAP – mean annual precipitation

MODIS – moderate resolution spectroradiometer

NDVI – normalized difference vegetation index

NNR – Nylsvley Nature Reserve

PPFD − Photosynthetic Photon Flux Density

SD – standard deviation

SE – standard error

SLA – specific leaf area

TRMM − Tropical Rainfall Measuring Mission

TS – *Terminalia sericea*

GLOSSARY

Carbon gain

The amount of carbon taken up by a leaf during photosynthesis

Compensatory growth

The continuous production and growth of new leaves throughout a growing season to combat disturbance by herbivory and leaf loss

Early-greening

The phenological phenomenon where deciduous savanna trees flush new leaves prior to the onset of seasonal rainfall. Species capable of this strategy can be referred to as early-greeners.

Facultative-greening

The pheno-strategy where deciduous trees will usually wait for the seasonal onset of rainfall prior to flushing new leaves, despite being able to produce new flush slightly ahead of seasonal rainfall onset under certain environmental conditions (i.e. late onset rainfall)

Green-up period

The period of the growing cycle when deciduous vegetation changes states from leafless, dormancy to fully-leaved, photosynthetically-functional canopies

Growing season

The period of an annual cycle in which plants have green leaves present which enable photosynthesis, nutrient cycling and growth to occur. The start of the growing season is when the first new leaves appear on the canopy irrespective of their functionality.

Late-greening

The pheno-strategy where vegetation will only commence growth after the onset of seasonal rainfall

Leaf flush

The start of new leaf production/growth

Leaf functionality

A functional leaf is able to photosynthesize and take up carbon – assessing leaf functionality involves determining at what stage of leaf development functional photosynthesis is able to occur

Phenology

The study of the timing of biological events such as the timing of when a deciduous tree flushes new leaves or produces flowers

Phenophase

The phenological stage of growth i.e. the new leaf phenophase occurs when a canopy is dominated by new leaves (> 50 % cover)

Rainfall onset

The start date of seasonal rainfall (based on a storm size greater than 15 mm) after several months of no precipitation during the dry season

Rainfall variability

The variation in both time (temporal variability) and space (spatial variability) of rainfall events

CHAPTER 1

INTRODUCTION

Background literature review, study rationale, study site and species descriptions, aims and objectives, thesis overview

1. General Introduction and Literature Review

Savannas cover more than 50 percent of southern Africa and approximately 20 percent of the terrestrial world (Huntley and Walker, 1982; Scholes and Walker, 1993). A large proportion of Africa's population relies on resources provided by these productive systems and it is therefore vital that every attempt is made to understand the different aspects of this complex biome (Scholes and Hall 1996; Jolly and Running 2004; Cleland *et al.* 2007). Climate change is now a globally recognised phenomenon and a renewed interest in how these changing conditions may impact vegetation dynamics has been growing over the last few decades (Chase *et al.* 1996; Menzel 2002; Jolly *et al.* 2005; Polgar and Primack 2011; Chambers *et al*. 2013). Particular attention has been given to how these changes may alter the timing of vegetative phenological events such as leaf flush and leaf fall (Menzel 2002; Buitenwerf *et al.* 2015; Adole *et al.* 2016). Research of these impacts in savanna systems falls short in comparison to that of other global systems, partly because of a lack of capacity and resources associated with the developing countries in areas such as the southern African region. The other major reason for the lack of knowledge in these systems is the complexity of the vegetative layer and its drivers (Archibald and Scholes 2007). This layer contains a variable mixture of tree and grass elements and a highly-variable inter-annual climate coupled with disturbances by fires, herbivores and frost (Childes 1989; Owen-Smith 1994; Holdo 2007; Whitecross *et al*. 2012). A prolonged dry season over the winter months has resulted in varied plant strategies for dealing with the lack of water in the system over this period (Childes 1989; Borchert 1994; Jolly *et al.* 2005). Deciduous savanna trees are an example of one of these life history strategies, losing their leaves over the dry period to avoid water loss and damage by fires (Huntley and Walker 1982; Williams *et al.* 1997; Elliott *et al.* 2006).

1.2. Phenology

The study of phenology has gone on for several thousand years linking most closely to the agricultural sector and the timing of vegetation development stages (Lieth and Schwartz 1997). The concept was formerly introduced into the scientific world in 1849 during a public lecture given by Charles Morren, a Frenchman. He defined it as the science surrounding natural phenomena, which appear continuously on the earth year-in and year-out. The term "phenology" was only introduced into the English language in 1875, when the Council of the Meteorological Society issued commands to record all phenological events that included: the monitoring of the first foliation and defoliation of trees, flowering and fruiting of plant species, as well as the arrivals, nesting periods and departures of bird species and other naturally occurring events. The most modern definition of phenology set out by the United States of America's Committee on Phenology is:

"the study of the timing of recurring biological events, the causes of their timing with regard to biotic and abiotic forces and the interactions among phases of the same or different species" (Lieth 1974, pg 4)

Vegetative phenology focuses on the study of developmental events in plants such as leaf flush or senescence in relation to the timing of environmental cues (Schwartz 1999; Badeck *et al.* 2004). Menzel (2002) made a strong case for the use of phenological studies in addressing global changes as the scientific community seeks to address future shifts under current climate change conditions. She argued that phenological records offer some of the longest historical biological records to the modern scientific community, such as the recording of cherry blossom flowering dates from 705 AD in Kyoto, Japan. By using the large array of phenological data collected across different global systems and biospheres (Reich and Borchert, 1984; Childes, 1989; Van Schaik *et al.*, 1993; De Bie *et al.*, 1998; Kramer *et al.*, 2000; Prior *et al.*, 2003; Archibald and Scholes, 2007; Polgar and Primack, 2011), scientists have a better chance of understanding and predicting the changes the world is likely to face in the future as global climates shift. There has been a clear increase in research efforts in this field over the last few decades as researchers have begun to utilise phenological attributes to try and answer questions relating to global modelling, monitoring and climate change impacts (Schwartz 1999; Sparks *et al.* 2000; Jolly and Running 2004; Cleland *et al.* 2007; Lindenmayer *et al.* 2015; February and Higgins 2016). Monitoring the timing of natural events enables climate change impacts to be quantified in terms of interannual variability of both meteorological events and subsequently the effects these changes have on the phenology of biotic components within different systems (Schwartz and Karl 1990; Running and Hunt 1993).

Phenological parameters are used to regulate the land-atmosphere energy-mass exchanges in global climate simulations and accurate data on phenological patterns is therefore needed to make these simulations as realistic as possible (Schwartz 1999). The development of remote sensing techniques has also contributed to the resurgence of this field: the normalised difference vegetation index (NDVI) and other spectral data provide far more spatial and temporal information than was available before, allowing leaf-out and green-up monitoring at an individual, ecosystem, landscape and regional scale (Tucker *et al.* 1985; Schwartz 1999, Polgar and Primack 2011).

1.3. Savanna phenology – a unique and complex paradigm

Savanna systems are often found in semi-arid regions where temperatures and rainfall can display high variability between seasons (Sarmiento *et al.* 1985; Scholes 1999; Mucina and Rutherford 2006). Savannas are characterised by the presence of both trees and grass at varying densities throughout southern Africa and competition between these two life forms can be intense (Walker and Noy-Meir 1982; Frost *et al.* 1986; Scholes and Archer 1997; Higgins *et al*. 2011). Many attempts to understand and model this co-existence have been developed, however, the complex nature and numerous contributing factors in this system have made it challenging to produce an all-encompassing model (Walker and Noy-Meir 1982; Higgins *et al*. 2000; Lehmann *et al.* 2009; Higgins *et al.* 2011). The tree-grass ratio in savannas is variable; however, one commonality throughout these systems is the difference in leaf phenology between the woody and herbaceous components (Scholes and Archer 1997; Shackleton 1999; Chidumayo 2001; Higgins *et al.* 2011).

Improvements in the phenological knowledge of the savanna system have been made over the past few decades with research into temperature, rainfall and soil moisture effects (Medina and Silva, 1990; Shackleton 1999; Chidumayo 2001; Jolly and Running 2004). Savanna systems are less dependent upon temperature and photoperiod, in comparison to northern hemisphere temperate systems; however, the reliance on the timing of rainfall events and the resulting increase in soil moisture appears to have major importance for savannas in general (Jolly and Running 2004; Archibald and Scholes 2007, Higgins *et al.* 2011). The start of the rainfall season in semi-arid southern African savannas is considered to be after the first rainfall event that is >20 mm within a 24 hr period (Hachigonta et al., 2008), however, several other definitions for the onset date of rainfall exist in the literature including Bate et al. (1982) who stated that 15 mm of rainfall is sufficient to commence grass growth in African savannas. Huxman *et al.* (2004) suggest that “pulses” or discrete-inputs of precipitation during the growing season may drive savanna ecosystems; however, much work is still needed in order to completely quantify how the systems respond. These pulse-dynamics are difficult to predict and a need for a clear understanding of them is vital as they may have the capacity to rapidly trigger biotic production within these systems which have a range of knock-on effects for all other ecosystem processes (Huxman *et al*. 2004; Xu *et al.* 2004; February and Higgins 2016). This raises the question of whether in systems where ideal conditions for growth are related to pulsed rainfall events; it is desirable to have patterns of leaf display linked to the main system driver – water – or to have more deterministic patterns and risk displaying leaves during unfavourable times?

Using life history strategies and phenological patterns as a way of classifying species has proven useful throughout the history of plant ecology. Plant functional trait categories that are broadly based on vegetation phenology have been used in the majority of global dynamic vegetation models (DGVMs), however, these are unable to fully describe the complete variation expressed by the plants they seek to model (Prentice *et al.* 2007).

Chapin *et al.* (1996) considered leaf phenology to be a vital factor in separating plant functional types. Examples of this include Borchert's (1994) classification of Costa Rican tropical dry forest species into evergreen, brevi-deciduous, deciduous and stem-succulent or Williams *et al.*'s (1997) description of Australian humid eucalypt savanna trees into evergreen, brevi-deciduous, semi-deciduous and deciduous, as well as Elliott *et al.'*s (2006) classification of Asian monsoon forest phenotypes such as spring-flushing, leaf-exchanging, deciduous and rain-induced. Within the southern African savanna system, several attempts to classify savanna trees into functional groups have been made (Walker and Noy-Meir 1982; Smith *et al.* 1992; Skarpe 1996). For most modelling purposes, savanna trees are grouped at the broadest scale as either evergreen or deciduous; where evergreen trees maintain their canopies throughout the dry season, and deciduous species have some period during which their canopies are not full (Skarpe 1996) – but within the deciduous type there is variability in the amount of leaf material lost (brevi-deciduous), the length of time canopies are bare, and the timing of leaf flush.

1.3.1. The early-greening phenological phenomenon

Some of the deciduous woody tree species in savannas have been observed to leaf-out ahead of the first rains at the end of the dry season (early-greeners), while others only begin leaf-out after the first rainfall event (late-greeners) (Chidumayo 2001; Archibald and Scholes 2007; Scheiter and Higgins 2009; Higgins *et al.* 2011). The growth in the grass component, however, is constrained to the wet season when there is moisture in the soil (Dye and Walker 1987; Chidumayo 1997). This difference in the timing of growth events has been suggested to be an important contributor to the coexistence of trees and grasses in this system: Temporal niche separation theory (Walker and Noy-Meir, 1982; Scholes and Archer, 1997) suggests that trees are active when grasses are dormant, allowing them access to nutrients without competition from their grassy competitors which only grow after the onset of seasonal rainfall (Scholes and Archer, 1997).

The early-greening strategy has been observed across a range of savannas systems including Australia, Asia, Africa and South America (Childes, 1989; Borchert, 1994; Williams *et al.*, 1997; Chidumayo, 2001; Elliot *et al.,* 2006; Archibald and Scholes, 2007; February and Higgins 2016). In some of these systems, for example South America, it is even seen as the dominant phenological strategy (Reich and Borchert, 1984; Rivera *et al.* 2002). Given the prevalence of this strategy across a range of climates and continents, it would suggest that these species must attain some benefit from employing this approach (Reich and Borchert, 1984; Childes, 1989; Borchert, 1994; Prior *et al.,* 2004; Hoffman *et al.,* 2005; Kushwaha and Singh, 2005; Elliot *et al.,* 2006). Little is known about the advantages or disadvantages of the early-greening strategy in southern African savannas. It is likely that it places notable costs on the trees; given that the plants are having to produce and maintain a full canopy when the environmental conditions are at their least favourable at the end of the dry season and risks of late fires or herbivory are high (Borchert, 1994; Chidumayo, 2001; Archibald and Scholes, 2007).

1.3.2. What influences savanna phenology?

Phenology is influenced by many environmental events including seasonal changes, rainfall, temperature and photoperiod (Childes, 1984; Borchert, 1994; Chidumayo, 2001). In the majority of northern hemisphere temperate systems, a good understanding of environmental phenological cues has been developed (Nizinski and Saugier, 1988; Chuine and Cour, 1999). Leaf-out is usually triggered by an increase in photoperiod (daylength) and temperature at the high temperate latitudes (Chmielewski and Rötzer, 2001). Phenological models have been developed to predict green-up events and growth periods for these well studied northern hemisphere systems (Schwartz, 1999). Given that the northern hemisphere holds the greatest proportion of terrestrial environment on earth, during its summer months the photosynthetically active vegetation is able to reduce overall global atmospheric carbon concentrations through uptake of available CO_2 (Myneni *et al.,* 1997; Canadell *et al.,* 2007). The northern hemisphere forests are responsible for the uptake of approximately 0.5±0.5 Gt C y^{-1} and this can be seen by the decrease in global atmospheric CO_2 concentrations over the northern hemisphere summer each year (Keeling and Whorf, 1998; Canadell *et al*., 2007). Scientists have struggled to make predictions of this nature for savanna systems, owing to the large knowledge gap with regard to the functionality and predictability of these complex systems. The seasonal variation in environmental variables, such as precipitation, coupled with the multifaceted interactions between the different layers of vegetation (trees and grasses) have further confounded modellers abilities to predict how these systems are actually functioning within the context of the broader, global carbon models (Poulter et al. 2014; Ahlström et al. 2015). Before any more realistic models can be built, there is a need to understand the growth patterns and timing of plant functionality within the savanna system (Lehmann et al., 2014; Poulter et al. 2014; Buitenwerf et al., 2015; Moncrieff et al., 2015). With the shift in climates and the alterations in the timing of phenological events, being able to predict these events will help us to understand how terrestrial plants (in particular savannas, which make up a large proportion of the southern hemisphere's terrestrial vegetation) are contributing to the global carbon cycle (Jolly et al. 2005; Ahlström et al. 2015). This will also aid in determining whether longer growing cycles may lead to increased CO_2 uptake in the future.

1.3.3. Drivers of leaf display in savannas

An accurate, process-based understanding of the drivers of leaf display in sub-tropical and semi-arid systems is not yet available – but developing this understanding and the ability to predict seasonal and inter-annual variation in carbon, energy and water exchange is certainly required (Jolly and Running, 2004; Scholes and Archibald, 2007). Early work by Reich and Borchert (1984) indicated that internal water status of Costa Rican lowland deciduous trees had a high correlation with timing of phenological events, whereas environmentally available water displayed less of a relationship with the trees' growth stages. Rivera *et al.* (2002) focused on the influence of photoperiod (daylength) as a driver of early-greening in tropical dry forests around the world, including Argentina, Cost Rica, Indonsesia, Thailand and Brazil. They found that leafing would begin after the spring equinox, as photoperiod began to increase, thus signalling the approach of the wet season (Rivera *et al.,* 2002). Some species in Asian monsoon forests access subsoil water reserves in the dry season and this enables new foliage to be produced ahead of the first rainfall event (Elliot *et al.,* 2006). Priyadarshini et al. (2016) corroborated this for southern Africa's savanna trees which make use of deep-soil water reserves when top-soil layers are dry. They also confirmed that these trees are able to rapidly switch back to using top-soil layer water after a rainfall event (Priyadarshini *et al.* 2016). February and Higgins (2016) also found that African savanna trees are able to store and recycle important nutrients, such as nitrogen, between seasons to assist in the rapid deployments of new leaves at the start of the growing season. Therefore, it is clear that rainfall is not necessarily the direct driver of tree phenology in these African savanna systems. Despite progress being made into understanding the early-greening phenomenon, very little is known about the costs and benefits of this strategy in relation to other leafing strategies found in the same systems. To date mostly speculative hypotheses have been suggested as explanations for the pros and cons of this strategy, but no clear assessments have been made to support any of them.

1.3.4. What are the benefits associated with an early-greening life history strategy?

At least four different hypotheses have been introduced to explain why trees might benefit from having an early-greening strategy in a savanna ecosystem:

According to Dye and Walker (1987), a benefit of utilizing the early-greening strategy is (1) an increase in the length of the growing season. Given that the wet season is only a few months long, trees which already have photosynthetically active canopies at the start of the growing season will be able to take better advantage of the available water (Elliot *et al.,* 2006). Another hypothesised benefit for this strategy is that by establishing an already expanded and hardened canopy of leaves, (2) the chance of nutrient leaching in young leaves (which lack a fully formed cuticle) by rainfall is decreased (Sarmiento *et al.,* 1985). The emergence of insect herbivores is driven by rainfall (Sinclair, 1978); hence an (3) early-greening tree affords itself time to develop mature leaves that can tolerate herbivory better than newly developing leaves (Sarmiento *et al.,* 1985; Wright, 1996). The first rain event of a season is associated with a large pulse of available nitrogen, at a time when grasses roots are not yet active (February and Higgins 2016). Scholes and Walker (1993) discuss the temporal separation of the tree and grass layers in a savanna, suggesting that (4) early-greening trees will have preferential access to any available nitrogen at the start of the season and can therefore better compete against their herbaceous competitors. None of these explanations is mutually exclusive, but they are very different. They also differ in the relative importance given to competition (for carbon and nitrogen) vs. disturbance (insect herbivory) as drivers of the early-greening strategy. This thesis will focus on testing hypotheses (1) and (3) and will return to the rest in the final discussion chapter.

1.3.5. What are the costs associated with an early-greening life history strategy?

Comparatively less, in relation to the literature on benefits, is known about the costs of this early-greening strategy employed by savanna trees. Most of the previous research has only focused on the advantageous elements this strategy has for early-greening trees (Sarmiento *et al.,* 1985; Borchert, 1994; Rivera *et al.,* 2002; Elliot, 2006). The time between the start of the first leaf-out and the commencement of the first rains is highly variable from year to year (Chidumayo, 2001; Do *et al.*, 2005). Trees which have produced a canopy ahead of the start of the rainfall cannot maintain it indefinitely without water, and (1) run the risk of having to shed that canopy should the delay between the onset of flush and the onset of rainfall be too great (Do *et al.*, 2005). After the rainfall begins, these trees are then able to reproduce a second canopy, but this would surely cost the tree in both metabolic energy and stored resources, while also (2) potentially decreasing the length of growing time it can utilize should it be forced to reflush a new canopy after the onset of rainfall. Another potential risk associated with this strategy is (3) the threat posed by disturbances such as fire, herbivory and frost (Owen-Smith, 1994; Wright, 1996; Holdo, 2007; Whitecross *et al.*, 2012). Late season fires may expose the new flush to burning or heating, whilst late frosts could be responsible for killing newly produced buds and stunt overall growth of the trees (Holdo, 2007; Ausperger, 2009; Whitecross *et al.*, 2012). Herbivory has the potential to pose the greatest threat to these newly flushing canopies that suddenly develop into islands of lush, green food at the end of the dry season when resources are at their lowest (Owen-Smith, 1994; Wright, 1996). Two other potential costs associated with the early-greening strategy may be (4) a loss of stored water through transpiration at the end of the dry season, or alternatively, (5) the over-heating of freshly produced leaves during the midday sunlight hours. Leaves absorb radiation, but can cool themselves due to evaporative cooling during transpiration (Lambers *et al.*, 2008). It is not totally clear whether newly flushed leaves at the end of the dry season (when temperatures can reach over 40°C) have fully functional stomata, or whether they make use of them for transpiration; however, these new leaves do run the risk of over-heating or dehydrating prior to the onset of rainfall (Lambers *et al.*, 2008). Unfortunately testing these consts fell outside of the scope of this study.

2. Study site and species

2.1. Study site

This study took place in the Nylsvley Nature Reserve (NNR), Limpopo Province, South Africa (24°39’ S, 28°42’ E) (Figure 1). The NNR is 3975 ha and falls within the upper reaches of the Nyl River floodplain. This floodplain is one of the largest inland wetlands in South Africa and hosts a high diversity of fauna and flora (Nobel and Hemens, 1978). The NNR is situated to the north of Modimolle (formerly Nylstroom) and the south of Mookgophong (Naboomspruit).

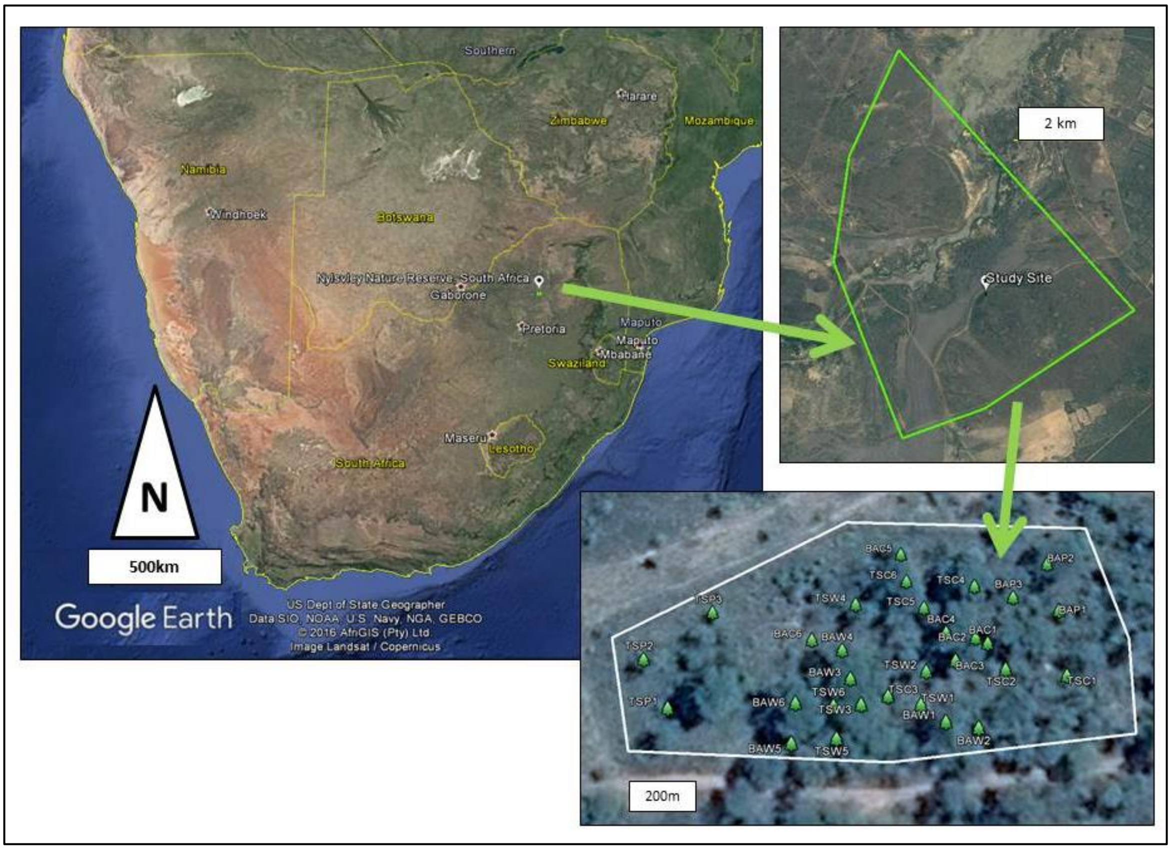

Figure 1. Map of the study site in the Nylsvely Nature Reserve as a regional, reserve and site scale. Tree codes have BA/TS for *Burkea africana* and *Terminalia serivcea* respectively, W/C/P for water, control or phenology only treatments respectively and the individual tree number.

The area was used for cattle grazing prior to the declaration of the reserve; however, this has had little impact on the natural vegetation within the area which has been mostly conserved (Coetzee *et al.*, 1977). The reserve's management strategy aims to conserve biodiversity within and surrounding the Nyl River floodplain, with particular focus on the avifauna which reside in and migrate to the reserve annually. The NNR was declared a Ramsar site in 1998; Ramsar is an international convention which aims at highlighting and protecting globally important wetlands and has over 1600 sites registered worldwide. Birdlife International, a conservation organization for the conservation of earth's avifauna has also declared the NNR as an 'Important Bird Area' or IBA due to the high numbers of migratory wetland bird species which migrate to the floodplain annually. Whilst the NNR's slogan states "Nylsvley is for the birds", the reserve also contains 77 mammal species such as roan antelope, brown hyena, aardwolf and serval, 58 species of reptiles and amphibians, approximately 12 fish species, as well as close to 10 000 species of insect, many of which have not yet been classified.

Prior to the establishment of the NNR in 1974, it is estimated that the Nylsvley area would burn approximately once every five years; however, these fires were mostly accidental and occurred mostly on the northern side of the reserve (Coetzee *et al.*, 1977). Today, the NNR management's policy has been to keep fires out of the reserve, often back-burning to stop any approaching fires from entering the reserve. NNR has not had a fire within the reserve for at least 20 years prior to the 2013 fire (N. Du Plessis - reserve manager *pers. comm.*).

The topography of the reserve ranges from undulating koppies (hills) to vast flat terrain across much of the floodplain itself, with an altitudinal range between 1080 to 1140 m. The NNR receives an average of 623 mm mean annual precipitation (69-year mean annual rainfall with a coefficient of variation of 24%). This rainfall is strongly seasonal, with 85% falling during the summer months (October – March) (Huntley and Morris, 1982). The mean annual temperature in the NNR is 19°C (Scholes and Walker, 1993). In the summer months (December/January) the mean maximum temperature is 29°C, whilst in the winter months (June/July) it is 21°C. The mean minimum temperature ranges from 17°C in the summer months to 4°C over the winter.

Savanna systems are one of the sunniest ecosystems in the world (Scholes and Walker, 1993). The NNR receives 75% of the potential annual total of 4371 sunshine hours and at the canopy level, radiation is 7316 MJ m^{-2}, approximately 61% of the radiation received above the atmosphere (Scholes and Walker, 1993). The total annual Photosynthetic Photon Flux Density (PPFD), which is the available energy that can be used for primary production with wavelengths between 300 – 700 nm and is expressed as quantum units, is 16 242 mol m^{-2} (Scholes and Walker, 1993).

Evaporation rates in the NNR are high due to the high radiation and resultant high temperatures. The Penman equation (Thom, 1975) was used to estimate the average evaporation for the NNR at 1897 mm per annum (Scholes and Walker, 1993).

Scholes and Walker (1993) recognized nine vegetation types within the Nyl River floodplain; some of which include the *Burkea africana* savanna; *Combretum* savanna; *Acacia karoo* savanna, as well as the seepline, floodplain and vertic soil grasslands. These vegetation types are grouped under the Central Sandy Bushveld Savanna vegetation type (Mucina and Rutherford, 2006). The area contains low mountains and ridges, where the lower-lying areas are dominated by *Burkea africana* open tree savanna and the midslopes are dominated by broad-leaved tree savanna with the dominant species being *Combretum apiculatum* (Mucina and Rutherford, 2006). This study took place in the *B. africana* savanna where high numbers of *B. africana* and *Terminalia sericea* occur, surrounded by grasses such as *Panicum maximum*, *Eragrostis pallens* and *Digitaria eriantha* (Coetzee *et al.*, 1977; Scholes and Walker, 1993).

The soils in the NNR are characteristically 1-2 m deep and infertile (Coetzee *et al.*, 1977; Scholes and Walker, 1993). They range from shallow soils derived from felsite to sandy soils derived from sandstone (Harmse, 1977). Alluvium lines the immediate area along the river and is considered to be nutrient-rich in comparison to the soils lying further away (Harmse, 1977). The specific site for this study had non-calcareous, well-drained sandy soils formed on felsite (Coetzee *et al*., 1977). These soils are shallow, with an orthic A-horizon underlain by apedal B-horizons on unconsolidated material or rock, belonging primarily to the Mispah series (Coetzee *et al.*, 1977). The *Burkea*-dominated savannas (where this study took place) grow in infertile loamy sands (Hutton form) which have been derived from Waterberg sandstone. Harmse (1977) reported the surface pH to be 4.7 and the cation exchange capacity to be ≈4 mmol/100 g soil. The total N stock for broad-leaved savanna soils has been estimated at ≈400 $gN.m^{-3}$ (D'Odorico *et al*., 2003). Scholes and Walker (1993) found a 44.6% more total soil N below tree canopies to a depth of 1 m, in comparison to soils found adjacent to canopies.

2.2. Study species

2.2.1. Burkea africana (Hook.) – Early-greener

Commonly known as the Wild Syringa, *B. africana* is a medium-sized leguminous tree, approximately 10-12 m in height, capable of reaching over 20 m. It is part of the Caesalpiniaceae family and is a monotypic genus found throughout central and southern Africa up to Ethiopia in the north and Nigeria in the west, and is a co-dominant species in Zambia. It is typically found on dry, sandy savanna and woodland soils as high as 1500 m.a.s.l. It tends to dominate nutrient-poor soils on the upper slopes of catenas, especially within the Nylsvley system. The fruits are pods, typically containing one large seed that weighs 104±29 mg (n=30) and they are produced between January to July (Wilson and Witkowski, 2003). Jackson (1974) reported *B. africana* as utilizing a cryptogeal germination strategy (also known as plumule burying), which is thought to have come about in response to frequent burning. This burial of the plumule enables the development of a root crown safely below the soil surface, thus offering buds protection from fire and other disturbances. From this safe beginning, *B. africana* is able to resprout after the above ground shoots are burnt off or damaged from fire, frost or herbivory (Wilson and Witkowski, 2003). *Burkea* *africana* has been shown to not support symbiotic nitrogen fixing bacteria, despite being a legume (Zietsman *et al*., 1988).

Burkea africana has an early-greening strategy and its flushing and green-up dates have been reported as early as September in southern Africa, before the commencement of summer rainfall (Childes, 1989). Rutherford and Panagos (1982) found an average of eight weeks for total green-up to take place in the *Burkea*-dominated savanna in the NNR starting in mid-September. Owen-Smith and Cooper (1987) reported that the available biomass of this species was high during November to June (summer to early-winter) relative to other palatable, deciduous species. The leaves are hairless, spineless and bipinnately compound and are often clustered at the tips of shoots. There are two to three pairs of pinnae with five to nine leaflets per pinna. Rutherford (1982) has estimated the total above-ground biomass for adult *B. africana* trees, as well as the contribution to this total by different parts of the tree (Table 1). The leaf area estimate ($m^2.ha^{-1}$) is also shown (Table 1).

2.2.2. Terminalia sericea (Burch. ex DC.) – Occasional early-greener

A member of the Combretaceae family, *T. sericea* is a small to medium-sized deciduous tree, commonly known as the Silver Cluster-leaf. It has an average height of 9 m, but an individual tree is capable of reaching 23 m. This species is found throughout southern Africa from Tanzania in the east, through the DRC and southwards into Angola and Namibia in the west, as well as through Botswana, Zimbabwe and the north-eastern region of South Africa. This species thrives in deep sandy soils and is found throughout grasslands, woodlands and wetland edges. *Terminalia sericea* is conspicuous in a savanna landscape due to the silvery sheen of its leaves and it tends to form a clear boundary along catenal seeplines between the broad- and fine-leaved savannas. It has single pink to red coloured fruits, 25-35 x 15-45 mm in size, that consist of an oval nut surrounded by a flat wing. Frequent parasitism of these fruits often results in the formation of deformed, thin round gall masses. Fruits are indehiscent and produced between January to May.

Terminalia sericea has been reported as flushing when the first seasonal rainfall begins if soil moisture is low (Childes, 1989). If soil moisture is high, this species has been known to flush just before the start of seasonal rainfall (Childes, 1989). The leaves of *T. sericea* are clustered at the branch tips and are narrowly obovate-elliptic with an entire margin, measuring 55-120 x 15-45 mm. A dense layer of silver, silky hairs covers the leaves, giving them their characteristic sheen. Leafing can occur several times in a season with new foliage being produced throughout the growing season. Rutherford (1982) has estimated the total above-ground biomass for adult *T. sericea* trees, together with the biomass contributions of different parts of the tree (Table 1). The leaf area estimates ($m^2.ha^{-1}$) are also shown (Table 1).

Table 1. The biomass estimates ($kg.ha^{-1}$) for adult *Burkea africana* and *Terminalia sericea* trees at Nylsvley Nature Reserve, as well as the leaf area ($m^2.ha^{-1}$), from Rutherford (1982).

Biomass ($kg.ha^{-1}$)	***Burkea africana***	***Terminalia sericea***
Total	8495	1631
Stem Wood	5560	1007
Branch Wood	2512	470
Current Twig	59	9
Leaf	364	145
Leaf Area ($m^2.ha^{-1}$)	2597	889

3. Research Rationale

Recently, a number of global reviews have emphasized the need for further understanding and investigation into tropical savanna tree phenology as part of a global agenda to reduce ambiguity in global models (Jolly and Running, 2004; Parmesan, 2006; Cleland *et al*., 2007; Lindenmayer *et al.* 2015; Chambers *et al.* 2013). The majority of the progress made in this field has been focused on understanding seasonal leaf display patterns in temperate ecosystems and significant breakthroughs in regards to explaining the variability in interannual global atmospheric carbon patterns have been achieved (Randerson *et al.,* 1999). Scientists now have a better grasp of the effects changing climates will have on temperate vegetation systems, but similar efforts need to be focused into improving the same knowledge about tropical and sub-tropical systems (Myneni *et al.,* 1997). This research aims to contribute towards that knowledge gap. At the regional-scale and even at a global-scale, numerous investigations have highlighted and explored the prevalence of the early-greening strategy in savannas (Sarmiento *et al.,* 1985; Chidumayo, 2001; Jolly and Running, 2004). Studies have shown that there are strong links to the relative dominance of this strategy in savannas and the extent of seasonality within them (Ryan, 2009).

Whilst the mechanisms for the early-greening strategy are well researched in several savanna systems (Borchert, 1994, 1999; Rivera *et al.,* 2002; Elliot *et al.,* 2006), the lack of a cost-benefit analysis of this strategy makes it difficult to establish a more generalised understanding of this phenomenon in southern African savannas in particular. Several studies assume that this life history strategy has emerged as a way to lengthen the growing season;

however, little evidence exists that demonstrates that early-greening species are actively photosynthesising prior to the onset of seasonal rainfall. There is, however, some evidence that their leaves are dysfunctional (stomates covered by a waxy cuticle) (Pantin *et al.* 2012). It is fundamental that the costs and benefits of this strategy are well understood before it can be successfully integrated into generalised phenological models. This research hopes to highlight and investigate some of the benefits associated with this life history strategy and to improve the overall knowledge about its occurrence in southern African savannas.

4. Aims and Objectives

The main aim of this study was to investigate the phenology of two broad-leaved savanna trees in relation to two proposed hypotheses regarding the benefits of early-greening in a southern African savanna: increasing the length of the growing season over late-greeners and grassy neighbours, as well as temporal avoidance of peak invertebrate herbivory periods during the growing season. This study also aimed to investigate how frequently early-greening events occur within southern Africa's broad-leaved savannas and how these phenological events may be influenced by changes in environmental conditions.

Using a watering experiment, this study compared the phenology, physiology and herbivory losses between three consecutive but very different Austral rainfall years (2012-2015) with the purpose of:

1. Describing the phenological responses of two savanna tree species in detail and assessing how flexible these responses were to changing environmental conditions.
2. Comparing the green-up of trees and grasses in relation to the addition of water through irrigation and natural rainfall.
3. Determining whether early-greening confers an advantage to trees using this strategy in terms of increased and/or extended photosynthetic activity.
4. Determining whether early-greening confers an advantage to the trees in terms of avoiding invertebrate herbivory when new leaves are at their most vulnerable stage of growth.

Using the ground-based data collected at the NNR in conjunction with remotely-sensed data collected by NASA's MODIS satellite, this study also aimed to:

5. Investigate whether detection of early-greening events was possible using remotely-sensed data, and if so,

6. Investigate the frequencies of early-greening events across southern Africa’s broad-leaved savannas between 2002 and 2014.
7. Assess the influence of environmental conditions such as rainfall, temperature and photoperiod on the onset of leaf flush.

4.1. Objectives relating to the tree and grass phenological investigation components:

- To compare green-up start dates (GUSDs) between the two selected species in response to watering treatments to understand the role of rainfall in cueing leaf-out and green-up in a semi-arid South African broad-leaved savanna over three consecutive seasons.
- To compare the green-up rates of trees receiving irrigation prior to the start of green-up and those receiving only natural rainfall.
- To assess the changes in leaf structure from bud-break, through leaf expansion to maturation in order to quantify different leaf phenophases during the green-up period.
- To observe differences in GUSDs and green-up rates between trees and their herbaceous competitors (grasses).
- To compare differences in GUSDs, green-up rates and growth rates of grasses situated below and between the tree canopies.
- To address the role of rainfall and soil moisture in relation to the growth cycles of each tree species and their neighbouring grasses.

4.2.Objectives relating to the physiological investigation component:

- To investigate changes in leaf chlorophyll content and functionality (photosynthesis, stomatal conductance and transpiration) from bud-break, through expansion to maturation.
- To assess how long it takes new leaves to become functional (photosynthetically active).
- To measure and compare the changes in rates of photosynthesis and transpiration between tree species over the green-up period.
- To investigate the influence of soil moisture on leaf photosynthetic rates.

4.3.Objectives relating to the herbivory investigation component:

- To compare how the rates of invertebrate herbivory change over two contrasting seasons – an early- vs. late-onset rainfall year.

- To assess how the rates of consumption change at a leaf and canopy scale over the season.
- To investigate how the consumption rates relate to leaf age – are new leaves preferred.
- To determine whether invertebrate herbivores show a preference for either species.
- To assess whether the trees compensate phenologically for the losses incurred through invertebrate herbivory.
- To determine whether early-greening species benefit from decreased herbivory on new leaves prior to the onset of seasonal rainfall.

4.4.Objectives relating to the remotely-sensed assessment of early-greening

- Investigate whether ground-based phenological monitoring can be used to calibrate NDVI estimates for early-greening events in a broad-leaved savanna.
- Assess how frequently early-greening events are occurring in broad-leaved savannas along a latitudinal gradient in southern Africa between 2002 and 2014.
- Determine whether the frequency of early-greening is influenced by temporal and spatial rainfall variability, i.e. savannas closer to the equator have higher occurrences of early-greening.
- Investigate the influence of potential environmental drivers such as rainfall, temperature and photoperiod on the commencement of leaf flush.

5. Thesis structure

The chapters for this thesis, excluding both the Introduction (Chapter 1) and Synthesis (Chapter 7), are independent and written in the format of scientific journal articles to be submitted for publication in internationally recognised scientific journals. Some of the chapters have already been submitted and published: Chapter 2 has been published in the *South African Journal of Botany*, Chapter 3 has been accepted for publication in the *South African Journal of Botany* and Chapter 6 has been accepted for publication in the journal *Austral Ecology*. The presentation of a thesis as a collection of papers designed for publication will inevitably result in the occurrence of some repetition – most often in the sections describing the study sites and species, as well as in the methods sections. Chapter 2 provides the detailed baseline phenology for the trees involved in this study and forms the base off of which the remaining research chapters were built. Chapters 3-5 handle the study objectives mentioned above with the aim of improving the current understanding of the benefits that an early-greening strategy can provide a semi-arid savanna tree. Chapter 6 addresses the prevalence of the early-greening strategy across southern Africa’s broad-leaved savannas in relation to the influence of different environmental drivers. Chapter 7 is the final synthesis chapter which will discuss and highlight the important findings of the study’s results in relation to the broader ecological context within the field of savanna phenology and modelling of global vegetation dynamics.

Chapter 2 forms the base chapter for the entire study, detailing the phenological changes in *B. africana* and *T. sericea* trees at the NNR over three seasons in relation to natural rainfall and a water manipulation experiment. The phenology of these trees was monitored at weekly intervals during the initial green-up periods and subsequently at monthly intervals for the remainder of the seasons.

Chapter 3 compares the phenology and growth of tress in relation to their neighbouring grasses during an early-rainfall-onset season and a late-rainfall-onset season which also included a severe fire. Aspects of the temporal niche hypothesis are discussed and the ground-work for the use of remotely-sensed data to detect early-greening is laid out ahead of Chapter 6.

Chapter 4 investigates how leaf functionality develops with leaf age over two seasons to address the key question of whether early-greening trees have functional leaves prior to the onset of seasonal rainfall. This would result in an extension of the growing season for early-greeners beyond that of late-greeners and grasses resulting in decreased competition for limited nutrients.

Chapter 5 focuses on answering the question of whether early-greening trees avoid herbivory damage on new leaves prior to the emergence of invertebrates with the onset of seasonal rainfall. It also considers other phenological defences such as compensatory growth during periods of high herbivore pressure.

Chapter 6 uses the ground-based phenology in Chapters 1 and 2 to assess the frequency of early-greening events across southern Africa’s broad-leaved savannas in relation to changing environmental conditions along a latitudinal gradient. By understanding how prevalent early-greening is across these savannas and what is potentially driving early-greening in certain savanna tree species, a better understanding of how those species may be benefiting from it can be achieved.

Chapter 7 synthesises the findings of the study as a whole through the exploration of the major results within the broader ecological context of savanna phenology and ecological modelling.

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CHAPTER 2

No two are the same: assessing variability in broad-leaved savanna tree phenology, with watering, from 2012 to 2014 at Nylsvley, South Africa.

This chapter has been published in the South African Journal of Botany

Whitecross, M.A., Witkowski, E.T.F., Archibald, S. (2016) No two are the same: Assessing variability in broad-leaved savanna tree phenology, with watering, from 2012 to 2014 at Nylsvley, South Africa. *South African Journal of Botany* **105**: 123–132. doi:10.1016/j.sajb.2016.03.016

Melissa Whitecross was responsible for the design of the study, collection and analyses of the data. She also wrote up the paper which was reviewed by her supervisors Prof. Ed Witkowski and Prof Sally Archibald prior to submission.

1. Abstract

Ecologists and modellers are yet to fully understand and design accurate models simulating the unpredictable green-up of savanna ecosystems, possibly due to the lack of long-term, phenological monitoring of this biome. In this study observations of the percentage of leaf age class (new, fully expanded and mature) phenology of two dominant, broad-leaved savanna trees at a weekly scale over the green-up period (August-November) and a monthly scale during the rest of the growing season (December-May) between August 2012 and May 2015 at the Nylsvley Nature Reserve, South Africa were made. An irrigation experiment, commencing in the dry season, to determine whether the simulation of a 20 mm rainfall event for several weeks prior to the start of rainfall, could influence the leaf phenology of an early-greener (*Burkea africana*) and a facultative-greener (*Terminalia sericea*) was used. Each observed season experienced different environmental conditions from early onset average rainfall (2012), to late onset high (2013) or low rainfall (2014). Irrigation of the facultative-greener in 2012 showed faster green-up rates and in combination with a late season fire during September 2013, resulted in a delayed green-up of watered *T. sericea* trees. Early-greening of *B. africana* was only observed during the 2014 season. *Terminalia sericea* showed faster maturation rates than *B. africana* which complemented its facultative-greening strategy, while *B. africana* had faster green-up rates after the fire when competition for available nutrients was low. This study has demonstrated that using fine-scale phenological measurements can provide a clearer picture of how trees are greening up in the early stages of the green-up period and showcases the high levels of variability in savanna tree phenology. Investment into long-term phenological monitoring across southern Africa's savannas is needed if future phenological preditions are to be made in the future.

Keywords: annual variation; broad-leaved savanna; early-greening; green-up period; water addition.

2. Introduction

Recent trends in modern ecological studies have tended towards broad-scale, synthesizing investigations (Parmesan, 2006; Cleland et al., 2007; Polgar and Primack, 2011; Klosterman et al., 2014); yet a lack of long-term, on-going, fine-scale studies hampers our understanding for many of the world's natural systems. The world faces an uncertain climatic future and numerous models and scientific techniques have been developed to assist in predicting and understanding what changes the world may experience going forward, however, many of these models still lack long-term baseline datasets, particularly for areas of the planet where it is difficult to fund or maintain long-term monitoring projects.

Phenology is the study of the timing of biological events, as well as the factors which influence them (Lieth, 1974). Phenological studies could be regarded as descriptive and mundane science - tracking the timing of natural events and often lacking any ecological contextualization, however, ecologists have begun to use phenological studies as a useful means for monitoring changes in ecosystems as future climatic conditions become less predictable (Schwartz, 1998; Menzel, 2002; Cleland et al., 2007). Consistent long-term phenological work has increased the understanding of the environmental cues and climate feedbacks in northern hemisphere temperate and boreal forests (Kramer et al., 2000b; Inouye, 2008; Augspurger, 2009; Polgar and Primack, 2011; Keenan et al., 2014; Vitasse et al., 2014). Comparatively less phenological work exists from tropical and subtropical ecosystems, with most of the work to date having occurred mainly in Australia (Wright and Van Schaik, 1994; Williams et al., 1997; Myers et al., 1998), South America (Reich and Borchert, 1984; Borchert, 1994; Rivera et al., 2002; Morellato et al., 2013) and southern Africa (Childes, 1989; Jolly and Running, 2004; Chidumayo, 2001; Ryan, 2009; Higgins et al., 2011). However, recent publications by Fitchett et al. (2015) and Lindenmayer et al. (2015) recognise the importance of quantifying phenological patterns in these complex, often water-limited ecosystems.

The advent of remotely sensed indices of vegetation greenness in the past few decades has revived interest in phenological research, and several useful studies that demonstrate the changes in growing season and seasonal leaf display using long-term AVHRR and MODIS data have been conducted (Myneni et al., 1997; Schwartz, 1998; Poulter and Cramer, 2009; Buitenwerf et al., 2015). However, remote sensing of phenology is most useful when integrated with field measurements which can validate and inform the vegetation indices acquired from satellite data (Fitchett et al., 2015). This is particularly important in systems with high ecosystem-level variation in phenological patterns, such as savannas, where the coarse scale of the remotely sensed data often obscures much of the system dynamics. In these situations long-term field measurements and phenological networks are still essential but are currently lacking in the majority of the world's savannas.

Savannas by definition are a variable mixture of discontinuous tree and continuous grass layers (Huntley and Walker, 1982; Scholes and Archer, 1997). The structure of these systems can be highly variable, so too can the phenology of these systems, with no two years displaying the same environmental conditions and phenology (Childes, 1989; Chidumayo, 2001; Higgins et al., 2011). The start of each growing season, when deciduous vegetation begins to flush new leaves, is known as the green-up period. The timing of green-up can vary between years as a result of various environmental and species-specific factors (Sarmiento and Monasterio, 1983; Childes, 1989; Chidumayo, 2001; Archibald and Scholes, 2007). The temporal niche separation theory describes how trees and grasses commence green-up at different times at the start of the growing season, potentially allowing for their coexistence in the savanna system (Simioni et al., 2004; Archibald and Scholes, 2007; Higgins et al., 2011). Grasses lack the storage capacity of their woody competitors (i.e. large stem and rooting systems) and are therefore heavily reliant on the arrival of seasonal rainfall to commence their growth after the dry season (Scholes and Archer, 1997). Savanna trees, however, are able to access deep water supplies through an extensive deep-root network and have the ability to store water in both their trunks and roots, and by using these stored reserves prior to rainfall onset, trees are able to produce green leaf material when grasses are brown (Chidumayo, 2001; Archibald and Scholes, 2007; Higgins *et al*., 2011). This means that trees are able to grow during periods of the season when grasses pose no competition for resources; i.e. during the dry season prior to the start of seasonal rainfall. This phenological phenomenon is called 'early-greening' and may provide trees with an advantage in out competing their herbaceous competitors at the start of the green-up period (Scholes and Walker, 1993). Not all savanna trees do this however. Little is known about the prevalence of this strategy in African savannas.

African savannas are regarded as semi-arid, water-stressed systems and the phenology of vegetation in this ecosystem is often closely linked to the availability of water (Huntley and Morris, 1982; Scholes and Walker, 1993). Moyo et al. (2015) investigated the impacts of additional water and nutrients on the phenology of resprouting *Terminalia sericea*, and found that treatments with the highest supplementation had longer growing seasons and higher leaf biomass. Chidumayo (2015) found that irrigating saplings during the Zambian dry season significantly increased their growth rates. Both of these studies are clear examples of the key role water plays in the growth cycles of some savanna trees. However, the early-greening strategy described above cannot be driven by the arrival of seasonal rainfall, indicating that some species must be responding to cues other than water availability (Childes, 1989; De Bie et al., 1998; Chidumayo, 2001; Archibald and Scholes, 2007). The fact that the environmental cues for seasonal leaf display vary between species within an environment complicates attempts to model savanna leaf phenology (Chidumayo, 2001; Archibald and Scholes, 2007; Higgins et al., 2011). Additionally, it limits the usefulness of remotely-sensed data which do not have the spatial resolution to describe this complexity. Thus, detailed field investigations of savanna phenology are important.

Through a fine-scale (weekly) assessment of broad-leaved savanna tree leaf phenology, this study aimed to observe changes in green-up dates and periods between two dominant tree species over three consecutive seasons (2012-2014). Changes in rainfall and soil moisture in relation to growth for each species were monitored (1) to determine links between water availability and green-up. In addition, an investigation into the role of water on tree phenology with the use of an irrigation experiment commencing prior to the start of natural rainfall during each growing season. (2) An investigation into whether the green-up rates of the trees receiving irrigation prior to the start of green-up and those receiving only natural rainfall were different was made. (3) Changes in leaf structure from bud-break, through expansion to maturation was monitored to quantify the different leaf phenophases during green-up. It was hypothesized that the phenology of the early-greening *B. africana* trees would not be affected by the early addition of water, but the more facultative-greening *T. sericea* trees would respond by showing earlier green-up. Testing for a carry-over effect of the watering experiment was conducted during the third season (2014) when irrigation did not occur but previously watered trees were still measured. During the second study season (2013), a severe fire burnt through the site and was incorporated into the assessment of phenology, as fires are a naturally occurring event in savannas.

3. Materials and methods

3.1.Study site

This study was conducted in the broad-leaved savanna of the Nylsvley Nature Reserve (NNR), Mookgophong, Limpopo Province, South Africa (24°39’ S, 28°42’ E; 3975 ha) which falls under the central sandy bushveld of the savanna biome (Rutherford et al., 2006). NNR has a rich history of research and is perfectly suited to test the tree phenology in comparison to previous studies in the same area (Coetzee et al., 1977; Huntley and Morris, 1982; Rutherford and Panagos, 1982; Scholes and Walker, 1993). The climate is seasonal with hot, wet summers during which an average of 85% of the 623 mm mean annual precipitation (MAP) falls between October and March (Huntley and Morris, 1982). Temperatures range from a mean maximum of 29 °C in summer to a mean minimum of 4 °C in winter (South African Weather Services 2013). The soils at the NNR are approximately 1-2 m deep and infertile, with loamy, sandy soils derived from Waterberg sandstone (Coetzee et al., 1977; Scholes and Walker, 1993). The study ran from the beginning of August 2012 until the end of May 2014.

On the 19th September 2013 a severe fire burnt 85% the NNR. The fire was accidentally started 14 km north of the study site on the previous day. The fire moved at an average speed of 25 km/h during the first day and burned for three days. The fire burnt from the northern boundary of the reserve and was driven by strong winds on a hot, dry day. The reserve had mostly excluded fire for the previous decade and high fuel loads had accumulated as a result. Residents in the area described it as the worst fire seen in over 45 years. Many adult trees were killed and the majority of saplings as well. Average scorch heights of ~4 m were observed on the trees in the study site, representing very high fireline intensities (Trollope et al., 2002).

3.2.Study species

Two of the dominant broad-leaved savanna trees in the NNR were selected for this study. *Burkea africana* (Hook.) (Caesalpiniaceae) is considered an early-greening species that has been known to flush its leaves prior to the start of seasonal rainfall (Childes, 1989; De Bie et al., 1998). It is distributed throughout central and southern Africa (Maroyi, 2010). It is a medium-sized deciduous, leguminous tree approximately 10-12 m in height (Maroyi, 2010). The green-up period in the NNR averages 8 weeks, usually commencing in late September (Rutherford and Panagos, 1982).

Terminalia sericea (Burch. ex DC.) (Combretaceae) is considered to be a facultative-greener, however, occasional early-greening may be observed in this species (Childes, 1989; Moyo et al., 2015). It is distributed throughout southern African savannas (Lemmens, 2009). It is a small to medium-sized deciduous tree with an average height of 9 m (Lemmens, 2009). Green-up generally occurs just before or immediately after the arrival of seasonal rainfall (Childes, 1989).

3.3.Experimental design and protocol

3.3.1. Irrigation experiment

A random selection of 14 *B. africana* and 14 *T. sericea* trees that were >4 m in height and >5 m apart took place. For each species, eight trees were allocated to the control (unwatered) and six to the watered treatment. At the start of the study, tree heights within species showed no overall differences between treatments (Wilcox tests: *B. africana*: W=21, d.f.=12, p=0.75: watered =8.58±0.44 (SE) versus control =8.08±0.47 m; *T. sericea*: W=37, d.f.=12, p=0.11: watered =6.41±0.52 m versus control =6.94±0.62 m). The stem diameters were not different between treatments in either species, however, *B. africana* stem diameters (watered: 32.9±3.76 cm; control: 28.31±2.14 cm) were significantly larger than the *T. sericea* diameters (watered: 17.92±1.82 cm; 19.39±1.37 cm) (Kruskal-Wallis: H=44.49, d.f.=3, p<0.001).

Each individual tree in the watered treatment group was fitted with a pressurised drip-irrigation system to ensure that water was distributed evenly around the base of the tree irrespective of the gentle slope (5.71 cm/m) effects (Netafim Uni-techline 0.3 m pressure regulated drip irrigation system, South Africa). Four consecutive, linked rings were placed at 1, 1.3, 1.6 and 2 m radii from the base of each tree trunk and connected to the main inlet valve. The start of the rainfall season in semi-arid southern African savannas is considered to be after the first rainfall event that is >20 mm within a 24 hr period (Hachigonta et al., 2008) and on average this occurs on the 14th October (±28 days (SD)) at Nylsvley with an average storm size of 27±12 mm (34 year average between 1980-2013). In order to test whether early green-up could commence in trees receiving additional water through the irrigation system prior to the start of the rainfall season, irrigation was planned at least one month prior to the start of natural rainfall and continued watering took place until the end of October which was approximately one month into the average green-up period at the NNR. In order to simulate a 20 mm rain event, each tree received ~250 L of water over the 12.6 m^2 area around their base. The study used ~3000 L per week of borehole water to irrigate all 12 watered trees. Irrigation commenced on the 2nd September 2012; however, that year the start of the rainy season began one week after (7th September 2012) the watering experiment had commenced – with an 80 mm rainfall event. Nevertheless, irrigation continued at a weekly interval until the end of October 2012. During the 2012 season water added via irrigation totalled 140 mm which was equivalent to 67.3% of the total water received during the green-up period (August – November) and 40.2% of the total annual water received. During the second season (2013), irrigation began on the 15th August 2013 at weekly intervals. The seasonal rainfall commenced on the 7th October 2013 and watering continued until the end of October 2013. The irrigation during 2013 totalled 180 mm which was 71.4% of the total water received during the green-up period and 22.7% of the total annual water received. No irrigation was applied during the 2014 season but previously watered trees were still considered a separate treatment for the analysis during that season.

3.3.2. Rainfall

Daily rainfall data were collected from the South African Weather Services' Bela-Bela station, approximately 60 km south of the NNR.

3.3.3. Soil moisture

Soil was collected every 3 days for two weeks at the start of irrigation and the start of the rainfall season during 2012 and 2013. Steel tubes were inserted into the soil at the bases of three trees per treatment and soil samples from the top 15 cm of soil were extracted. Samples were then weighed to obtain wet mass, after which samples were dried for 48 hours at 70 °C and then reweighed to obtain dry mass. The difference between wet and dry mass was then calculated as a percentage of total soil moisture.

3.3.4. Phenological scores

All estimates were conducted from the beginning of August 2012 to the end of May 2015. Phenological observations for each austral growing season (July-June) between 2012 and 2014 were divided into two parts: 1) an intensive estimation of weekly phenology between August to November and 2) a monthly estimation of phenology from December to May. This was to allow for detailed measurements of the different leaf age class phenophases during the green-up period. A classification for leaf age similar to that of Williams et al. (1997) was used. This classification has three leaf age classes: new (<0.5*length of adult leaf), fully expanded (>0.5*the length of adult leaf) and mature (a sun-drenched, adult leaf) (Williams et al., 1997). Sun-drenched leaves are adult leaves which have been exposed to sunshine and have developed a tough, outer layer on the surface of the leaf, also known as a waxy cuticle. The Walker aerial cover scale was used as a baseline to estimate canopy fullness of each leaf age class, where 0: 0%, 1: 1-10%, 2:10-25%, 3: 25-50%, 4: 50-75%, 5: 75-90%, 6: 90-99% and 7: 100% (Walker, 1976). Standardised photographs were taken of each individual tree at weekly intervals using a Nikon D3100 with an 18-55 mm lens for comparison. Sampling of the 2014 growing season phenology was only conducted every two weeks during the green-up period.

3.4.Data analyses

All analyses were conducted using the open-source R statistical software (R Core Team 2014, version 0.98.1102). The differences between tree heights within each species were compared using Wilcoxon rank sum tests and stem diameters were analysed using a Kruskal-Wallis test to compare the watered and control treatments from both species. Daily rainfall data collected from the South African Weather Services was totalled for each month and then for each austral year (July to June). The green-up period rainfall (and irrigation) was totalled for August to November each year. Wilcoxon independent tests were used to compare soil moisture between each species and treatment. The phenological scores for each species and treatment's leaf age classes were averaged to calculate the mean±SE for each week or month. These were then plotted using the ggplot2 in R. The means±SE green-up start dates (GUSDs) for each treatment and year were calculated from the date of the first day during the week when a tree had more than 5% new leaves on the canopy. These were then compared between treatments using Wilcoxon Rank Sum Tests. The means±SE for the green-up periods – time taken from date of first flush to >50% mature canopy coverage – of each treatment were compared using a Kruskal-Wallis test and Kruskal Multiple Comparisons (kruskalmc) post-hoc analysis from the pgirmess package in R. The length of leaf phenophases between treatments were also compared using the Kruskal-Wallis and kruskalmc tests. Mature leaf phenophases were estimated as the number of days from the date of the first week of 50% mature canopy coverage to the middle of the last month when >50% mature canopy coverage was present. Phenological scores for each species' leaf age class were converted to percentages and then analysed between treatments, weeks and years using repeated measures ANOVAs. Regression analyses were compared between the individual tree's GUSDs and the seasonal rainfall start dates for all species and treatments and regression lines were presented with the means and standard errors for each species and treatment.

4. Results

4.1.Rainfall

The total annual precipitation (TAP) during the study period was highly variable with comparatively wet and dry seasons (Figure 1). During 2012 (July-December 2012 + January-June 2013), seasonal rainfall arrived five weeks earlier than average (7th September) and had the second highest TAP of 596 mm, while in 2013 rainfall commenced a week earlier than average but a month later than the previous season (7th October), with the highest overall TAP of 791 mm (Figure 1). The lowest TAP of 491 mm was during 2014 with the latest starting time for seasonal rainfall (11th October) (Figure 1). Rainfall totals for the green-up period (August-November) were 208 mm, 252 mm and 103 mm for 2012 to 2014 respectively.

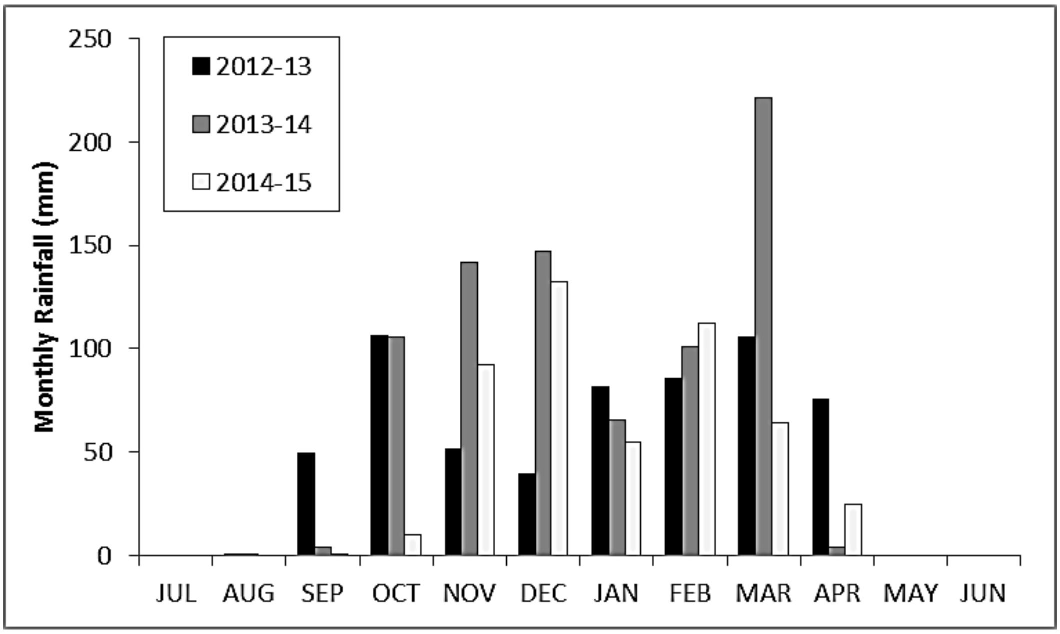

Figure 1. The monthly rainfall of the austral years (July-December + January-June) for 2012-2014.

4.2.Soil moisture

A clear increase in soil moisture was visible after irrigation in both 2012 and 2013 (Figure 2). Watered trees had a 4.45% and 5.84% higher soil moisture for *B. africana* and *T. sericea* respectively during the beginning of the irrigation periods for both years (Wilcoxon Test: W=8, p=0.027). The arrival of seasonal rainfall was earlier in 2012, resulting in a rapid increase in control tree soil moisture (Figure 2) meaning that from the 7th September there was no difference between treatments during this year (Wilcoxon Test: *B. africana*: W=21, p=0.71; *T. sericea*: W=17, p=0.38). The later arrival of rainfall during 2013, resulted in differences in soil moisture between treatments with watered trees experiencing higher soil moisture at the start of irrigation (Wilcoxon Test: *B. africana*: W=9, p=0.048; *T. sericea*: W=8, p=0.027). No differences were observed between species for either year (Wilcoxon Test: Watered: 2012: W=22, p=0.80; 2013: W=13, p=0.99; Control: 2012: W=22, p=0.80; 2013: W=66, p=0.75).

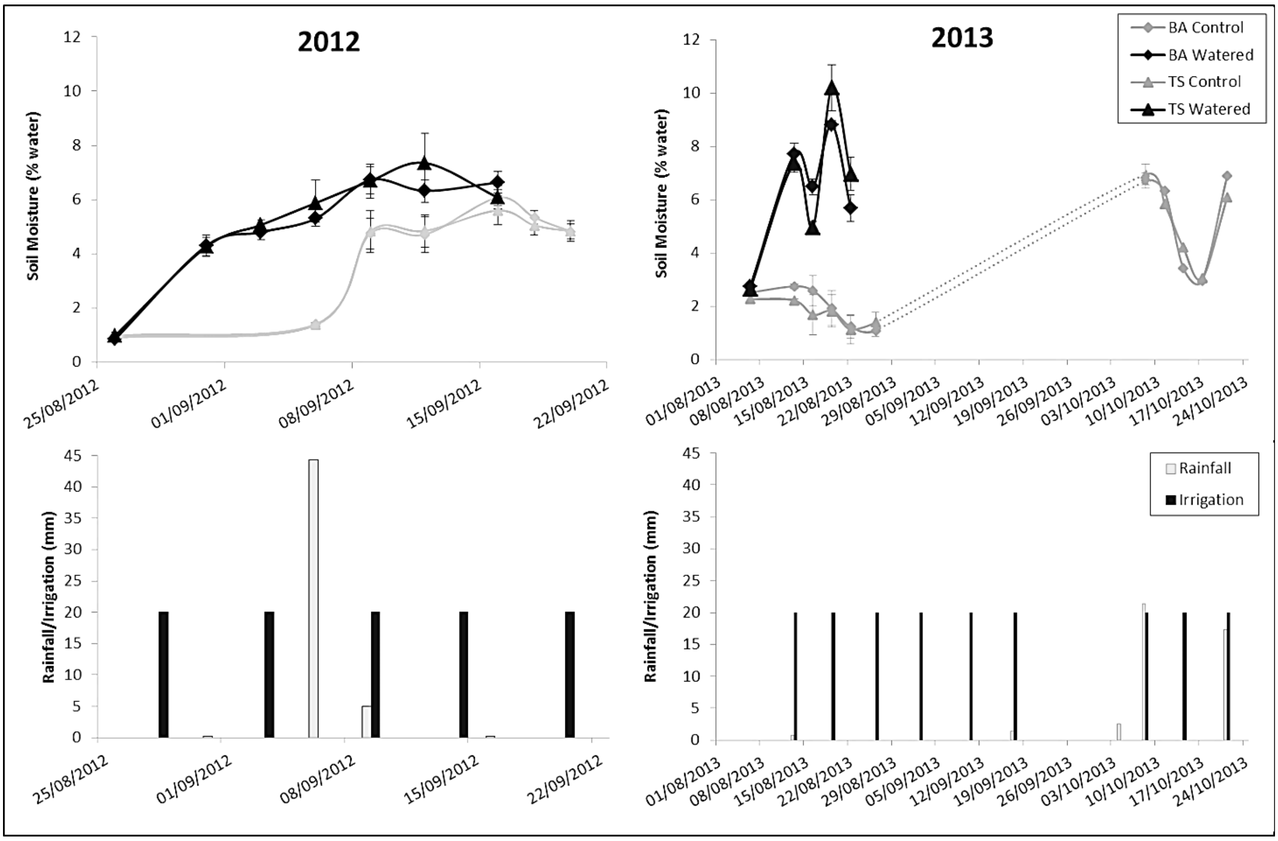

Figure 2. The soil moisture (top row) and water input via rainfall and irrigation (bottom row) at the start of the growing seasons of 2012 and 2013. Soil moisture under watered and control (unwatered) *Burkea africana* (BA) and *Terminalia sericea* (TS) trees was measured in relation to natural rainfall and irrigated trees.

4.3.Tree phenology

The phenology of the watered and control *B. africana* (Figure 3) and *T. sericea* (Figure 4) trees were highly variable between seasons. Mean phenological scores for each leaf age class showed a generalized trend for each phenophase across seasons, however the commencement and duration of these phenophases shifts from year to year (Figures 3 and 4). There is also evidence that *T. sericea* is producing new leaves throughout the growing season (Figure 4), while *B. africana* maintains a single set of leaves which mature without replacement (Figure 3).

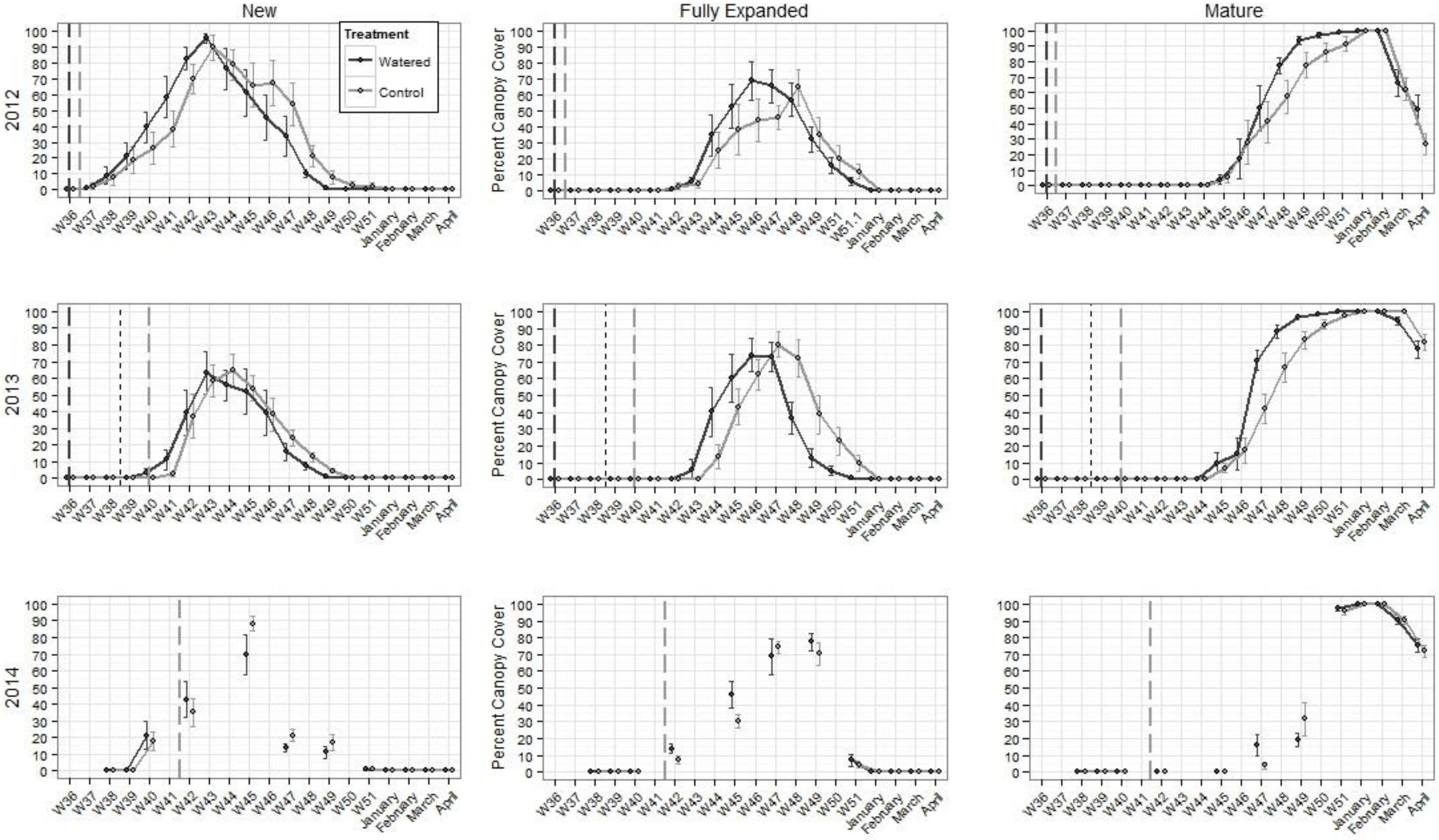

Figure 3. Three seasons of *Burkea africana* phenology (means±SE) of watered (black) and control (grey) treatment trees with new, fully expanded and mature leaves measured for percent canopy coverage at weekly intervals during the green-up period (September – November) and monthly intervals during the second half of the growing season (December – April). Time in week number and months is indicated on the x-axis. Each row corresponds to the year written on the far left axis. Grey long-dashed lines indicate the start of seasonal rainfall, black long-dashed lines indicate the start of irrigation, with the black dotted line indicating when a severe fire occurred in 2013.

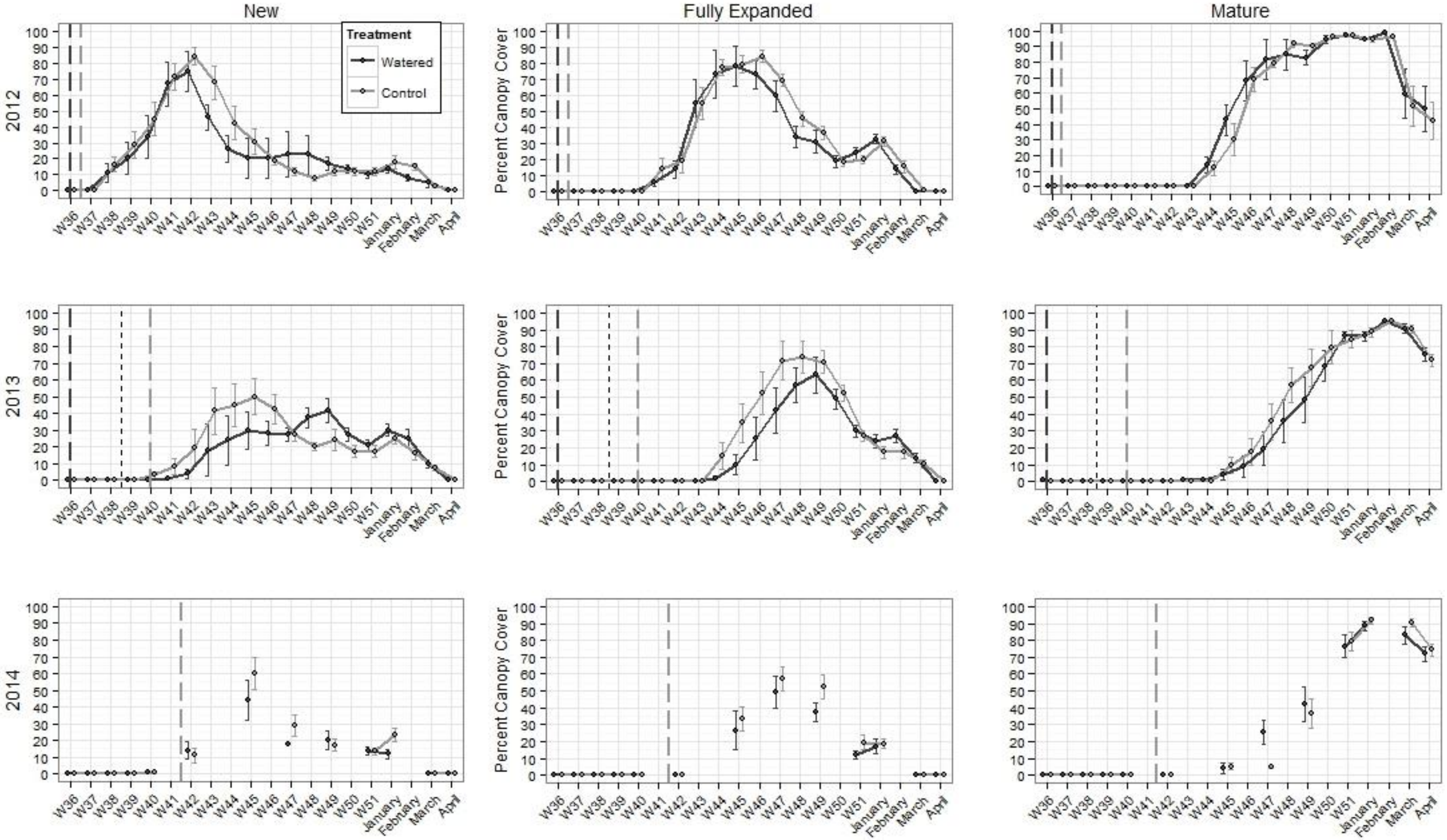

Figure 4. Three seasons of *Terminalia sericea* phenology (means±SE) of watered (black) and control (grey) treatment trees with new, fully expanded and mature leaves measured for percent canopy coverage at weekly intervals during the green-up period (September – November) and monthly intervals during the second half of the growing season (December – April). Time in week number and months is indicated on the x-axis. Each row corresponds to the year written on the far left axis. Grey long-dashed lines indicate the start of seasonal rainfall, black long-dashed lines indicate the start of irrigation, with the black dotted line indicating when a severe fire occurred in 2013.

4.4.Summarizing the green-up periods over three seasons

No differences between treatments within years was found for mean green-up start dates (GUSDs) (Wilcox Tests: $p>0.05$, Table 1). A difference within and between species was found between years, with *B. africana* trees experiencing significantly later GUSDs in 2013 (Kruskal-Wallis: H=21.44, d.f.=2, $p<0.001$) and *T. sericea* trees experiencing significantly earlier GUSDs in 2012 (Kruskal-Wallis: H=25.67, d.f.=2, $p<0.001$, Table 1). The watered *T. sericea* trees had the latest average GUSDs during the 2013 season possibly due to the fire in September 2013.

The average green-up period for all trees in each treatment over the three seasons showed no differences (Kruskal-Wallis: H=1.14, d.f.=3, p=0.77) with watered *B. africana* taking 8.4±0.5 weeks and control 7.9±0.4 weeks. The watered *T. sericea* trees took 7.9±0.3 weeks relative to control trees which took 8.0±0.3 weeks to reach maturity (Table 1). A difference between treatments and years was found (Kruskal-Wallis: H=45.26, d.f.=11, $p<0.001$), but this was only specifically between *B. africana* watered trees in 2012 and 2013 ($p<0.05$), as well as 2013 and 2014, and control trees in 2013 and 2014 ($p<0.05$), all other treatments showed no overall difference (Table 1). The 2013 green-up periods were noticeably faster in both species possibly due to effects from the fire. Slow green-up in both species was observed in 2014, possibly as a result of the late arrival and low amounts of rainfall during that season (Figure 1, Table 1).

Differences between years and treatments were observed in the new leaf phenophase (Kruskal-Wallis: H=45.93, d.f.=11, $p<0.001$), but specific differences were only detected between the 2012 and 2013 control *B. africana* trees, the 2012 and 2013 watered *B. africana* trees, as well as the 2013 and 2014 *T. sericea* trees. The phenophases for new leaves lasted the longest in 2012 for watered *B. africana* trees and in 2013 for watered *T. sericea* trees (Table 1). An overall difference was found between treatments and years in the fully expanded leaf phenophase (Kruskal-Wallis: H=25.96, d.f.=11, p=0.007), however, no specific differences were found in the post-hoc analyses (Table 1).

Table 1. Phenological summaries of the watered and control *Burkea africana* and *Terminalia sericea* trees at Nylsvley Nature Reserve between 2012-2014. Green-up start dates were recorded when all trees in a treatment had at least 5% canopy cover of new leaves. Phenophases were calculated for new leaves as the first day of leaf flush to >50% canopy cover of fully expanded leaves and then for fully expanded until there was >50% canopy cover of mature leaves. Estimates of the mature leaf phenophase were calculated based on the number of days in months where >50% canopy cover of mature leaves was present. No irrigation took place during 2014 but the same *watered* trees were still analysed separately.

Species	Year	Treatment	Rainfall start date	Green-up start date	Average total number of weeks to reach 50% mature canopy	Phenophase (No. of days)		
						New to Fully Expanded	Fully Expanded to Mature	Mature to Senescing (Estimate)
Burkea africana	2012	Watered	7-Sep	20-Sep±2.9	9.2±0.4	56±4.78	22.2±2.15	141
		Control		21-Sep±3.8	8±0.6	52.5±3.74	19.3±1.75	141
	2013	Watered	7-Oct	06-Oct±5.7	5.7±0.21	31.5±1.87	18.67±2.33	155
		Control		07-Oct±1.7	6.1±0.35	31.5±3.5	22.75±1.15	134
	2014	*Watered*	11-Oct	28-Sep±1.7	10.3±0.21	39.67±2.95	22.17±2.95	157
		Control		30-Sep±2.2	9.6±0.56	42.9±1.58	25.4±1.84	157
Terminalia sericea	2012	Watered	7-Sep	18-Sep±3.9	7.7±0.42	37.3±3.46	22.2±2.81	155
		Control		14-Sep±1.1	8.4±0.32	42±2.65	29.8±1.15	124
	2013	Watered	7-Oct	20-Oct±4.2	7.8±0.65	53.7±2.95	24.5±2.39	157
		Control		11-Oct±4.2	6.9±0.55	42±4.27	30.6±2.63	166
	2014	*Watered*	11-Oct	11-Oct±2.5	8.2±0.6	32.7±2.33	22.2±1.17	157
		Control		14-Oct±3.1	8.8±0.53	35.9±2.45	23.6±2.27	157

4.5.How variable was phenology between seasons?

Variability between seasons was measured by comparing the percent canopy cover of different leaf age classes within a species over time. Differences between years were significant for all leaf age phenophases (Table 2). There was a highly significant interaction between weeks within each season and between years for both species, indicating that the phenology from each leaf age class showed a difference during a specific week in each year (Table 2).

4.6.Did the addition of water affect tree phenology?

The mean heights of trees were measured before the addition of water in 2012 and then after the addition of two seasons of irrigation and three seasons of rainfall in 2015 (Figure 5A). All treatments showed an increase in height after three years with the most significant increase in the control *T. sericea* trees (1.07±0.25 m, Wilcox Test: W=13, d.f.=14, p=0.05). The watered *T. sericea* did not show a significant increase (0.99±0.17 m, Wilcox Test: W=11, d.f.=10, p=0.31), nor did either of the *B. africana* treatments (Watered: 0.92±0.39 m, Wilcox Test: W=11, d.f.=10, p=0.31; Control: 1.17±0.3 m, Wilcox Test: W=19, d.f.=14, p=0.19, Figure 5A). None of the stem diameters showed any significant changes between 2012 and 2015 (Wilcox Tests: p>0.05, Figure 5B). The watered trees, however, did show an average increase in both species, with the *B. africana* trees having the largest average increase (1.79±1.7 cm, Figure 5B).

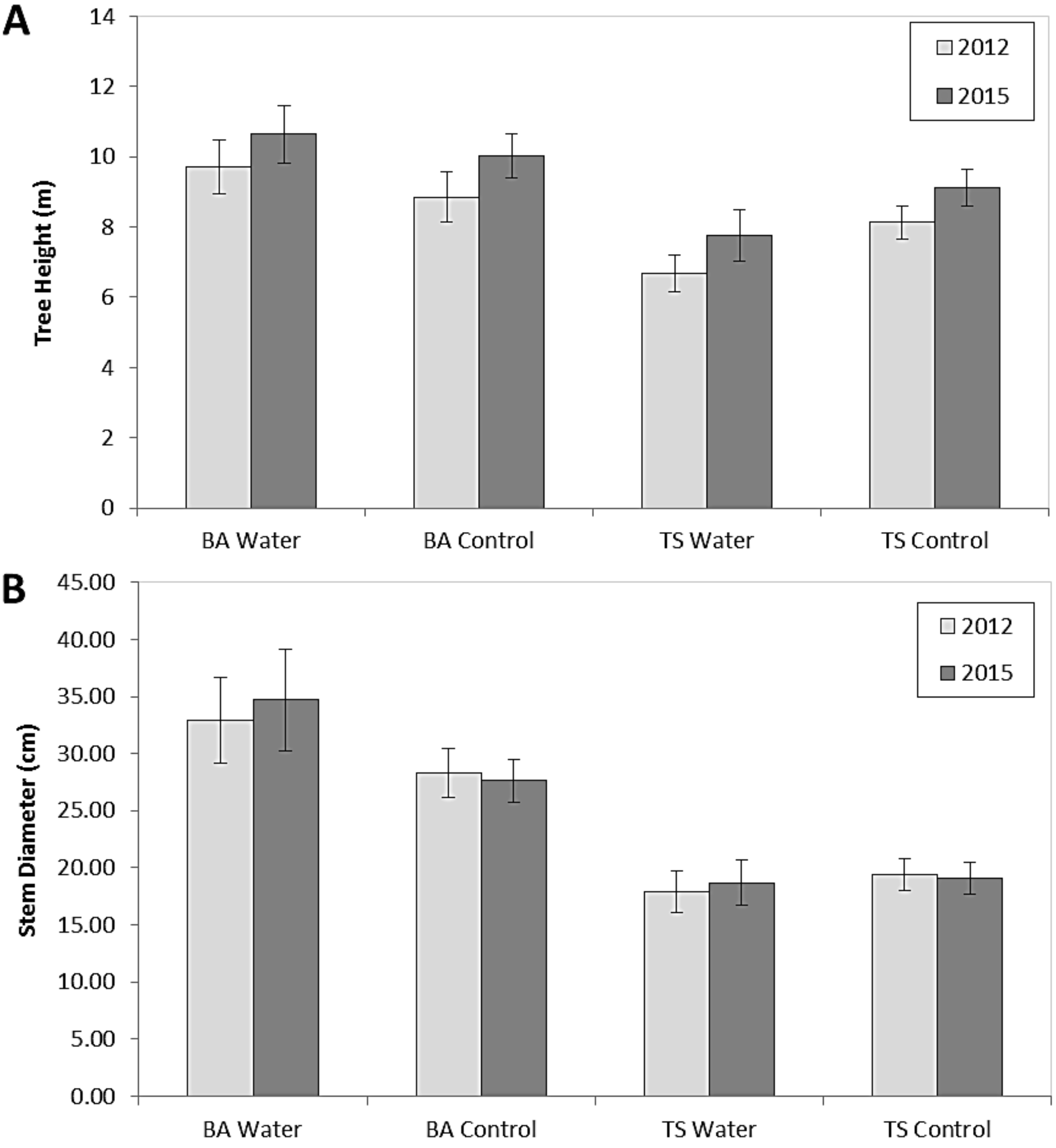

Figure 5. The mean±SE tree heights (A) and stem diameters (B) measured during Week 35 of 2012 and 2015 for the watered and control (unwatered) *Burkea africana* (BA) and *Terminalia sericea* (TS) trees in the Nylsvley Nature Reserve.

There was a significant interaction between treatment and week in both the new and fully expanded *T. sericea* leaf age classes, confirming that through the addition of water, the study was able to shift green-up in this facultative-greening species. Differences in *B. africana* were only observed in the fully expanded and mature leaf age classes, which may indicate that this early-greening species has unlinked the cue for commencing green-up of new leaves from water availability (Table 2). Somewhat counter-intuitively in 2013 the control *T. sericea* trees commenced green-up a week earlier than the watered trees and reached full maturity almost a week faster on average (Figure 4, Tables 1 and 2). This difference may be related to the effects of the 2013 fire – as the watered trees had potentially already become physiologically active and were thus more vulnerable to the damaging effects of the fire.

4.7.Did rainfall influence green-up start dates?

The *T. sericea* trees GUSDs showed a strong positive relationship with rainfall arrival, possibly as a result of this species largely facultative green-up strategy (Regression analysis: Control: R^2=0.74, $F_{1,22}$=61.22, $p<0.001$; Watered: R^2=0.64, $F_{1,16}$=28.29, $p<0.001$, Figure 6). The *B. africana* trees GUSDs were also positively, but less strongly correlated with rainfall start dates indicating that this species phenology is not totally-independent of rainfall, particularly in years when rainfall arrives early as seen in 2012 (Regression analyses: Control: R^2=0.31, $F_{1,22}$=9.8, p=0.0049; Watered: R^2=0.39, $F_{1,16}$=10.51, p=0.0051, Figure 6).

Table 2. The outputs of two-way repeated measures ANOVAs for the different leaf age classes of two broad-leaved savanna trees in the Nylsvley Nature Reserve, South Africa between the 2012 to 2014 growing seasons. Factor variables describe Treatment as watered or control trees, Week as the week number of each observation within a growing season and Year as the three growing seasons, 2012-2014. (**p<0.01, ***p<0.001, NS-not significant).

Species	**Leaf Age Class**	**Factor Variables**	**F-value**	**D.F.**	**p-value**	**Significance**
Burkea africana	New	Treatment	0.391	1	0.544	NS
		Year	35.861	2	<0.001	***
		Week	70.187	18	<0.001	***
		Treatment*Year	0.072	2	0.931	NS
		Treatment*Week	0.943	18	0.527	NS
		Year*Week	5.626	28	<0.001	***
		Treatment*Year*Week	0.346	28	0.999	NS
	Fully Expanded	Treatment	1.316	1	0.274	NS
		Year	8.116	2	0.002	**
		Week	74.037	18	<0.001	***
		Treatment*Year	2.493	2	0.1038	NS
		Treatment*Week	2.027	18	0.0096	**
		Year*Week	4.173	28	<0.001	***
		Treatment*Year*Week	0.838	28	0.7072	NS
	Mature	Treatment	2.421	1	0.146	NS
		Year	14.994	2	<0.001	***
		Week	433.097	18	<0.001	***
		Treatment*Year	0.602	2	0.556	NS
		Treatment*Week	2.231	18	0.0037	**
		Year*Week	19.264	28	<0.001	***
		Treatment*Year*Week	1.087	28	0.352	NS
Terminalia sericea	New	Treatment	8.651	1	0.0123	*
		Year	10.287	2	<0.001	***
		Week	28.756	18	<0.001	***
		Treatment*Year	0.105	2	0.900	NS
		Treatment*Week	2.026	18	0.0111	*
		Year*Week	13.773	28	<0.001	***
		Treatment*Year*Week	0.467	28	0.979	NS
	Fully Expanded	Treatment	2.715	1	0.125	NS
		Year	59.027	2	<0.001	***
		Week	87.406	18	<0.001	***
		Treatment*Year	0.082	2	0.922	NS
		Treatment*Week	2.088	18	0.0085	**
		Year*Week	14.647	28	<0.001	***
		Treatment*Year*Week	0.646	28	0.882	NS
	Mature	Treatment	0.011	1	0.918	NS
		Year	23.167	2	<0.001	***
		Week	130.68	18	<0.001	***
		Treatment*Year	1.858	2	0.178	NS
		Treatment*Week	0.47	18	0.968	NS
		Year*Week	24.219	28	<0.001	***
		Treatment*Year*Week	1.294	28	0.174	NS

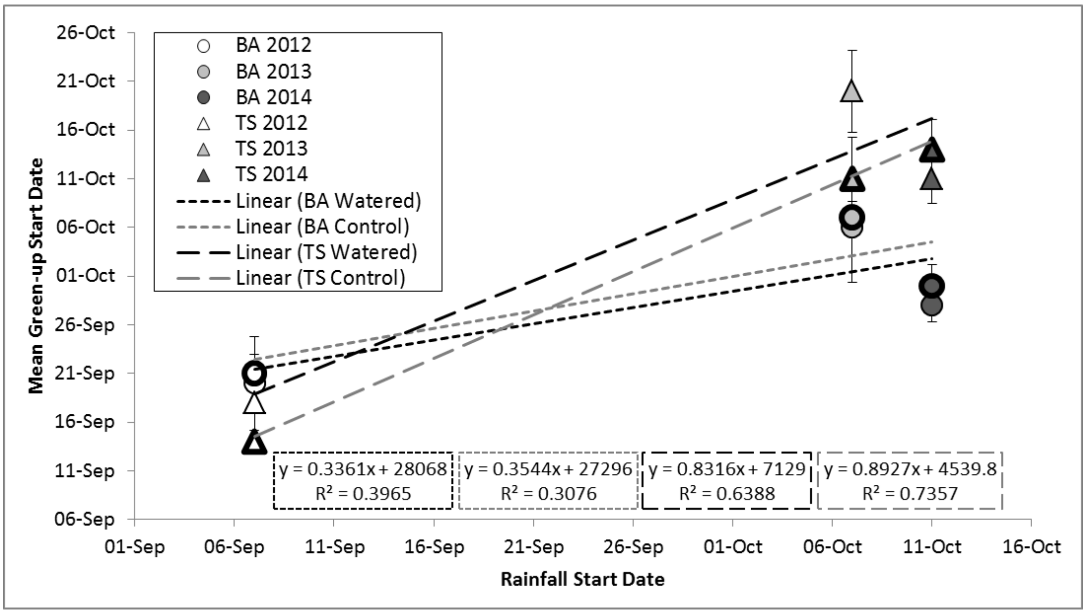

Figure 6. The start date of seasonal rainfall in relation to the mean±SE green-up start dates for watered (symbols with thin outline) and control (thick outline) *Burkea africana* (BA) and *Terminalia sericea* (TS) trees between 2012-2014. Linear regressions (calculated from the full set of data) for each treatment are illustrated with corresponding equations and regression values in boxes at the bottom of the figure.

5. Discussion

5.1.The variability of savanna tree phenology

High variability in broad-leaved savanna tree phenology over the three study seasons was shown, with changing rainfall regimes and fire disturbances further contributing to the inconsistency in leaf phenology observed. Few studies have shown this level of phenological variability in savanna systems despite several mentions of it in the literature (Huntley and Walker, 1982; Scholes and Archer, 1997; Poulter and Cramer, 2009; Higgins et al., 2011; Buitenwerf et al., 2015). High variability was detected in the new leaf phenophase at the beginning of the green-up periods for both species. *Burkea africana* commenced green-up consistently earlier than *T. sericea* during seasons when rainfall began during October (2013 and 2014); however, during the 2012 season when rainfall began early in September, the *T. sericea* trees were able to green-up faster. *Burkea africana* is often reported as an early-greening species in studies from Zimbabwe (Childes, 1989), Zambia (Chidumayo, 2001) and throughout West African savannas (De Bie et al., 1998). Early-greening in *B. africana* trees was only recorded during the 2014 season when rainfall arrived later than the previous two seasons. This may indicate that the cue for *B. africana* green-up to commence is unlinked from the main savanna system driver of water availability (Chidumayo, 2001; Elliott et al., 2006; Archibald and Scholes, 2007). Do et al. (2005) have investigated the influence of different atmospheric conditions on green-up in savannas and suggest that possible links to vapour pressure deficit in the atmosphere may be responsible for the onset of early-greening. During the 2012 early-rainfall season, *B. africana* green-up occurred shortly after the onset of rainfall confirming that early-greening species are able to adapt to suitable environmental conditions if needed, however, the green-up of this species was slower in comparison to the more facultative *T. sericea* trees. There is evidence to suggest that early-greening trees commence green-up ahead of seasonal rainfall in order to outcompete faster growing facultative-greening trees and grasses for available water and nutrients when seasonal rainfall begins (Elliott et al., 2006; Scholes and Walker, 1993). The rapid green-up seen in *T. sericea* during the 2012 season supports this theory. *Terminalia sericea* is described as a facultative-greener with the ability to green-up rapidly after the onset of seasonal rainfall but occasionally greening up to two weeks prior to the arrival of rainfall (Childes, 1989; Moyo et al., 2015). During the 2014 season when rainfall arrived late, the previously watered trees – despite not receiving irrigation during that season – commenced green-up simultaneously with the arrival of seasonal rainfall, while control trees greened-up four days thereafter. Green-up of *T. sericea* trees was facultative in both the 2012 and 2013 seasons confirming the importance of water availability in driving the green-up of this species.

5.2.The influence of water on the variability of phenology

An irrigation experiment was used to test the effects of water on green-up. The data showed differences between the watered and control trees for all *T. sericea* treatments and leaf age classes over time, however, only differences in the fully expanded and mature age classes of *B. africana* treatments were detected (Table 2). No significant carry over effects of the watering were observed during the 2014 season when trees were not irrigated prior to the onset of rainfall (Table 2), however, slower green-up rates were observed in both species treatments compared to the previous seasons (Table 1). The irrigation system was installed close to the base of the tree (within 2 m) and the low direct treatment effect (Table 2) suggests that insufficient water was added to surpass the rooting systems of the neighbouring grasses (discussed in Whitecross et al. 2017 – *see Chapter 3*), to influence the overall phenology of the trees during the 2012 season. Rutherford (1983) conducted an extensive study into the rooting systems of both *B. africana* and *T. sericea* in the NNR and found that *B. africana* has an extensive surface root system which occurs at approximately 0.5-0.6 m below the surface and can reach distances of 20 m from the base of the tree. The *T. sericea* root systems mainly grow in the upper layers of the soil and are particularly well developed, ranging from 12-35 cm deep and 3.6-6.6 m in length from the base of the tree (Rutherford 1983). The network of shallow, lateral roots in both species are designed to capture moisture in the upper layers of soil in conjunction with deep soil tap-roots that are able to access the water table well below the surface of the soil when rainfall is scarce (Rutherford and Panagos, 1982; Rutherford, 1983; February and Higgins, 2010; Verweij et al., 2011). *Terminalia sericea* has been shown to adapt its rooting strategies based on the environmental conditions of an area, increasing its shallow-root network when growing in more arid conditions (Hipondoka and Versfeld, 2006; Verweij et al., 2011). These shallow-root networks enable *T. sericea* to compete effectively with grasses at the onset of seasonal rainfall, while *B. africana* trees rely more on their deeper root networks to provide them with the necessary water needed for early-greening (Rutherford and Panagos, 1982; Rutherford, 1983; Hipondoka and Versfeld, 2006).

Chidumayo (2015) found that irrigation of broad-leaved tree saplings during the dry season had a significant positive effect on the monthly and annual growth rates of watered saplings compared to control plants in a Zambian savanna, due to their higher proportion of lateral roots and possible lack of access to deep, subsoil water. However, not all of the irrigated species responded with earlier onset of the growing season when additional water was provided (Chidumayo, 2015). Given that this study focused on established adult trees, it is not surprising that irrigation had smaller effects on the overall phenology of the trees. Moyo et al. (2015) tested the effects of irrigation on coppicing *T. sericea* in the lowveld and found that water additions in conjunction with additional nutrients increased the presence of leaves on the canopy at the end of the growing season by ~30%. Water additions also doubled the fruit yield and significantly increased leaf production during the growing season. While irrigation during the 2012 season in this study did not appear to affect green-up of *T. sericea*, during the 2013 season, a difference in the onset and rate of green-up was observed between the two treatments with the watered trees taking ~10 days longer to commence green-up and over a week longer to reach full maturity than the control trees (Table 1). Given that *T. sericea* has been shown to react to irrigation (Moyo et al., 2015), it is possible that the watered trees had already commenced the internal physiological processes required for the flushing of new leaves, rendering them vulnerable to the effects of the severe 2013 fire, while the control trees - still in the dormant phase - were less at risk in comparison.

5.3.Fire's effect on savanna phenology

Fire is a well-studied driver in southern African savannas (Higgins et al., 2007; Holdo, 2007; Bond, 2008; Lehmann et al., 2014), however, many of these studies tend to focus on the impacts that fire has on vegetation structure and composition, rather than its potential effect on plant phenology. Wilson and Witkowski (2003) showed that larger *B. africana* individuals are able to tolerate the effects of fire by having thicker bark, whilst Higgins *et al*. (2007) showed that *T. sericea* trees are not excluded from an area if fire intensity and frequency are too high, but will tend to remain small and coppice. Nefabas and Gambiza (2007) showed that *B. africana* and *T. sericea* differ in their tolerance strategies for disturbances by fire, with *B. africana* relying on thick bark and *T. sericea* relying on a rapid resprouting strategy. The majority of the *B. africana* trees included in this study had stem circumferences >400 mm, placing them in the fire resistant size class, which is possibly why this species showed less impact during green-up after the fire. The larger average heights of *B. africana* trees relative to *T. sericea* trees are a probable explanation for their lower overall damage post-fire as well. The high intensity of the fire inferred from the scorch heights suggests that the *T. sericea* canopies may have had up to two thirds of their canopies directly affected by the flames and that is excluding the additional heat damage caused above the flame height (Trollope et al., 2002). The stem circumferences of *T. sericea* were also small in comparison to the *B. africana* trees, placing them under higher risk of fire damage to their main stems. In South Africa, fires have been shown to occur mainly in the dry season months of July, August and September with slightly later fires burning in October in the southern-most parts of the country (S. Archibald et al., 2010). In protected areas, particularly those such as Nylsvley where management have actively excluded fire from the system, large quantities of fuel biomass have accumulated and have the potential to facilitate high intensity burns if environmental conditions on the day are suitable (Govender et al., 2006). The occurrence of fires in protected savanna systems tend to be most frequent in the late dry season and this has associated risks for vegetation employing an early-greening strategy (Govender et al., 2006; Holdo, 2007; S. Archibald et al., 2010). New flush of early-greening species may be exposed to direct burning or heat damage if caught in a late season fire and this could have disadvantageous physiological consequences for the affected trees.

Rutherford (1981) monitored woody biomass during a controlled burn at the NNR and found that fires did have the ability to affect canopy growth the following season in small individuals (<3 m) of both species, but not for larger trees. Rutherford (1981) observed little mortality of adult trees and no mortality was recorded during this study either, confirming once again the theory that savanna trees need to invest in fire tolerance/resistance to survive in Africa. The green-up rates of both the watered and control *B. africana* in 2013, the fire year, were the fastest observed during the study period. This may be a result of the decrease in above-ground biomass, especially herbaceous biomass, and the rapid release of available nutrients after the fire with little competition from grasses and other woody biomass until the arrival of rainfall a few weeks later (Rutherford, 1981; Gandar, 1982; Scholes and Archer, 1997; S. Archibald et al., 2010).

5.4.Phenology for the future

Two recent publications have called for long-term ecological monitoring of southern hemisphere ecosystems (Fitchett et al., 2015; Lindenmayer et al., 2015). There are few consistent, long-term savanna monitoring projects in southern Africa focused on phenology, apart from the odd citizen scientist's garden observations (Dave Thompson *pers. comm.*) and a handful of two to three year studies spread out across the continent over the last four decades (Rutherford, 1983; Owen-Smith and Cooper, 1987; Childes, 1989; Chidumayo, 2001; Archibald and Scholes, 2007; Ryan, 2009; Moyo et al., 2015). Phenological responses have been shown to be one of the most easily measured and precise indicators of the effects that changing climatic variables may have on vegetation across the planet (Schwartz, 1998; Menzel, 2002; Cleland et al., 2007). From a vegetation modelling point of view, a comprehensive understanding of what is driving leaf phenology of southern Africa's savannas is lacking and through consistent monitoring of these phenological changes, ecologists stand a much greater chance of improving that understanding in the future. Examples of long-term monitoring and experimental sites do exist in South Africa – such as the >60 year replicated burn plots in the Kruger National Park (Higgins et al., 2007) and the >50 year multiple-catchment studies at Cathedral Peak (Scott et al., 2000), and there are plans to develop a network of terrestrial observation sites across the country. This study strongly advocates that long-term phenological monitoring be incorporated into this proposed network.

5.5.Conclusions

The results of this study confirm published information that the leaf phenology of *T. sericea* is generally driven by rainfall patterns, whereas *B. africana* leafing is somewhat independent of the seasonal patterns of rainfall. However, large differences in the green-up between the *T. sericea* treatments during the 2013 season, when a late season fire occurred, further emphasizes the complex environmental interactions vegetation undergoes in savannas. Both species showed the ability to adapt to early onset of rainfall during the 2012 season, with *B. africana* showing early-greening during the 2014 season when rainfall arrived late. The high variability observed in the savanna tree phenology over the three study seasons emphasizes the need for more, long-term phenological studies to enable ecologists to predict with more certainty, how this system is currently responding to climatic conditions and may respond to future climatic changes.

6. Acknowledgements

We would like to thank the following institutions for their financial support of this project: the Centre for Tree Health Biotechnology (CTHB), the National Research Foundation (NRF) and the Centre for Scientific and Industrial Research (CSIR). Thanks to Natasha du Plessis and the staff at the Nylsvley Nature Reserve. Thanks to Mainline Irrigation and Gert Le Roux for assistance with the planning, development and support of the irrigation experiment. Thanks to Working on Fire South Africa for the loan of their ‘bakkie sakkie’ water tank and pump. Thanks to the following individuals for their help and support in the field: Robert Whitecross, Helenor Whitecross, Caroline Howes, Wesley Hattingh, Giuseppe Venturi, Kirstin Olsen, Marcelle Johnson, Vidosava Jakovljevic, Isabelle Inder, Ryan Thomas, Nadine Cronk and Roxy for many hours spent by my side.

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CHAPTER 3

Savanna tree-grass interactions: a phenological investigation of green-up in relation to water availability over three seasons.

This chapter has been published in the South African Journal of Botany

Whitecross, M.A., Witkowski, E.T.F., and Archibald, S. (2017) Savanna tree-grass interactions: a phenological investigation of green-up in relation to water availability over three seasons. *South African Journal of Botany*, 108: 29-40.

Melissa Whitecross was responsible for the design of the study, collection and analyses of the data. She also wrote up the paper which was reviewed by her supervisors Prof. Ed Witkowski and Prof Sally Archibald prior to submission.

1. Abstract

Phenology of African savannas is considered to have high temporal variability, yet few studies have quantified this variation between seasons. This study assessed the weekly green-up phenology of trees, as well as below- and between-canopy grasses in a broad-leaved savanna woodland in the Nylsvley Nature Reserve (NNR), South Africa over three growing seasons (2012-2014). During September 2013 a severe fire burnt through the study site. Tree green-up start dates were highly variable in comparison to the grasses, whose green-up showed close ties to the availability of water, particularly rainfall. Early green-up of *Burkea africana* trees occurred if rainfall onset was after mid-October, thus long-term rainfall records indicate that trees would benefit from early-greening approximately 46% of the time in the NNR. The effects of tree canopies on the growth and biomass accumulation of below-canopy grasses showed that during periods of irrigation when water was not limited, light availability became the limiting factor for grass growth, with grasses below the higher shading of *B. africana* producing significantly lower biomass than those below the less shaded *Terminalia sericea* canopies. Access to higher light conditions at the start of the growing season potentially drives the 2-5 days faster green-up of below-canopy grasses compared to the between-canopy grasses. A comparison of the above phenological data to the remotely sensed normalized difference vegetation index (NDVI) was made, to determine if it was possible to detect an early-greening signal from the trees, which the sensor was able to effectively distinguish. This study highlights the variability in temporal separation between tree and grass phenology in an African savanna. Trees take advantage of periods of low competition from herbaceous neighbours at the end of the dry season prior to the onset of seasonal rainfall, and potentially at the end of the growing season when seasonal rainfall concludes through the uncoupling of their green-up cue from seasonal water availability.

Keywords: canopy effects, fire, phenology, tree-grass interactions, water addition

2. Introduction

Understanding and investigating phenology of ecosystems is important for identifying any future changes in response to changing climates (Menzel, 2002; Cleland et al., 2007; Polgar and Primack, 2011; Chambers et al. 2013; Buitenwerf et al., 2015; Fitchett et al., 2015). Phenology is the study of the timing of biological events, for example the date at which trees commence the flushing of new leaves each year (Lieth, 1974). The phenology of African savannas has been shown to be unpredictable, partly due to the high variability in seasonal rainfall which acts as one of the major environmental drivers of green-up in this ecosystem (Eamus et al., 1999; Lehmann et al., 2011, Whitecross et al., 2016 - *see Chapter 2*). Savannas are most broadly defined as a mixture of a homogeneous grass layer with a discontinuous tree layer which can vary in density (Huntley and Walker, 1982; Scholes and Archer, 1997). Tree/grass ratios in savannas are driven by several abiotic factors including climate, geology and topography, as well as disturbances such as fire, herbivory and frost (Huntley and Walker, 1982; Scholes and Walker, 1993; Whitecross et al., 2012; Lehmann et al., 2014). Numerous studies have investigated the physical impacts of disturbances such as fire on savanna vegetation (Gandar, 1982; Higgins et al., 2000; Kennedy and Potgieter, 2003; Wilson and Witkowski, 2003; Furley et al., 2008; Ryan and Williams, 2011), however, only a few studies have shown the impacts of these disturbances on savanna plant phenology (Coutinho, 1982; Trollope 1982; Whitecross et al. 2016 – *see Chapter 2*). Wade and Johansen (1986) proposed the 'tree physiology hypothesis' wherein seasonal changes in tree physiology play an important role in influencing trees' susceptibility to fire. Kennedy and Potgieter (2003) supported this hypothesis when they showed that in the dry season (May to August) dormant broad-leaved deciduous trees were more tolerant of fires, whereas in the early growing season (September-October) when physiological activity increased, so too did the risk of fire-related damage. Understanding the phenology of savannas is difficult as the two dominant life-forms – trees and grasses – show different seasonal dynamics and seem to respond to different environmental cues (Rutherford and Panagos, 1982; Childes, 1989; De Bie et al., 1998; Archibald and Scholes, 2007; Higgins et al., 2011; Buitenwerf et al., 2015).

The phenology of semi-arid savanna vegetation is closely linked to the availability of water, with deciduous vegetation flushing new leaves after the onset of the first substantial (>15 mm) seasonal rainfall event (Huntley and Walker, 1982; Scholes and Archer, 1997; Chidumayo, 2001; Jolly and Running, 2004; Archibald and Scholes, 2007; Hachigonta et al., 2008). The arrival of these storm systems is highly variable both temporally and spatially between seasons (Huntley and Walker, 1982). Although the first rains represent the end of the “dormant season” in these tropical and sub-tropical systems, some tree species are able to flush new leaves prior to the arrival of seasonal rainfall which enables them to grow during a period of limited competition for resources from their herbaceous and non-early-greening woody neighbours (Childes, 1989; Scholes and Walker, 1993; De Bie et al., 1998; Chidumayo, 2001; Higgins et al., 2011; February and Higgins 2016; Whitecross et al., 2016). Unlike trees, the phenology of savanna grasses is closely linked to water availability (Dye and Walker, 1987; Prins, 1988; Scholes and Walker, 1993; Archibald and Scholes, 2007; February et al., 2013).

The patchy distribution of trees across the savanna landscape results in grasses growing either below or between tree canopies. Grasses found below canopies benefit from a concentrated source of nutrients through litterfall and an improvement in water use as temperatures are lower under the canopy’s microclimate (Belsky, 1994; Durr and Rangel, 2000; Ludwig et al., 2001). However, below-canopy grasses face reduced light availability through shading and direct competition with trees for available resources (Knoop and Walker, 1985; Scholes and Archer, 1997; Ludwig et al., 2001; February et al., 2013). Light-limited grasses growing below canopies have shown differences in growth rates, biomass accumulation and expansion of leaf area compared with between-canopy grasses (Belsky, 1994; Durr and Rangel, 2000; Ludwig et al., 2001). Grasses growing between canopies experience less competition from trees and compete directly with other grasses for limited nutrients and space to grow (Rutherford, 1983; Belsky, 1994). Few studies have investigated the effect of tree canopies on the phenology of below- and between-canopy grasses (Dye and Walker, 1987; Prins, 1988), with the majority of the research tending towards investigations into growth and biomass accumulation (Scholes and Walker, 1993; Belsky, 1994; Ludwig et al., 2001). Understanding the effect that canopies have on grass phenology can assist in furthering knowledge about phenological dynamics in the complex savanna ecosystems.

Many studies demonstrate the usefulness of long-term remotely sensed indices of vegetation greenness from AVHRR and MODIS data for tracking phenological shifts (Myneni et al., 1997; Schwartz, 1998; Poulter and Cramer, 2009; Richardson et al., 2013; Buitenwerf et al., 2015). However, remote sensing of phenology is most useful when integrated with field measurements which can validate and inform the vegetation indices acquired from satellite data (Fitchett et al., 2015). This is particularly important in systems with high ecosystem-level variation in phenological patterns, such as savannas, where the coarse scale of the remotely sensed data often obscures much of the system dynamics such as the specific greening of trees or grasses (Scholes and Walker, 1993; Chidumayo, 2001;

Scanlon et al., 2002; Jolly and Running, 2004; Simioni et al., 2004; Archibald and Scholes, 2007; Higgins et al., 2011). In these situations long-term field measurements and phenological networks are still essential but are currently lacking in the majority of the world's savannas, particularly those in Africa (Chambers et al., 2013).

Without detailed ground-based studies it will be challenging to develop generalised theories on the phenology of savannas and their different life forms. Few studies have shown the phenological changes which occur in savannas at more than a monthly temporal scale, which often obscures complex processes occurring rapidly at the start of the season (Childes, 1989; De Bie et al., 1998; Scholes and Walker, 1993; Chidumayo, 2001). The aim of this study was to investigate the relative responsiveness of trees and grasses in a broad-leaved savanna to the timing of water availability at a weekly scale over the 2012, 2013 and 2014 green-up periods (August – December). WeThis study compared differences in growth onset and rates of below- and between-canopy grasses and tested the response of grasses when additional water was provided through irrigation. It was hypothesized that additional water would result in an earlier onset of grass growth and faster growth rates. A comparison of the onset of green-up between the trees and grasses was conducted and the findings were compared to remotely sensed normalized difference vegetation index (NDVI) data to determine whether the onset of green-up was accurately detected by the satellite imagery. Through this study it is hoped that a further the understanding of how these coexisting floral life-forms are competing at various temporal and spatial scales will be developed.

3. Materials and methods

This study was run in conjunction with Whitecross et al.'s (2016) study and conducted from the beginning of August 2012 until the end of May 2014. Full details regarding the layout of the irrigation and tree phenology aspects of the study are reported in Whitecross et al. (2016).

3.1.Study site

This study was conducted in the broad-leaved savanna of the Nylsvley Nature Reserve (NNR), Mookgophong, Limpopo Province, South Africa (24°39' S, 28°42' E; 3975 ha). The vegetation type is classified as central sandy bushveld within the savanna biome (Rutherford et al., 2006). The NNR has a seasonal climate with hot, wet summers in which an average of 85% of the 623 mm mean annual precipitation (MAP) falls between October and March (Huntley and Morris, 1982). Further details on the rainfall during the study period are presented below. On the 19th September 2013, a severe unplanned fire burnt 85% the NNR, damaging many adult trees and incinerating all aboveground grass (and herbaceous) biomass (more details in Whitecross et al. 2016). The irrigation system was also destroyed, but reinstalled one week thereafter. The study area was fenced to remove the effects of mammalian herbivory.

3.2.Study species

3.2.1. Trees

Two of the dominant broad-leaved savanna trees in the NNR were chosen for this study. *Burkea africana* (Hook.) (Caesalpiniaceae) has been recorded as an early-greener – flushing new leaves prior to the start of seasonal rainfall (Childes, 1989; De Bie et al., 1998). Its green-up period in the NNR is recorded as an average of 8 weeks, generally commencing in late September (Rutherford and Panagos, 1982). It is a medium-sized deciduous, leguminous tree and stands at an average of 10-12 m in height (Maroyi, 2010). Its distribution is vast, ranging throughout central and southern Africa (Maroyi, 2010).

Terminalia sericea (Burch. ex DC.) (Combretaceae) occasionally demonstrates early-greening as new leaves are flushed just before or immediately after the arrival of seasonal rainfall (Childes, 1989; Moyo et al., 2015). It is a small to medium-sized, deciduous tree with a mean height of 9 m and is distributed throughout southern African savannas (Lemmens, 2009).

3.2.2. Grasses

Grass plots were marked out under *B. africana* and *T. sericea* canopies and also between these tree canopies with no shade from woody plants. The dominant grasses associated with the *B. africana* plots were *Setaria sphacelata var. sphacelata* (Schumach.), *Eragrostis curvula* (Schrad.) and *Sporobolus africanus* (Poir.). Under the *T. sericea* canopies, plots were dominated by *Trachypogon spicatus* (Kuntze) and *Sporobolus africanus. Digitaria eriantha* (Steud.) and *Panicum maximum* (Jacq.) were dominant under both tree species. Plots selected between the tree canopy cover were dominated by *Panicum maximum, Stipagrostis uniplumis* (Licht.)*, Eragrostis pallens* (Hack.) and *E. racemosa* (Thunb.).

3.3.Experimental design and protocol

A random selection of 14 *B. africana* and 14 *T. sericea* trees for this study was made and eight trees from each species were then assigned to the control (unwatered) treatment and six trees to the watered treatment. Between-canopy grasses received no watering during the study. This study design formed part of a broader study by Whitecross et al. (2016) (*see Chapter 2*) conducted at the NNR, hence the uneven number of trees in each treatment group.

Underneath each tree canopy in a northerly direction, two 0.5 m x 0.5 m plots were marked out with four flags each at 0.5 m and 1.5 m from the base of the trunk to form grass monitoring plots (56 plots in total – 24 of which were irrigated and 32 of which were not). No differences were observed between the 0.5 m and 1.5 m grass plots in terms of their growth rates or cumulative heights and they were thus combined for all analyses. All grass within a 2 m radius of the tree trunk base was cut on the 26th August 2012 prior to the start of irrigation and sampling to allow for the same starting conditions across all treatments. Four grass species were identified as dominant between-canopy species and three 0.5 m x 0.5 m per species (12 plots in total) were setup at least 10 m from the nearest tree canopy to compare the growth of below- and between-canopy grasses. The between-canopy grass plots were also cut on the 26th August 2012.

3.3.1. Irrigation, soil moisture and rainfall

The base of each watered tree was fitted with a pressurised drip-irrigation system (Netafim Uni-techline 0.3 m pressure regulated drip irrigation system, South Africa). Four consecutive, linked rings were placed at 1, 1.3, 1.6 and 2 m radii from the base of each tree trunk and connected to the main inlet valve. An early-greening response was simulated by irrigating prior to the start of seasonal rainfall to end of October at weekly intervals. Each tree and its sub-canopy grass plots received ~250 L of water per week over the 12.6 m^2 area around their base to simulate a 20 mm precipitation event. In 2012, irrigation commenced on the 2nd September 2012, however, the early arrival of rainfall (7th September) led to only one week of irrigation prior to the start of seasonal rainfall. The total irrigation dispensed during the 2012 green-up period (August-November) was 140 mm, or 40% of total water received (rainfall and irrigation) over this time and 19% of total annual water received in the 2012 growing season (July 2012-June 2013) (Figure 1). Irrigation again commenced on the 15th August 2013, allowing for an extended period prior to the onset of natural rainfall, and terminated at the end of October 2013. Irrigation during 2013 added 180 mm of water, or 42% of total water received in the green-up period and 18.5% of the total annual water input in 2013. The entire irrigation system had to be replaced after the 19th September 2013 fire, however, only one week of watering was missed as a result of this and an extra watering session was included in September 2013 to account for the missed session.

Daily rainfall data were collected from the South African Weather Services' Bela-Bela station, approximately 60 km south of the NNR (Figure 1). The average date of first rainfall at this site is 14 October ± 28 days (SD) (34 year average between 1980-2013, South

African Weather Services). Rainfall in 2012 arrived early (7th September) with a total austral year (1st July - 30th June) precipitation of 597 mm, 27 mm less than the MAP for NNR (Figure 1). Irrigation inputs totalled 140 mm during the 2012 green-up period. The rainfall during 2013 arrived 30 days later (7th October) with lower initial amounts in comparison to 2012 but totalled 791 mm, 1.2x the mean annual rainfall (Figure 1). Irrigation inputs during the 2013 green-up period were 180 mm and were the only water inputs to the system for two months prior to the start of rainfall (Figure 1). Rainfall arrived on the 11th October 2014, with a total precipitation of 491 mm for the austral year, 21% lower than the MAP for NNR (Figure 1).

Soil moisture was monitored at 5cm depth using eight EnviroSMART probes and a Campbell Scientific CR1000 data-logger set to record the percentage of soil moisture in the soil at five minute intervals. These data were then averaged to indicate a daily trend over the year. The 2012 soil moisture illustrates a close relationship with rainfall and an increase in soil moisture is observed as soon as rainfall commences (Figure 1). The baseline soil moisture was slightly lower in early 2013 possibly because it remained drier for longer into the later part of the year following a long dry season (Figure 1). The fire did not interfere with the irrigation treatment in 2013, but the soil moisture probes were irreplaceably destroyed (Figure 1).

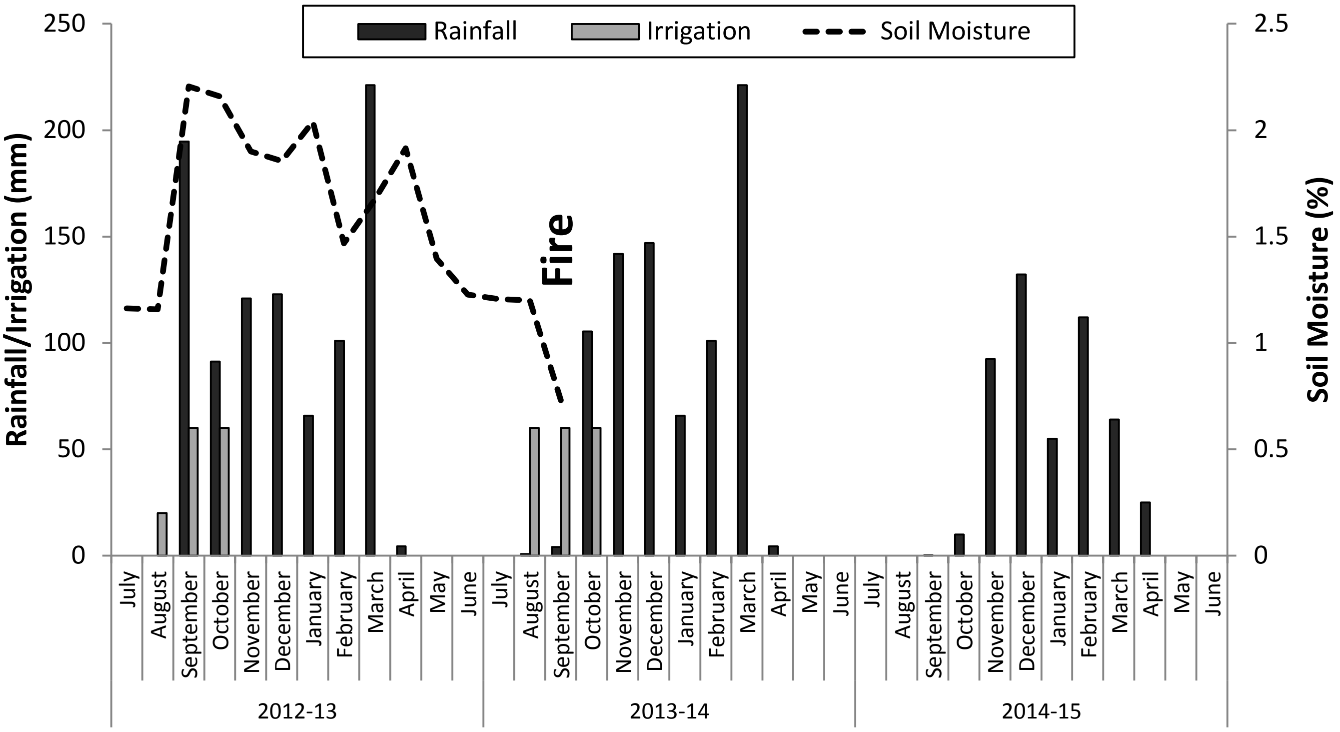

Figure 1. Rainfall and irrigation inputs and daily soil moisture excluding irrigation, throughout the 2012-13, 2013-14 and 2014-15 growing seasons, at the Nylsvley Nature Reserve. A fire destroyed the soil moisture probes in September 2013, indicated by the word 'Fire'; hence the missing soil moisture values thereafter. However, the irrigation system was repaired post-fire and continued to be used.

3.3.2. Grass growth

The grass green-up start dates (GUSDs) were recorded when the first green grass shoots were observed within the 0.5 x 0.5 m plots. The species with more than 60% coverage in each plot was recorded. Grass growth was measured at weekly intervals in conjunction with tree phenology estimates between August and November each year. To estimate the grass growth within each plot, photographs were taken from one meter above the centre of each plot on a weekly basis. To estimate grass height on a weekly basis, a meter long ruler was placed on the western edge of the grass plot and a camera (Nikon D3100 with an 18-55 mm lens) was setup one meter away from this ruler, on the eastern side of the plot in line with the maximum height of the grass. Grass height was then determined from the photographs. A disc pasture meter (DPM) was used to estimate the grass biomass below and outside the tree canopies. DPM measurements were taken at marked 1 m and 2 m points from the tree base on the north, east, south and west sides of the tree to give a total of eight measurements below each canopy. Three DPM measurements of outside canopy grass plots were taken per grass species. The DPM calibration equation specific to the NNR was used to convert the disk height measurements to grass biomass (kg ha^{-1}) (Dörgeloh, 2002).

3.3.3. Summary of tree phenology

Estimations of tree phenology for each austral growing season starting in 2012, 2013 and 2014 were divided into two periods: 1) a weekly sampling of phenology between August to November and 2) a monthly sampling from December to May. This allowed for detailed measurements of different leaf age class phenophases during green-up. A phenophase is the period during which a specific age class of leaf dominates >50% of total canopy area. The three leaf age classes were: new (<0.5*length of adult leaf), fully expanded (>0.5*length of adult leaf, not yet sun-drenched) and mature (sun-drenched, adult leaf) (based on Williams et al., 1997). Sun-drenched leaves are fully mature, adult leaves which have hardened and developed an outer waxy cuticle layer after exposure to sunlight. The Walker aerial cover scale was used as a baseline to estimate canopy fullness of each leaf age class, where 0: 0%, 1: 1-10%, 2: 10-25%, 3: 25-50%, 4: 50-75%, 5: 75-90%, 6:90-99%, and 7: 100% (Walker, 1976). Refer to Whitecross et al. (2016) for further details on tree phenology.

3.3.4. Comparing ground-based phenology with NDVI

Moderate Resolution Imaging Spectroradiometer (MODIS) vegetation index data (MOD13Q1) for the NNR on tile h20v11 for dates spanning 27th July 2012 to 10th April 2015 were obtained from NASA Land Processes Distributed Active Archive Centre (LPDAAC). The MOD13Q1 product is produced with a 250 m spatial resolution and a 16-day composite image of NDVI – a measure of vegetation “greenness” – which can be used to infer phenological trends in deciduous vegetation (Fisher and Mustard, 2007; Poulter and Cramer, 2009; Polgar and Primack, 2011; Klosterman et al., 2014). All NDVI data were extracted for the pixel which covered the ground-based study site during the specified period and these values were compared to rainfall, and tree and grass green-up.

3.4.Data analyses

All analyses were conducted in RStudio (R Core Team, 2014, version 0.98.1102). To calculate the mean grass height per treatment/grass species per week, the mean±SE of the measured heights taken from the weekly photographs was calculated. The mean±SE grass biomass values per week were determined by averaging all samples within each treatment group (below-canopy) or species (between-canopy) per week. Paired Wilcoxon tests were used to compare between the post-rainfall or post-irrigation GUSDs of 2012 and 2013 for each treatment and the species within each treatment. Kruskal-Wallis Rank Sum Tests

(Kruskal-Wallis) were used to compare the GUSDs and growth rates between below-canopy treatments for each year in conjunction with a Kruskal Multiple Comparisons Post-hoc Test (Kruskalmc) from the package ‘pgirmess’ when a significant difference was detected (Giraudoux, 2014). Kruskal-Wallis was also used to compare the grass heights and biomass at the end of each green-up period. Comparisons between the relevant mean±SE GUSDs for trees and grasses were conducted to determine which plant life-forms were commencing green-up earlier in relation to rainfall and fire. The coefficients of variation (CVs) for the GUSDs of the trees and grasses were compared using an independent student’s t-test. The CVs were then recalculated using the austral scale where day 1 is the 1st July to illustrate the variation around the GUSDs more clearly. Rainfall start dates (storms >15 mm after the 1st July) from the South African Weather Services Bela-Bela station in comparison to the latest possible green-up start date for *B. africana* (day 288 – 15 October) was used to determine the probability of early-greening. The earliest date of rainfall at the Bela-Bela station was used to re-examine the CVs on a scale which represented the earliest possible start for the NNR growing season. The rate of change in NDVI from the lowest value pre-rainfall to the subsequent value (~2 weeks) was calculated for each season. The average maximum NDVI was calculated from the average values from the beginning of January to the end of February each year.

4. Results

4.1.Summary of grass phenology

4.1.1. Green-up start dates and growth rates

It took 2-10 days after water was applied for grass to start greening (Table 1), with the greening of the between-canopy grasses responding slightly slower than those below the tree canopies. The addition of water through irrigation resulted in a ~4 day earlier onset of green-up in below-canopy grasses associated with *B. africana* than the unwatered grasses which only received rainfall (Wilcoxon: W=152, d.f.=26, p=0.002). However, *T. sericea* below-canopy grasses showed no difference between watered and unwatered grasses during 2012, the year with early rainfall (Wilcoxon: W=102, d.f.=26, p=0.42, Table 1). In 2013 when rainfall commenced much later, watered below-canopy grasses showed significantly earlier green-up than the unwatered grasses below *B. africana* (Wilcoxon: W=1, d.f.=11.7, p<0.001) and *T. sericea* (Wilcoxon: W=0.5, d.f.=13.2, p<0.001, Table 1). Overall, watered grasses took longer to respond to irrigation during 2013 than in 2012, but green-up onset was ~28 and ~33 days earlier than the start of rainfall for watered grasses below *B. africana* and *T. sericea* respectively during 2013 (Table 1). During 2012, on average unwatered below-canopy grasses took ~4.7 (mean) days less to commence green-up post-rainfall than between-canopy grasses (Wilcoxon: W=100.5, d.f.=19, p=0.005, Table 1). In 2013, on average unwatered below-canopy grasses were ~2 days faster than the between-canopy grasses (Wilcoxon: W=24, d.f.=18.5, p=0.006, Table 1). Differences between grass species found below different tree species were not consistent across treatments. The majority of the grass species showed slower green-up responses during the 2013 season when rainfall commenced later in the year and the dry season (19 September) fire had already occurred, than in 2012 when rainfall onset was early and there was no fire. *Panicum maximum* was represented across all treatment groups and showed the fastest green-up response below the unwatered *T. sericea* during 2012 and the slowest response between the canopies during 2013 (Table 1). *Digitaria eriantha* was represented in all below-canopy treatments and had the fastest green-up response below the watered *B. africana* in 2012 and the slowest below the watered *T. sericea* in 2013 (Table 1).

The watering did not significantly increase the growth rates of below-canopy grasses during 2012, whether under *B. africana* or *T. sericea* (Table 1). However, the growth rates of below-canopy *B. africana* grasses were higher for the unwatered than the watered grasses in 2013, an unexpected result, but this was probably an effect of the fire (W=145; d.f.= 22; p<0.05, Table 1). This was not mirrored below *T. sericea* canopies in 2013, where there were no differences in growth rates between watered and unwatered grasses (Table 1). At the grass species level, a significant increase in growth rates between years for *E. curvula* in both the watered and unwatered *B. africana* plots was observed (Table 1). *Panicum maximum* showed no overall differences between the treatments for its growth rates during the 2012 (Kruskal-Wallis: H=4.75, d.f.=4, p=0.31, Table 1) or 2013 seasons (Kruskal-Wallis: H=2.70, d.f.=4, p=0.61, Table 1). A comparison of the mean growth rates of *Digitaria eriantha* under *B. africana* and *T. sericea* trees showed no differences during 2012 (Wilcox Test: W=0.94, d.f.= 12, p=0.31) or 2013 (Wilcox Test: W=22, d.f.= 12, p=0.80). *Setaria sphacelata* under watered *B. africana* trees had slower growth rates than the unwatered grasses during both seasons (Table 1). The watered *S. africanus* below *B. africana* had higher growth rates than the unwatered grasses, as well as the *T. sericea* watered grasses (Table 1). *Trachypogon spicatus* was the only species found under the unwatered *T. sericea* that had a higher growth rate than the previous season (2012) after the fire during 2013 (Table 1). *Eragrostis racemosa* had the highest growth rate of the between-canopy grasses in 2012, while *E. pallens* had the highest overall growth rate after the fire in 2013 (Table 1).

Table 1. The mean±SE green-up start days and growth rates for grasses below and between tree canopies in the Nylsvley Nature Reserve during the 2012 and 2013 green-up periods. Certain below-canopy grasses received irrigation and are listed under the watered treatment, while grasses exposed to only natural rainfall are listed under the unwatered treatment. Averages for all below-canopy grasses combined in bold-type. Asterisks show the results of paired t-tests between years with *=p<0.05, **=p<0.01 and ***=p<0.001.

Tree Species	Treatment	Start dates of rainfall and irrigation*		Grass species	Number of days post-rainfall for green-up to start			Number of days post-irrigation for green-up to start			Growth rate (cm/week)		
		2012	2013		2012	2013		2012	2013		2012	2013	
Below-canopy grasses													
Burkea africana	Unwatered	7th September	7th October	**All species**	**7±1.0**	**8.4±0.38**					**4.08±0.37**	**3.72±0.21**	*****
				Eragrostis curvula	4±<0.1	8±<0.1	*				1.8±0.2	3.6±0.2	*
				Setaria sphacelata	4±<0.1	8±<0.1	*				5±<0.1	5.3±<0.1	
				Panicum maximum	7±1.6	8±<0.1					4.2±0.4	3.2±0.3	*
				Digitaria eriantha	7±2.0	9.2±1.2					4.3±0.7	3.1±0.5	*
				Sporobolus africana	12±<0.1	8±<0.1	*				3.8±<0.1	3.2±<0.1	
Burkea africana	Watered	2nd September*	15th August*	**All species**	**2±0.85**	**-28±2.16**	*******	**7±0.85**	**25.4±2.16**	*******	**3.19±0.33**	**3.06±0.27**	
				Sporobolus africana	1±<0.1	-23±<0.1	***	6±<0.1	31±0<0.1	***	4.9±<0.1	3.8±<0.1	*
				Digitaria eriantha	1±3.0	-20±<0.1	***	6±3.0	33±<0.1	***	2.7±0.7	3.6±0.5	
				Setaria sphacelata	2±3.0	-27±2.5	***	3±3.0	26±2.5	***	2.2±0.8	3.4±0.4	*
				Panicum maximum	3±1.0	-32±3.1	***	8±1.0	21±3.1	***	3.5±0.4	3.6±0.4	
				Eragrostis curvula	5±<0.1	-25±<0.1	***	9±<0.1	28±<0.1	***	2.4±<0.1	3.3±<0.1	*
Terminalia sericea	Unwatered	7th September	7th October	**All species**	**4.5±0.50**	**8.4±0.38**	*******				**3.73±0.28**	**4.02±0.36**	
				Trachypogon spicatus	4±<0.1	8±<0.1	**				4.4±0.5	4.9±0.3	
				Panicum maximum	4±<0.1	8.6±0.6	***				3.2±0.3	3.6±0.4	
				Digitaria eriantha	8±4.0	8±<0.1					4.2±0.7	4.7±0.7	
Terminalia sericea	Watered	2nd September*	15th August*	**All species**	**4±<0.1**	**-33±1.19**	*******	**9±<0.1**	**19.6±1.19**	*******	**3.30±0.33**	**3.33±0.27**	
				Sporobolus africana	4±<0.1	-35±<0.1	***	9±<0.1	18±<0.1	***	2.3±<0.1	2.2±<0.1	
				Digitaria eriantha	4±<0.1	-33±1.6	***	9±<0.1	19.6±1.6	***	2.5±0.3	3.3±0.4	*
				Panicum maximum	4±<0.1	-33±2.1	***	9±<0.1	19.8±2.1	***	4.1±0.4	3.6±0.4	
Between-canopy grasses													
		7th September	7th October	**All species**	**9.6±1.9**	**9±0.6**					**4.26±0.4**	**3.96±0.5**	
				Eragrostis pallens	4±<0.1	8±<0.1	*				4.67±0.79	5.10±0.83	
				Panicum maximum	9.3±2.7	10±2.0					3.44±0.97	2.75±0.19	
				Stipagrostis uniplumis	12±<0.1	8±<0.1	*				3.67±0.53	4.33±0.29	
				Eragrostis racemosa	12±<0.1	10±2.0					5.33±0.72	3.67±0.36	

4.1.2. Grass heights

The height of below-canopy watered and unwatered grasses under both tree species showed no significant differences during the 2012 season when rainfall began early in the season (Figure 2). During the 2013 season, however watered grasses commenced green-up two weeks after the start of irrigation and again one week after the fire prior to unwatered grasses which only commenced green-up two weeks after the start of rainfall (Figure 2). Between-canopy grasses had slightly higher grass heights than below-canopy grasses; however, these were reduced to similar heights after the fire in 2013 which burnt all of the aboveground herbaceous biomass (Figure 2). There were no significant differences observed between the heights of any of the below-canopy grass treatments at the end of the growing seasons (Kruskal-Wallis: 2012: $H_{3,52}$ = 2.67, p=0.44; 2013: $H_{3,52}$ = 0.90, p=0.83, Figure 2). The between-canopy grasses also showed no overall differences in height during the final week in 2012 (Kruskal-Wallis: $H_{3,52}$ = 7.27, p=0.06) or 2013 (Kruskal-Wallis: $H_{3,52}$ = 7.22, p=0.06). When comparing the final cumulative growth of grasses below- and between-canopies in 2012, between-canopy grasses had significantly higher cumulative growth than both the watered and unwatered below-canopy grasses (Kruskal-Wallis: H=15.49, d.f.=2, p<0.001, Kruskalmc: p<0.05). In 2013, however, the impacts of the fire resulted in no overall difference in cumulative growth heights for either the below- or between-canopy grasses (Kruskal-Wallis: H=0.13, d.f.=2, p=0.94).

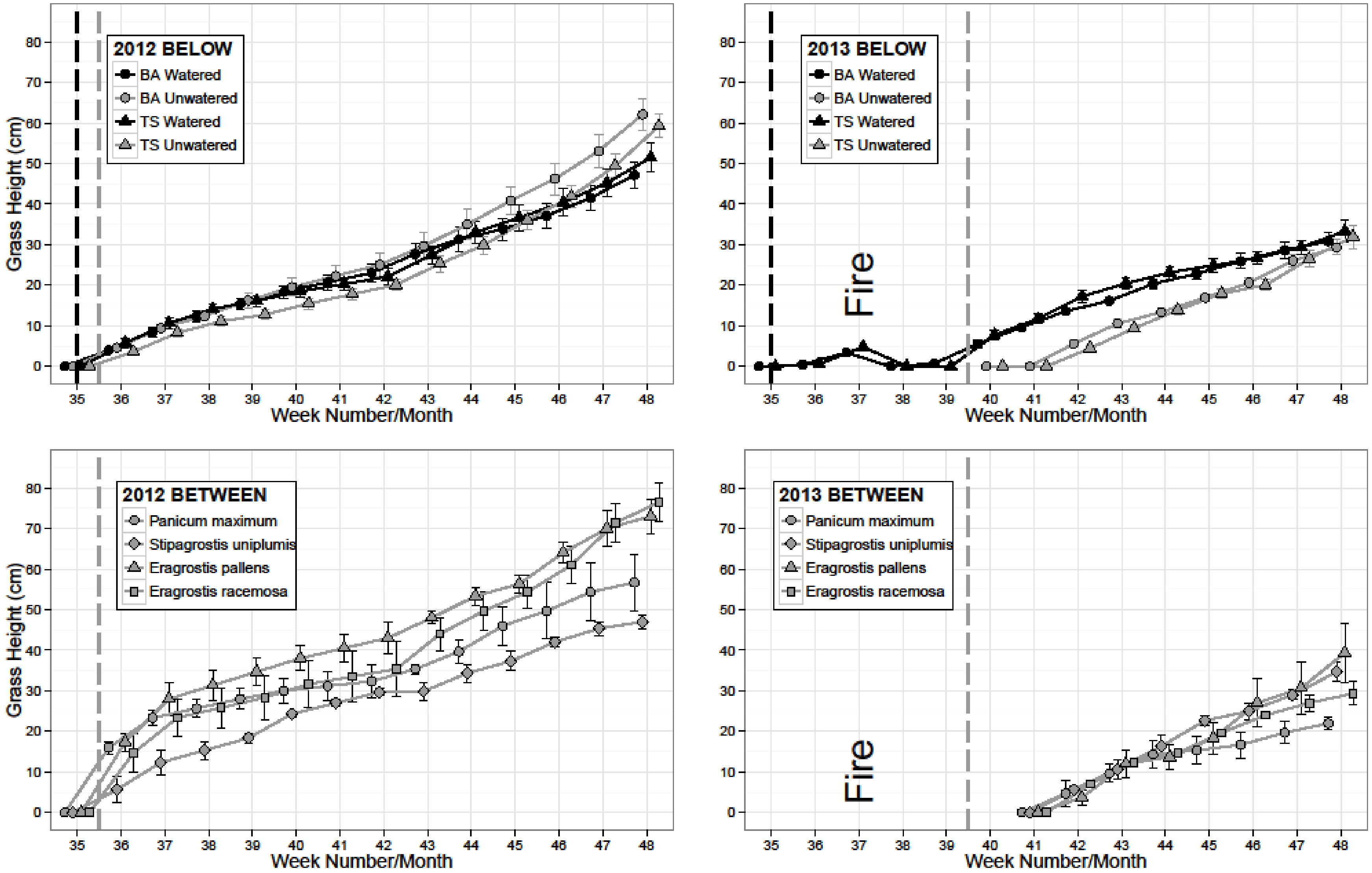

Figure 2. The mean (±S.E.) grass height within 0.5 x 0.5 m grass plots below the canopies of watered and unwatered *Burkea africana* (BA) and *Terminalia sericea* (TS) and between canopy plots at least 10 m from the nearest tree canopy during the 2012 and 2013 growing seasons. The black vertical dashed lines indicate the start dates of irrigation of the watered treatments and the grey line indicates the start dates for seasonal rainfall. In 2013 a severe wildfire occurred in the study site which burnt all of the standing grass biomass, indicated by the word ‘Fire’.

4.1.3. Grass biomass

A comparison of the 2012 biomass of the below-canopy grasses showed that the combined *T. sericea* associated grasses had higher overall biomass than the *B. africana* associated grasses (Kruskal-Wallis: H=11.21, d.f.=3, p=0.01, Figure 3), but posthoc analyses indicated that a specific difference was only observed between the unwatered *B. africana* and watered *T. sericea* grasses (Kruskalmc: p<0.05). Measurements taken during November 2013 showed an overall difference between the below-canopy grasses (Kruskal-Wallis: H=42.22, d.f.=3, p<0.01), with higher biomass in the watered compared to the unwatered grasses (Kruskalmc: p<0.05, Figure 3). A final grass biomass measurement (when they peaked) in August 2015 showed differences between unwatered *T. sericea* grasses and the watered and unwatered *B. africana* grasses, but no differences between treatments within each species were observed (Kruskal-Wallis: H=20.86, d.f.=3, p<0.001, Kruskalmc: p<0.05, Figure 3).

Biomass of the between-canopy grasses only showed a clear difference between *P. maximum* and *S. uniplumis* during January 2013 (Kruskal-Wallis: H=7.41, d.f.=3, p=0.05, Figure 3). During November 2013, two months after the fire, no differences were observed across the four between-canopy grass species (Kruskal-Wallis: H=0.19, d.f.=3, p=0.98, Figure 3). Between-canopy grasses were remeasured in August of 2015, but no overall differences were found, despite *P. maximum* continuing to have the highest overall biomass (Kruskal-Wallis: H=5.97, d.f.=3, p=0.11, Figure 3).

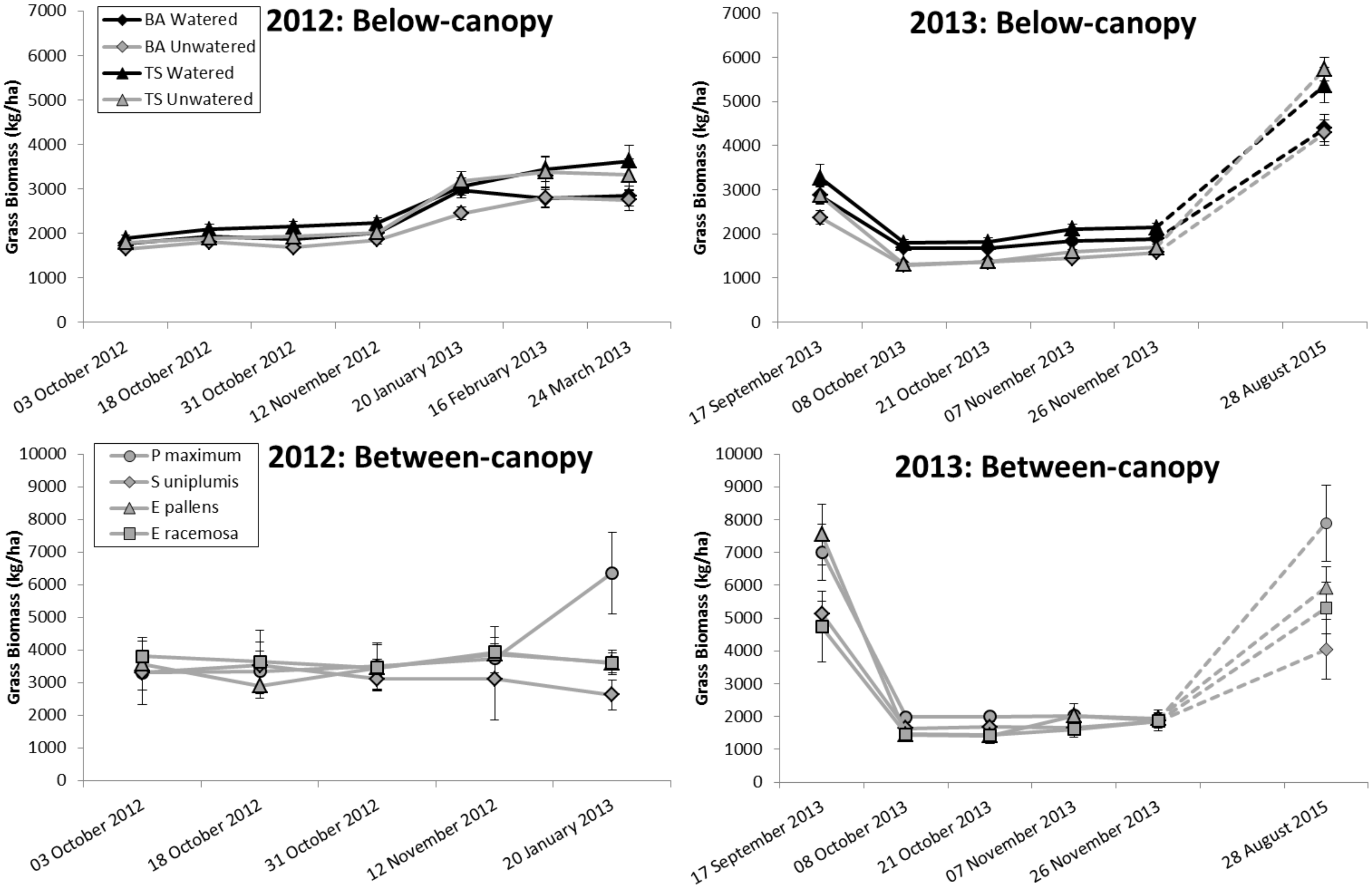

Figure 3. Grass biomass (mean ±S.E.) measured using a disc pasture meter below and between the canopies of watered and unwatered *Burkea africana* (BA) and *Terminalia sericea* (TS) trees during the 2012 and 2013 growing seasons. A final biomass measurement was taken at the end of August 2015 to determine any long term changes. A fire burnt all of the aboveground biomass on the 19th September 2013.

4.2. Summary of tree phenology

The start of green-up in all unwatered trees was closely linked with the arrival of seasonal rainfall during both seasons. Green-up of watered *B. africana* trees was 2 weeks faster than the unwatered trees in 2013 when irrigation had occurred for several weeks (Figure 4). The watered *B. africana* trees in 2013 greened-up a week prior to the fire and unwatered trees greened-up 1.5 weeks after the first rainfall (Figure 4). Watered *T. sericea* trees took 11 weeks to reach >50% canopy cover after the fire, while unwatered trees only took 8 weeks. This difference is potentially a result of an early engagement of the little known physiological processes that cue leaf flush (Polgar and Primack 2011), such as the redeployment of stored carbon and nitrogen to distal branches (February and Higgins, 2016), relating to green-up after watering. This leaves them vulnerable to the effects of the fire (Kennedy and Potgieter, 2003) (Figure 4). For more details and explanations of the tree phenology, refer to Whitecross et al. (2016).

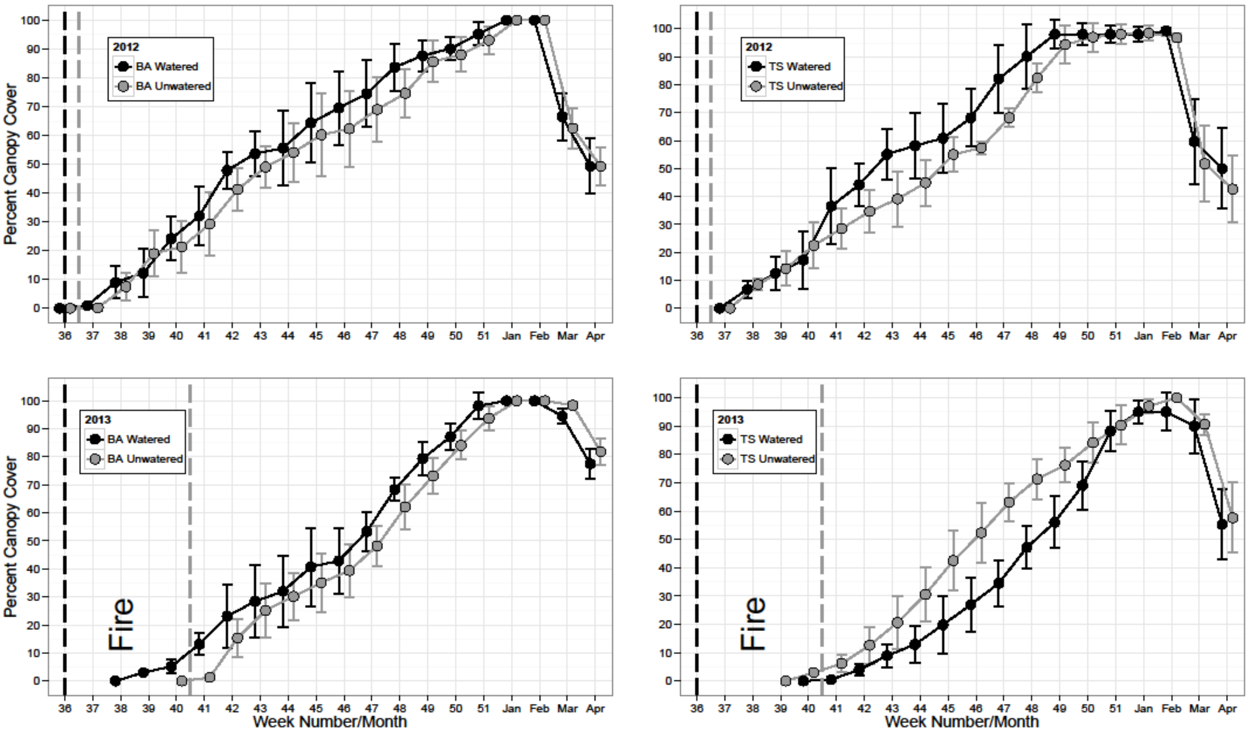

Figure 4. The mean±SE percent canopy cover of leaves of watered and unwatered *Burkea africana* (BA) and *Terminalia sericea* (TS) trees during the 2012 and 2013 growing seasons. Week numbers (from start of year) and months are indicated on the X-axis. Vertical dotted lines represent the start dates of irrigation (black) and rainfall (grey) and the word 'Fire' indicates the date a fire burnt through the study site.

4.3. Comparison of tree to grass green-up

The grasses responded approximately 6 days faster to the onset of the unusually early seasonal rainfall in 2012 than either tree species, with watered and unwatered grasses below *T. sericea* responding 7 and 2 days faster respectively than the trees (Figure 5a). Similarly, the watered and unwatered grasses below the *B. africana* trees were 9 and 8 days faster than the trees respectively in commencing green-up post-rainfall (Figure 5a). During the 2013 green-up period, watered grasses commenced green-up 26 and 44 days before their associated watered *B. africana* and *T. sericea* trees respectively (Figure 5a). In the same year, the watered grasses greened-up 35 and 41 days earlier than unwatered grasses under *B. africana* and *T. sericea* respectively (Figure 5a). However, after a severe fire burnt through the site, green-up of the watered grasses had to begin again as seen by the post-fire GUSDs, which commenced 13 and 15 days post-fire for the watered *B. africana* and *T. sericea* grasses respectively (Figure 5a). Green-up of unwatered grasses occurred 26 days after the fire and 8 days after the onset of rainfall (Figure 5a). Watered grasses showed significantly faster green-up after the fire than unwatered grasses, which only commenced green-up after the start of rainfall (Figure 5a).

The variation in starting dates for trees was consistently higher than their grassy competitors as shown by the higher CVs across the majority of treatments (paired T-test: t=4.62, d.f.=11, p=0.018, Figure 5b). The mean±SE CVs for all the tree treatments when using the 1st January as day 1, was 2.7±0.3%, while the grasses were lower at 0.9±0.2%. The mean±SE CV for *B. africana* across the three seasons was 2.4±0.4%, while *T. sericea* was higher at 3.0±0.3%. If consideration is made that the austral growing season, in theory, commences on the 1st July and thus this study used this as day 1 of the austral year, the mean±SE CVs across all three seasons increases to 8.0±0.9% for the trees (with 7.3±1.3% for *B. africana* and 14.4±1.3% *T. sericea*) and 3.2±0.9% for all of the grasses (Figure 5c).

Burkea africana green-up did not occur later than mid-October (~day 289) when no rainfall had occurred. An assessment of rainfall start dates from 1980 showed that 46% of years had rainfall onset after the 15th October which would likely result in *B. africana* showing an early-greening response (Figure 5d). The earliest start date for rainfall in this area since 1980 was the 7th August 1983 (day 220). If this is used as the earliest possible green-up date and re-examination of the CVs for the NNR site occurs, it is found that the mean±SE for all trees becomes 13.6±1.7% (with 12.8±2.5% for *B. africana* and 14.4±2.3% for *T. sericea*), while the grass CVs increase to 5.7±1.6%.

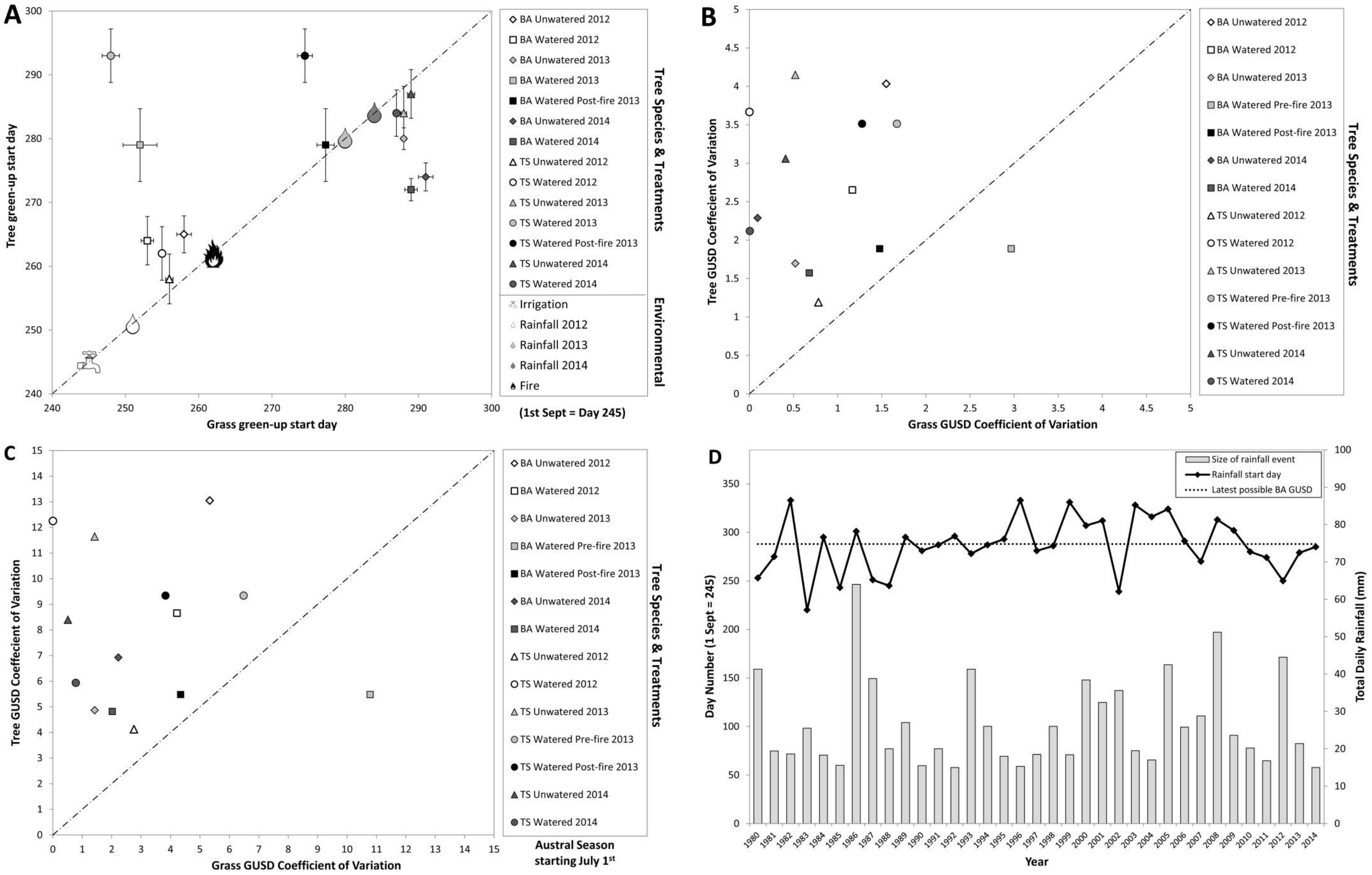
A
Tree green-up start day
Grass green-up start day
(1st Sept = Day 245)
BA Unwatered 2012
BA Watered 2012
BA Unwatered 2013
BA Watered 2013
BA Watered Post-fire 2013
BA Unwatered 2014
BA Watered 2014
TS Unwatered 2012
TS Watered 2012
TS Unwatered 2013
TS Watered 2013
TS Watered Post-fire 2013
TS Unwatered 2014
TS Watered 2014
Tree Species & Treatments
Irrigation
Rainfall 2012
Rainfall 2013
Rainfall 2014
Fire
Environmental
B
Tree GUSD Coeffecient of Variation
Grass GUSD Coefficient of Variation
BA Watered Pre-fire 2013
TS Watered Pre-fire 2013
C
Austral Season starting July 1st
D
Size of rainfall event
Rainfall start day
Latest possible BA GUSD
Day Number (1 Sept = 245)
Total Daily Rainfall (mm)
Year

Figure 5. (A) The mean±SE green-up start dates (GUSDs) of trees (*Burkea africana* [BA] and *Terminalia sericea* [TS]) and their associated grass plots in watered and unwatered treatments over the 2012 (light grey), 2013 (darker grey) and 2014 (darkest grey) growing seasons at the Nylsvley Nature Reserve. The start dates of rainfall and irrigation are indicated, as well as the occurrence of a severe fire which burnt all standing grass (and other herbaceous) biomass in September 2013. Post-fire, watered treatment grass green-up is shown in black. (B) Coefficients of variation (CVs, SD/mean*100) measured using the Julian day scale (1st January-31st December) for the paired tree and grass GUSDs over three years, as well as pre- and post-fire CVs for the 2013 watered treatments. (C) The previous CVs measured using an austral season scale (1st July-30th June) where day 1 is the 1st July. (D) The rainfall start dates for each year at the South African Weather Service's Bela-Bela station from 1980-2014, as well as their corresponding amount of precipitation, in relation to the latest possible green-up day for *B. africana* at Nylsvley Nature Reserve (day 289).

4.4. Comparing ground phenology to NDVI

When comparing the three season's green-up periods, it is clear that the NDVI signal is sensitive to greening from both the trees and grasses, with combined green-up of both life-forms resulting in a stronger signal (Figure 6). The change in NDVI over the first two weeks of green-up was 0.126, 0.129 and 0.075 for 2012, 2013 and 2014 respectively. During 2014, when early-greening of the trees occurred, the NDVI increase was smaller than the previous two seasons (Figure 6). The average maximum NDVI (not illustrated below) for 2012, 2013 and 2014 was 0.589, 0.574 and 0.598 respectively, with the fire the likely cause of the lower NDVI value during the 2013 season.

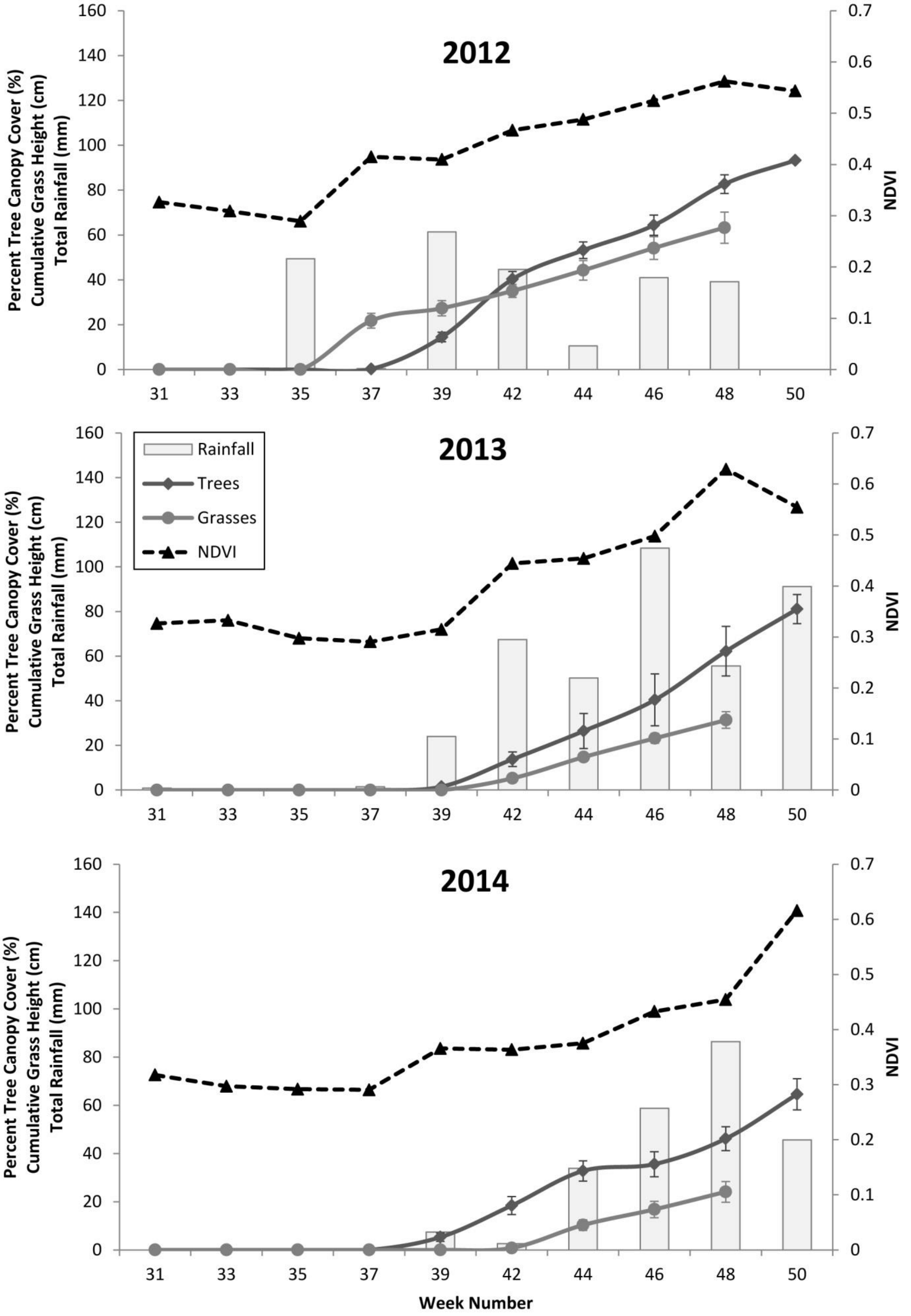

Figure 6. A comparison between the green-up of trees (% canopy fullness) and grasses (cm height above ground) in the Nylsvley Nature Reserve during the 2012, 2013 and 2014 green-up periods (early-August to mid-December) in relation to weekly rainfall (mm) and NDVI.

5. Discussion

5.1. When does the austral growing season truly commence?

This study has quantified the temporal variability associated with the phenology of both trees and grasses in an African savanna over three seasons. However, the scale at which this variation is considered played a major role in clearly illustrating this variability. The vast body of phenological literature which has emerged from the northern hemisphere uses the Julian calendar (1st January = day 1) to measure the temporal changes in their growing seasons (Schwartz, 1998; Polgar and Primack, 2011; Richardson et al., 2013; Buitenwerf et al., 2015). This, however, results in green-up start dates (GUSDs) well into the 200-300 range for most southern hemisphere systems (including southern Africa's savannas). These high numbers can skew results, such as the coefficient of variation (CV), into appearing smaller than they are in reality. When the CVs were reanalysed in relation to the austral growing season, the values more than doubled those measured on the Julian day scale (Figure 5). This analysis was taken further by assessing the CVs in relation to the earliest possible GUSD for the NNR based on historical rainfall records, which consequently almost doubled the CVs yet again. This raises the question, when does the austral summer truly begin? Owing to the high temporal variability across southern hemisphere ecosystems, this study suggests that all studies assessing phenology of austral systems be conscious of the effects of the abovementioned temporal scales and hence use the 1st July as day 1 of the southern hemisphere growing season.

5.2. Below- vs. between-canopy grasses

Numerous studies have compared the differences between grasses that grow below tree canopies and grasses with no association to tree canopies and it is widely concluded that trees can have negative or positive effects on their neighbouring grasses (Dye and Walker, 1987; Belsky, 1994; Scholes and Archer, 1997; Ludwig et al., 2001; Treydte et al., 2008; February et al., 2013). Light-access is an important resource for grasses and while this was not assessed as part of this study, the dense canopy of *B. africana* (leaf area: 2840 m^2ha^{-1}, Rutherford, 1979) casts a higher degree of shading on the below-canopy grasses than those under the more open *T. sericea* (leaf area: 984 m^2ha^{-1}, Rutherford, 1979) canopies (Belsky, 1994; Ludwig et al., 2001, Witkowski *pers. obs.*). Mbatha and Ward (2010) found no effect of irrigation on the biomass of savanna grasses; this study concurs with this finding, suggesting that access to light has a greater influence on grass biomass than water availability. It is possible that by increasing their response time to water availability, the below-canopy grasses are able to take advantage of the higher light levels below the tree while canopy leaves are still developing. During the green-up periods when irrigation was provided, watered below-canopy grasses showed higher biomass than the unwatered below-canopy grasses. No watering was provided during the 2014-15 growing season however, yet by the end of August 2015 the grasses below *T. sericea* canopies had higher biomass than those below *B. africana*. This suggests that water has an influence on biomass accumulation in below-canopy grasses in the initial weeks of green-up when shading from developing canopies is minimal. However, if water is limited, light availability will have a larger influence. The influence of light will be more clearly observed at the end of the growing season (when grasses have had more time for growth), as indicated by the higher biomass below *T. sericea* (sparser canopy) than *B. africana* (denser canopy) in August 2015.

The between-canopy grasses showed higher overall biomass than the below-canopy grasses, once again reaffirming that access to light is important (Ludwig et al., 2001). This difference can also be attributed to the physical structure of the between-canopy grasses, for example *Eragrostis pallens* forms a large tussock with thick, lengthy shoots that enables it to effectively compete for available light over other open-air grasses (Grunow et al., 1980; Randall and Cresswell, 1983). This species had the fastest growth rates overall, which is most likely a result of its large size. *Digitaria eriantha* – one of the common below-canopy grasses – grows in a more compact manner with softer leaves and thinner stems and had one of the faster average growth rates (Randall and Cresswell, 1983). *Panicum maximum* also has softer leaves and was found below both tree species and between the canopies, but showed no overall difference in growth rates for either season. Differences in responses to water availability were observed between the watered and unwatered below-canopy grasses. The addition of water to the below-canopy grasses resulted in a five week earlier flush of new leaves during the late-onset rainfall year (2013), however, the growth rates of watered grasses were lower than the unwatered grasses, which was unexpected (Table 1). As this study did not account for belowground activity in the plants, it is possible that the increased irrigation resulted in greater tillering, reproduction or storage within these grasses; however, this fell outside the scope of the study (Belsky, 1994; Scholes and Archer, 1997; Ludwig et al., 2001; Treydte et al., 2008).

5.3. Variability in tree and grass green-up

This study has highlighted the different and variable phenological responses of coexisting savanna trees and grasses to the availability of water – one of the key environmental drivers of African savannas (Huntley and Walker, 1982; Jolly and Running, 2004; February et al., 2013; Lehmann et al., 2014). Trees and grasses are representatives of two different plant life-forms; varying in life-span, height, shape, size, rooting structure, root:shoot ratios and phenology; however, these coexisting groups depend on and compete for the same set of limited resources within the savanna system (Belsky, 1994; Scholes and Archer, 1997; Sankaran et al., 2004; Verweij et al., 2011). Whitecross et al. (2016) clarified the variability observed over the three seasons within the tree component and this study has contextualised this variability in relation to the phenology and growth of the below- and between- canopy grasses. The grasses showed far narrower variation in GUSDs over the three seasons (as expected) given that water availability has been shown to be a strong driver of grass green-up (Dye and Walker, 1987; Scholes and Archer, 1997). The growth form of grasses provides them with less long-term storage capacity than their woody competitors, thus linking their growth directly to the arrival of seasonal rainfall (Scholes and Archer, 1997). A delay in the onset of seasonal rainfall provides trees with an opportunity to commence green-up during a period when grasses remain dormant, and through the early onset of growth, trees are able to have faster acquisition of nutrients at the start of seasonal rainfall (Scholes and Walker, 1993). The higher variability observed in the tree GUSDs is indicative of the disconnection of water availability as a cue for the onset of green-up as suggested by previous studies (Childes, 1989; De Bie et al., 1998; Chidumayo, 2001; Do et al., 2005; Archibald and Scholes, 2007; February and Higgins 2016; Whitecross et al., 2016). Because of variable rainfall, early-greening did not occur in all years for all species – for example *B. africana* only had a significant period of time with leaves present before the rains started in one of the three study seasons (2014), although it always greened up on or before week 42. From the results it appears that tree greening occurs earlier than grasses when rainfall arrives after mid-October, thus *Burkea africana* appears to benefit from an early-greening strategy approximately half of the time in the NNR when assessing the first rains in long-term rainfall records. It was assumed that only the unwatered *B. africana* would show earlier green-up than their below-canopy grasses; however, early-rainfall in 2012 resulted in earlier flushing of all grasses (watered and unwatered). While the fire in 2013 did complicate the greening signals, watered grasses commenced green-up significantly earlier than unwatered grasses and regrew faster post-fire as well. Watering appeared to disadvantage the *T. sericea* as the unwatered individuals commenced green-up earlier than the watered trees post-fire, although the fire may have disrupted their potential early growth onset. The later arrival of rainfall in 2014 resulted in an early-onset of green-up in both *B. africana* and *T.*

sericea. Given the complexity shown across the three seasons of phenology, attempts were made to compare the ground-based measurements to a remotely-sensed product to determine how changes in tree and grass green-up may be represented at a landscape scale.

5.4. Relating ground-based phenology to remotely sensed imagery

Remote sensing has become a popular technique for assessing large-scale ecosystem features such as phenology, which in turn can be used to infer ecosystem productivity and carbon sequestration (Eamus et al., 1999; Fisher and Mustard, 2007; Grace et al., 2007; Ryan, 2009). Some success has been achieved in predicting and modelling phenology of the more homogeneous systems such as temperate deciduous forests in the northern hemisphere (Botta et al., 2000; Cramer et al., 2001; Poulter and Cramer, 2009; Klosterman et al., 2014; Buitenwerf et al., 2015). Given the structural complexity and spatial heterogeneity of savannas, however, remote sensors have struggled to tease apart the mixture of tree and grass signals detected by satellites (Chidumayo, 2001; Jolly and Running, 2004; Simioni et al., 2004; Archibald and Scholes, 2007; Higgins et al., 2011; Whitecross et al. 2016 *in press - see Chapter 6*). The majority of studies where specific canopy phenology has been monitored have only been conducted for a season or two and given the variability in phenology observed within the savannas (Whitecross et al. 2016), it is difficult to draw conclusive evidence from a limited number of seasons (Simioni et al., 2004; Sally Archibald et al., 2010; Higgins et al., 2011). Grasses are often treated as a uniform aspect in savannas and it is important to acknowledge the complexity associated with the grass layer in this savanna, as shown by the varying results in green-up and growth rates. Nevertheless, attempts were made to use the collective results to determine if the on-the-ground study could be coupled with remote sensing techniques to ascertain the dates of green-up in this heterogeneous landscape. This study has shown that during three variable seasons, NDVI sensors are sensitive enough to detect greening of both the tree and grass layers separately (if tree green-up occurs prior to the rainfall onset) or in unison (post-rainfall green-up of both trees and grasses). Further work is needed to ascertain the specific contributions of both life-forms at different stages of the growing season; however, the lower initial increase in NDVI during the early-greening season (2014) suggests that grasses have a larger influence on the overall NDVI value (Scanlon et al., 2002; Archibald and Scholes, 2007; Fisher and Mustard, 2007). The furthering of this work will improve the ability of researchers to accurately monitor the phenology of these two lifeforms within broader savanna ecosystems using remote sensing techniques.

5.5. Conclusions

This study compared the relative responsiveness of savanna trees and grasses to water availability and found that tree green-up is less coupled than that of grasses, based on the broad temporal variability associated with tree green-up dates at the start of each growing season. The influence of trees on below-canopy grasses was seen by higher levels of biomass under *T. sericea* canopies, where shading was lower than under *B. africana* canopies. However, increased water availability due to irrigation caused grass biomass to increase temporarily under watered trees during the start of each growing season. By comparing the field measurements with MODIS NDVI values, this study was able to show that sensors are capable of detecting early-greening in the tree layer, but this signal is subtler than that of the grass layer. Further fine-scale monitoring of tree and grass phenology will provide beneficial datasets that can assist in monitoring future expected increases in growing season lengths within African savannas.

6. Acknowledgements

Thank you to these institutions for their financial support of this study: the Centre for Tree Health Biotechnology (CTHB), the National Research Foundation (NRF) and the Centre for Scientific and Industrial Research (CSIR). Thanks to Natasha du Plessis and the staff at the Nylsvley Nature Reserve. Thanks to Mainline Irrigation and Gert Le Roux for assistance with the planning, development and support of the irrigation experiment. Thanks to Working on Fire South Africa for the loan of their ‘bakkie sakkie’ water tank and pump. Thanks to the following people for their assistance and support during fieldwork: Robert Whitecross, Helenor Whitecross, Caroline Howes, Wesley Hattingh, Giuseppe Venturi, Kirstin Olsen, Marcelle Johnson, Vidosava Jakovljevic, Isabelle Inder, Ryan Thomas, Nadine Cronk and Roxy.

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8. Appendix

Species specific responses of grass height (Figure S1) and biomass (Figure S2) to watering treatments, below *Burkea africana* and *Terminalia sericea* trees.

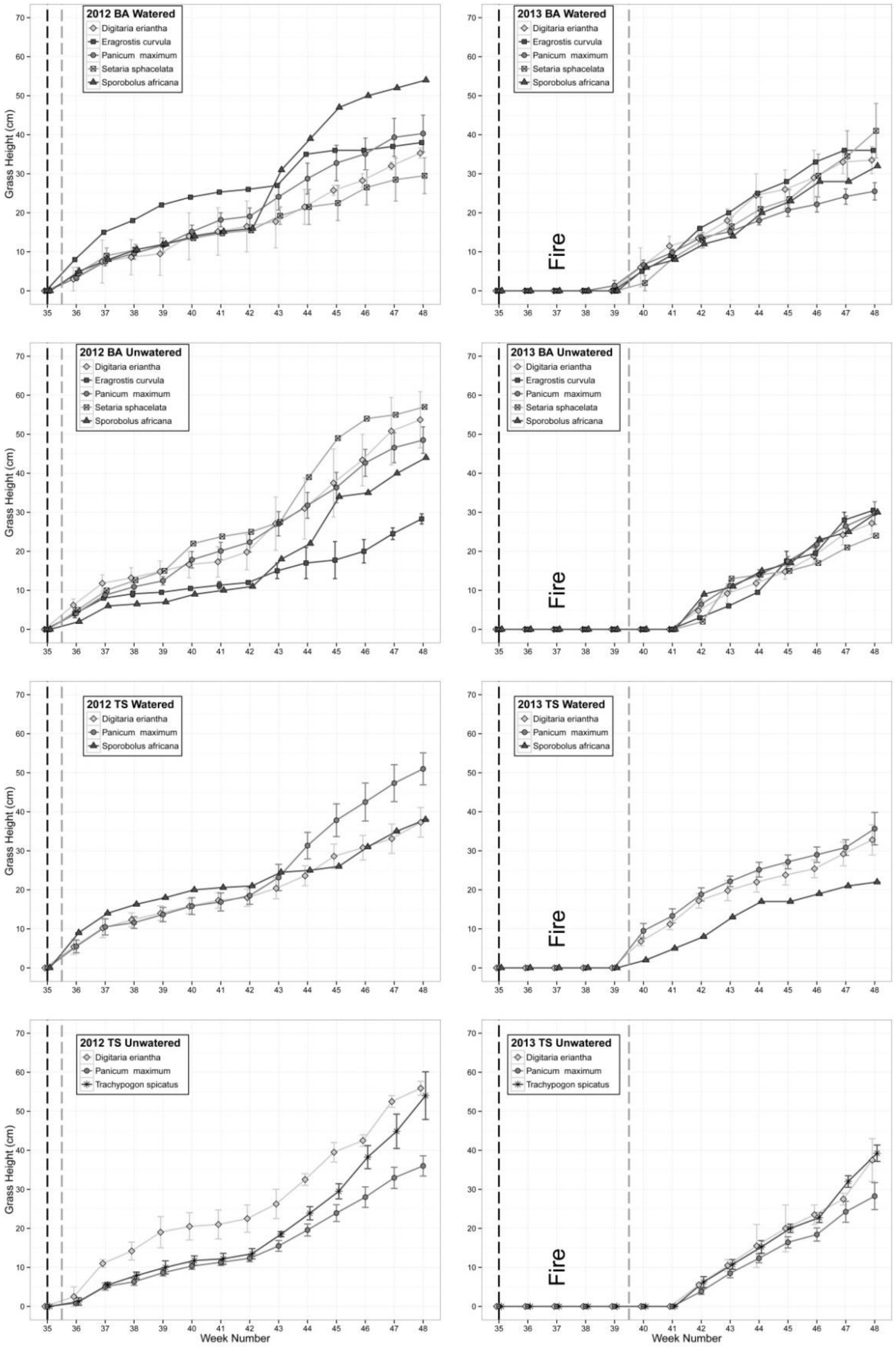

Figure S1. The mean (±S.E.) height of 0.5 x 0.5 m grass plots below the canopies of watered and unwatered *Burkea africana* (BA) and *Terminalia sericea* (TS) during the 2012 and 2013 growing seasons. The black vertical dashed lines indicate the start dates of irrigation of the watered treatments and the grey line indicates the start dates for seasonal rainfall. On 19th September 2013 a severe wildfire occurred in the study site which burnt all of the standing grass biomass, indicated by the word ‘Fire’.

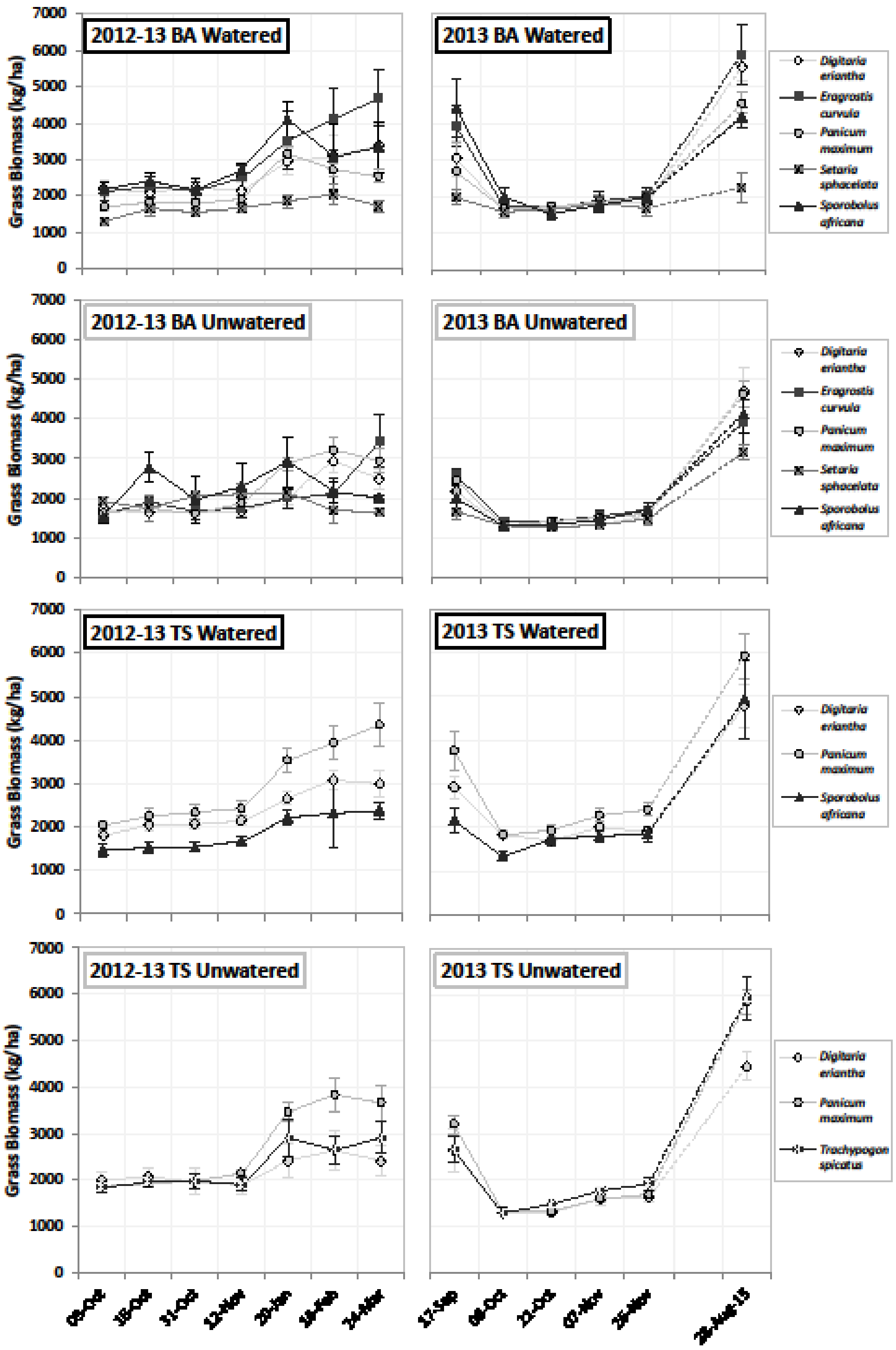

Figure S2. Grass biomass (mean ±S.E.) of specific species measured using a disc pasture meter below and between the canopies of watered and unwatered *Burkea africana* (BA) and *Terminalia sericea* (TS) trees during the 2012 and 2013 growing seasons. A final biomass measurement was taken at the end of August 2015 to determine any long term changes. A fire burnt all of the aboveground biomass on the 19th September 2013.

CHAPTER 4

Assessing leaf development and functionality in a South African broad-leaf savanna

This chapter has been prepared for submission to Plant Ecology

1. Abstract

At what stage of a savanna tree leaf's development does it become a contributor to whole plant carbon gain, rather than a drain on its stored resources? This study aimed to assess the development and functionality of leaves of two semi-arid savanna tree species (*Burkea africana* and *Terminalia sericea*) in relation to their seasonal phenology in the Nylsvley Nature Reserve (NNR), Limpopo, South Africa. Over two growing seasons we measured leaf chlorophyll content using a SPAD 502-meter and leaf gas exchange using a LI-6400 XT portable photosynthesis system. New leaves of both species had low levels of chlorophyll content and little photosynthetic activity within the first two weeks of bud-break, however, transpiration was occurring in the new leaves. Both chlorophyll content and photosynthetic activity rapidly increased with leaf age. *Burkea africana* had lower overall photosynthetic rates and maintained the same set of leaves throughout the growing season, while *T. sericea* had high photosynthetic rates and continued to produce new leaves with shorter lifespans throughout the growing season. It is unclear whether the new leaves of early-greening trees are functional prior to the onset of seasonal rainfall in the NNR, given the low photosynthetic rates of new leaves post rainfall onset. However, early-greening does provide trees with a head-start on leaf-expansion after rainfall onset, where new leaves (carbon sinks) expand into carbon contributors to whole plant growth faster than the dormant grasses and late-greening trees.

Keywords: early-greening, leaf development, leaf functionality, phenology, photosynthesis

2. Introduction

Savanna climates are characterised by an intense wet season during which the majority of the annual precipitation falls, followed by a lengthy dry season that usually experiences very little precipitation (Huntley and Walker, 1982). This stark climatic contrast has enabled certain species of deciduous savanna tree to evolve a seemingly unusual phenological strategy: they lose their leaves at the end of the wet season and then produce new flush during the driest period of the year up to one month ahead of the onset of the following season's rainfall (Elliott et al., 2006; Whitecross et al., 2016 - *see Chapter 2*). This phenological phenomenon is called early-greening and it has been observed in deciduous savannas around the world, including Africa (Childes, 1989; De Bie et al., 1998; Chidumayo, 2001; February and Higgins, 2016; Whitecross et al., 2016 - *see Chapter 2*), Australia (Williams et al., 1997; Prior et al., 2003), South and Central America (Sarmiento et al., 1985; Borchert and Rivera, 2001), and Asia (Elliott et al., 2006). Early-greening has not been recorded in any other terrestrial ecosystems. Numerous proposed benefits for this strategy include an extended growing season, a reduced threat from invertebrate herbivores on new flush at the start of the growing season, quicker uptake of nutrients made available when the rains commence, and decreased competition from other late-greening tree species (Sarmiento et al. 1985; Dye & Walker 1987; Scholes & Walker 1993; Elliot et al. 2006; *see Chapter 5).* Unfortunately, many of these theories have not yet been quantified, and very few phenological studies in African savannas have assessed the phenological changes which occur at the leaf scale. In particular, the degree to which early greening can extend the growing season has never been quantified.

Leaves form the principle site for the exchange of carbon, water and energy between terrestrial vegetation and the surrounding atmosphere (Lambers et al., 1998; Wright et al., 2004; Pantin et al., 2012). Their emergence at the start of the growing season in deciduous

African savannas is vital for driving ecosystem functionality through the uptake of nutrients such as nitrogen and phosphorus, as well as the conversion of carbon gases into plant structural matter (Lambers et al., 1998; Wright et al., 2004). However, as leaves develop they can potentially face numerous abiotic and biotic stresses (Coley, 1988; Crawley, 1989; Coley and Kursor, 1996; Bryant et al., 1991; Nabity et al., 2009; Pantin et al., 2012). Abiotic stresses that negatively affect leaf development and longevity include limited nutrient and water availability, temperature, and light limitations through shading by neighbouring vegetation (Huntley and Walker, 1982; Coley et al., 1985; Sarmiento et al., 1985; Smith and Goodman 1987; Bertness and Callaway, 1994; Scholes and Archer, 1997; Eamus et al., 2001; Baldocchi et al., 2004; Kgope, 2005). In African savannas the impacts of invertebrate and mammalian herbivory on leaf development and survival is well documented (Scholtz, 1982; Cooper and Owen-Smith, 1986; Cooper et al., 1988; Bryant et al., 1991; Sankaran et al., 2008; Hempson et al., 2015; Davies et al., 2016). New leaves have to develop rapidly if they are to survive the impacts of invertebrate herbivores, the effects of which can be restricted in older leaves through the production of tannins and secondary compounds which make the leaf unpalatable (McKey et al., 1978; Coley et al., 1985; Bryant et al., 1991; Scogings et al., 2004; Nabity et al., 2009). Many African plants also rely on structural defences, such as thorns or prickles, to protect their leaf matter from the onslaught of mammalian herbivores (Huntley and Walker, 1982; Cooper and Owen-Smith, 1986; Bazzaz et al., 1987; Milewski et al., 1991; Midgley et al., 2001; Gowda and Palo, 2003). In order to grow and enhance these structural defences, savanna plants need to ensure that their leaves are able to develop and function optimally as soon as possible so that excess carbon can be rapidly diverted to defence sites (Bazzaz et al., 1987; Coley, 1988; Coley and Kursor, 1996; Gowda and Palo, 2003).

Leaf development is made up of two main components which include: an increase in volume through expansive growth and an increase in structure through an accumulation of dry matter (Dale, 1985; Bazzaz et al., 1987; Lambers et al., 1998; Pantin et al., 2012). Leaves are initially carbon sinks as they first begin to grow, relying on stored reserves of the tree to produce and expand the initial buds (Pantin et al., 2012). As a leaf grows, it transitions from a net carbon importer (new leaf) to an exporter (mature, functional leaf), at which point stomatal and photosystems are fully functional (Turgeon, 1989). No photosynthesis is observed in new leaves across many northern hemisphere species and it is likely that this is observed across a wider array of species globally as well. However, as the new leaves begin to expand, transpiration commences, their photosynthetic apparatus matures and eventually carbon gain is observed (Turgeon 1989; Pantin et al. 2012). Choinski and Johnson (1993) measured photosynthetic rates and the water status of developing *Brachystegia spiciformis* (an early-greening tree) leaves in Zimbabwe and found that pre-rain new leaves showed non-existent to very low levels of photosynthesis, however, as the leaves expanded these rates increased rapidly and reached a peak when the leaves became mature after the onset of rainfall. Other studies on early-greening savanna trees, such as *Ochna pulchra*, showed no gas exchange taking place in young leaves due to underdeveloped stomata or occlusion by the cuticle (Bate and Ludlow, 1978; Cresswell et al., 1982; Ludlow, 1991). Transpiration rates in new *B. spiciformis* leaves were low and increased as leaves expanded, which is likely to have assisted this species in limiting water loss through the new leaves during the driest part of the season (Choinski and Johnson, 1993). Key to understanding the benefits of early-greening for savanna tree species is determining how functional these early leaves are, and at what point transpiration and carbon gain occur. If leaves are not transpiring they run the risk of overheating in the hot dry days at the end of the dry season. If they are not photosynthesising then this strategy may only extend the growing season of early-greening trees through providing the advantage of faster leaf expansion after rainfall onset.

The aims of this study are to investigate (1) how the photosynthetic rates change as the leaves of two dominant broad-leaved savanna trees in Nylsvley Nature Reserve (*Burkea africana* and *Terminalia sericea*) mature – at what point do they start being a benefit rather than a cost to the tree? As well as (2) how photosynthetic rates and transpiration are linked to the onset of rainfall through an increase in soil moisture? For example, in early-greening species, can any sign be detected that the pre-rain leaves are photosynthetically active, thus suggesting that trees are experiencing a longer growing season than their herbaceous competitors are?

3. Material and Methods

3.1. Study site

The study took place in the Nylsvley Nature Reserve (NNR), Mookgophong, Limpopo Province, South Africa (24°39’ S, 28°42’ E; 3975 ha) broad-leaved savanna between August 2012 to February 2014. NNR is part of the central sandy bushveld of the savanna biome classified by Rutherford et al. (2006). The NNR is a well-researched area which offers the opportunity to compare this study’s findings with extensive literature on the same area (Coetzee et al., 1977; Huntley and Morris, 1982; Rutherford and Panagos, 1982; Scholes and Walker, 1993; Whitecross et al., 2016). The climate in NNR is seasonal with hot, wet summers and cool dry winters (Huntley and Morris, 1982). An average of 85% of the ~623 mm mean annual precipitation falls between October to March during intense thunderstorms (Huntley and Morris, 1982). The mean maximum summer temperature is 29 °C, with a mean minimum winter temperature of 4 °C (South African Weather Services 2013). The NNR soils are infertile and approximately 1-2 m deep, with loamy, sandy soils derived from Waterberg sandstone (Coetzee et al., 1977; Scholes and Walker, 1993). For detailed analyses of the phenology of the *B. africana* and *T. sericea* trees during this study please refer to Whitecross et al. (2016).

3.2. Study species

Two of the dominant broad-leaved savanna trees in the NNR were selected for this study. *Burkea africana* (Hook.) (Caesalpiniaceae) is a medium-sized deciduous, leguminous tree that has the ability to flush its leaves prior to the onset of seasonal rainfall and is known to green early over much of its range (Childes, 1989; De Bie et al., 1998; Maroyi, 2010). However, at the NNR (far south of its range) it only greens before the rains ~20% of the time (Whitecross et al. 2016 *in press – see Chapter 6* – rainfall onset is highly variable in this system). It takes an average of 8 weeks to reach full canopy, commencing in approximately late September (Rutherford and Panagos, 1982; Whitecross et al. 2016 – *see Chapter 2*).

Terminalia sericea (Burch. ex DC.) (Combretaceae) usually greens-up simultaneously with the onset of rainfall (Childes, 1989; Moyo et al. 2015), however, early-greening has been observed for this species (Childes, 1989; Moyo et al., 2015). It is a small to medium-sized deciduous tree with an average height of 9 m and a widespread distribution across southern Africa's savannas (Lemmens, 2009).

3.3. Experimental design and protocol

3.3.1. Tree and leaf characteristics

Ten *B. africana* and ten *T. sericea* trees in the NNR broad-leaved woodland were selected for this study. The heights and stem diameters (1.5 m from the base of the trunk) were measured to the nearest 0.5 m and 0.1 cm respectively. The specific leaf area (SLA) for 20 mature leaves each from five selected individual *B. africana* and *T. sericea* trees was calculated using the one sided area of a fresh leaf divided by its oven-dry mass ($m^2.kg^{-1}$), which is the SLA standard procedure in Cornelissen et al. (2003). Leaves were collected and analysed at the end of November 2012. Leaf moisture content was calculated as the difference between the wet and dry mass of the leaf in relation to the total wet mass of the leaf. Leaf thickness was measured using Vernier callipers placed adjacent and parallel to the right-side of the mid-vein of the leaf and a measurement in mm (0.1 mm) was taken (these results can be found in the appendix).

3.3.2. Leaf lifespans

A leaf lifespan assessment was set up on six trees from each species. Three branches per individual canopy were tagged and on each individual branch, three leaves (*B. africana*) or leaf clusters (*T. sericea*) were also tagged using white cable ties. These leaves were monitored each week from the start of leaf flush in early September until the end of November during the green up period of 2012 after which they were monitored once a month until the end of the growing season (late April). At the end of the green-up period (November) any new flush was separately marked with black cable ties and in the peak growing season (February) new flush was marked with yellow cable ties. The number of leaflets (*Burkea africana*) and leaves (*Terminalia sericea*) per leaf/cluster tagged, were sequentially numbered and counted each week and the leaf lifespan was calculated based on the number of days each leaflet/leaf was present during the season.

3.3.3. Tree phenology

The total percentage of canopy leaf area was estimated for each austral growing season starting in 2012 and 2013. Sampling was divided into two periods: 1) weekly estimates of percent canopy area between September to November and 2) monthly estimations from December to April. Three leaf age classes were measured: new (<0.5*length of adult leaf), fully expanded (>0.5*length of adult leaf, not yet sun-drenched) and mature (sun-drenched, adult leaf) (based on Williams et al., 1997). The Walker aerial cover scale was used to estimate canopy cover of each leaf age class, where 0: 0%, 1: 1-10%, 2: 10-25%, 3: 25-50%, 4: 50-75%, 5: 75-90%, 6:90-99%, and 7: 100% (Walker, 1976). A phenophase is the duration of which a specific leaf age class dominates >50% of total canopy area. Refer to

Whitecross et al. (2016 – *see Chapter 2*) for further details on tree phenology during this study.

3.3.4. Chlorophyll content

Chlorophyll is required for active photosynthesis and positive carbon gain (Evans, 1989; Wu et al., 2008), and the chlorophyll content of new leaves is an indication of their maturity and ability to photosynthesise optimally. The SPAD-502 chlorophyll meter (Minolta Camera Co. Ltd., Japan) can measure the amount of chlorophyll within a leaf based on the measurement of the absorption of red and infrared spectra. Samples from ten new, fully expanded and mature leaves from each of the sample trees' canopies (10 *B. africana* and 10 *T. sericea* trees) were measured from two weeks after the first flush (start of the growing season, ~mid-October). Sampling was conducted once a month from October 2012 to April 2013 during the 2012-13 growing season. Samples during the 2013-14 growing season were measured during November and December 2013, as well as February 2014.

3.3.5. Gas exchange

From the first date of flush, the net CO_2 and H_2O exchange was measured on new, fully expanded and mature leaves using a LICOR portable open-path infra-red gas analyser (IRGA) (LI-6400XT: LI-COR Bio-sciences, Lincoln, NE, USA). The light conditions within the IRGA during measurements were set to 1200 µmol m^{-2} s^{-1} using a blue-red LED light source (6400-02B). The temperature on the IRGA block was maintained at 25°C and the flow of air through the IRGA was maintained at 400 µmol s^{-1}. The CO_2 concentration was set to 400 µmol mol^{-1} using a 6400-01 CO_2 mixer. All measurements were recorded once a month between 07:00 am and 12:00 pm from October to February of both seasons (2012-13 and 2013-14). During the 2012-13 season, six individual trees of *B. africana* and *T. sericea* were measured and during the 2013-14 season, five individuals were measured from each species (photosynthetic rates for each individual season can be found in Figure S1). Between three and five leaflets/leaves were measured on each individual canopy during each session. The net photosynthesis (*A*), stomatal conductance (g_{st}) and transpiration (*E*) were recorded and calculated according to von Caemmerer and Farquhar's (1981) equations. Leaf age and area was estimated prior to the insertion of the leaf into the chamber and entered into the system accordingly. Leaves which did not completely fill the IRGA chamber were accounted for by adding in a correction factor that accurately represented the size of the area filled in the chamber by the leaf. A sensitivity analysis ensured that the leaf area estimates were not biasing the gas exchange calculations at both 5% and 10% of the leaf area estimates. Data were logged after a minimum of 60 seconds, if the changes in sample CO_2 and H_2O showed a slope of <0.2, the flow rate slope remained <0.5 and the leaf temperature slope was <0.1 for a minimum of 30 seconds.

3.3.6. Soil moisture

Daily soil moisture was recorded using eight EnviroSMART probes and a Campbell Scientific CR1000 data-logger set to record the percentage of water content in the soil at five minute intervals. Soil moisture was recorded at a depth of 5cm below the surface. Data were averaged to attain daily measurements between August 2012 and October 2013.

3.4. Data analysis

All photosynthesis data were pooled into one dataset for both seasons and analysed using RStudio (version 0.99.896). The leaf chlorophyll content over the season was analysed using a multilevel linear model from the package *nlme* (Pinheiro et al., 2016) and a Tukey posthoc analysis from the package *multcomp* (Hothorn et al., 2008). This model is able to deal with correlated dependent data and therefore allows us to successfully compare the leaves from the same trees through time. A Wilcoxon rank sum test was used to compare specific leaf age classes’ chlorophyll over the season between *B. africana* and *T. sericea*. A Kruskal-Wallis rank sum test was used to compare the mean photosynthetic rates between leaves of different age classes in conjunction with a Kruskal multiple comparisons posthoc analysis from the package *pgirmess* (Giraudoux, 2014). The relationships between photosynthesis and both transpiration and conductance between species and leaf age classes were tested using linear regressions (R Core Team 2014). Differences in the slopes of the regressions were tested using ANCOVAs (R Core Team 2014). Mixed effects models from the package *lme4* (Bates et al., 2015) were used to assess the effects of soil moisture, leaf age and species on leaf photosynthetic rates and model efficacy was then compared using the anova() function from the package ‘car’ (Fox and Weisberg 2011).

4. Results

4.1. Phenophases and leaf lifespans

Whitecross et al. (2016 – *see Chapter 2*) summarised the lengths of phenophases in *B. africana* and *T. sericea* at the NNR during the 2012-13 and 2013-14 growing seasons and the pooled averages for this period are presented below (Table 1). *Burkea africana* leaves tagged at the start of the growing season had an average leaf lifespan of 175±2 days. Unlike *B. africana* which produced and maintained the same set of leaves throughout the growing season, *T. sericea* continued to produce new flush over the course of the growing season. *Terminalia sericea* leaves produced and tagged at the start of the growing season had an average leaf lifespan of 128±5 days, while leaves produced during the middle of the growing season (January) had an average lifespan of 70±4 days.

Table 1. Mean (±SE) phenological summaries taken from Whitecross et al. (2016) for *Burkea africana* and *Terminalia sericea* trees at Nylsvley Nature Reserve during the 2012-13 and 2013-14 austral growing seasons. Phenophases were calculated for new leaves as the first day of leaf flush to >50% canopy cover of fully expanded leaves and then for fully expanded until there was >50% canopy cover of mature leaves. Estimates of the mature leaf phenophase were calculated based on the number of days in months where >50% canopy cover of mature leaves was present.

Species	Average total number of weeks to reach 50% mature canopy	Average Phenophase (No. of days)		
		New to Fully Expanded	Fully Expanded to Mature	Mature to Senescing (Estimate)
Burkea africana	7.1±0.5	42±3.6	21.0±1.5	138
Terminalia sericea	7.7±0.5	42±3.0	30.2±1.9	145

4.2. Temporal and ontogenic changes in leaf physiology

4.2.1. Chlorophyll content

The chlorophyll content showed a significant increase from new leaves to fully expanded to mature leaf age classes with a subsequent decrease into the senescent leaf age class over the course of the growing season for both *B. africana* ($\chi^2_{(6)}$=471.68, p<0.001, Figure 1) and *T. sericea* ($\chi^2_{(6)}$=68.08, p<0.001, Figure 1). The majority of leaf age classes showed little to no overlap in chlorophyll content (see posthoc results on Figure 1); however, senescent leaf chlorophyll content was reduced to levels equivalent to those of new leaves at the end of the growing season in both species (Figure 1). The mean±SE chlorophyll content for new *B. africana* leaves (21.88±0.49 SPAD Units) showed no difference to the new *T. sericea* leaves (21.24±0.20 SPAD Units) (t=1.21; d.f.=376; p=0.23). The mean±SE chlorophyll content for mature *B. africana* leaves (48.20±0.23 SPAD Units) was higher than the mature *T. sericea* leaves (46.91±0.18 SPAD Units) (t=4.42; d.f.=1968; p<0.001).

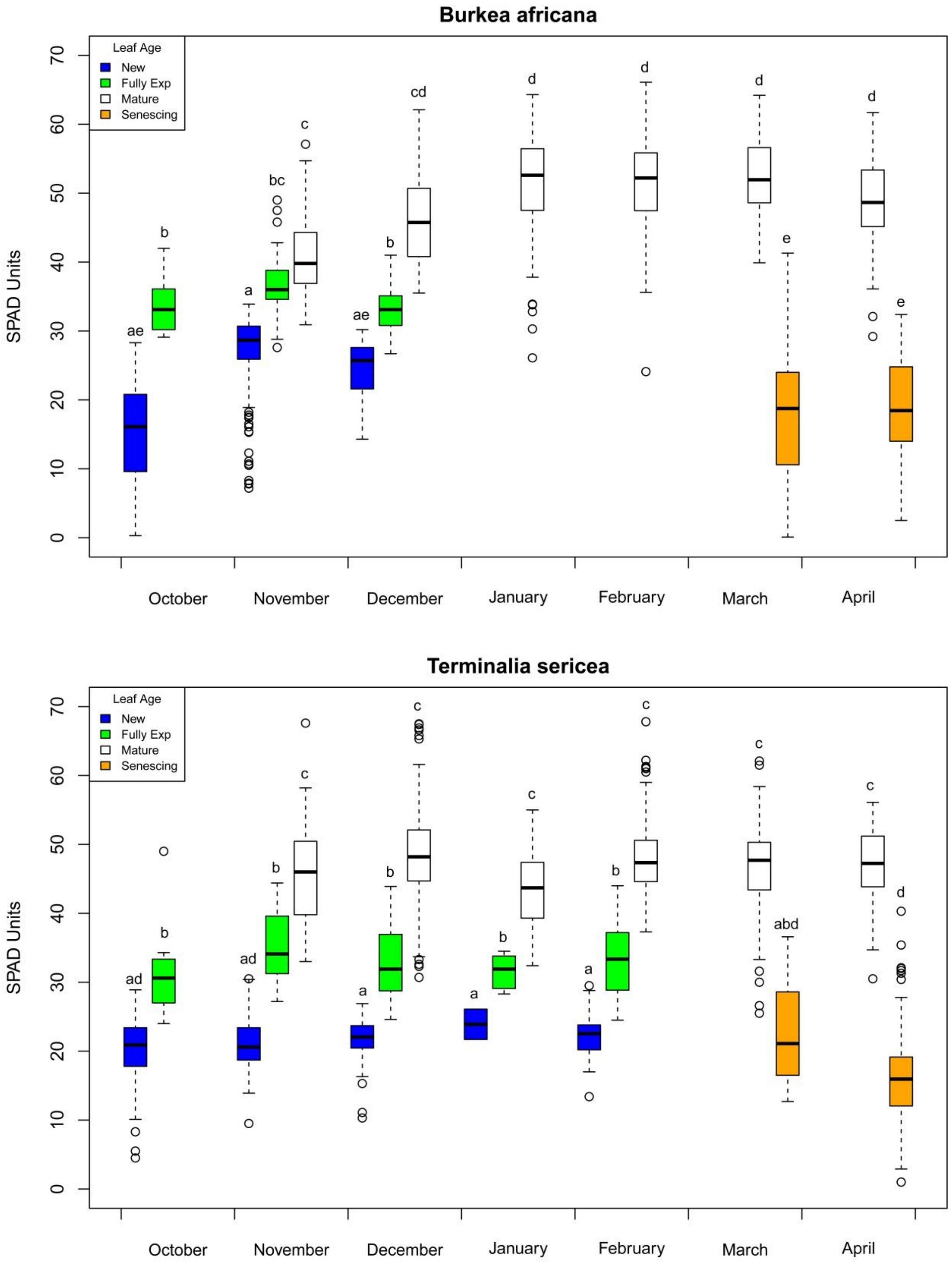

Figure 1. The monthly chlorophyll content (indicated in SPAD units) of new, fully expanded, mature and senescing *Burkea africana* and *Terminalia sericea* leaves over the course of a growing season (October to April). Letters represent the results of multiple comparison posthoc tests.

4.2.2. Gas exchange

4.2.2.1 Does leaf age affect photosynthetic rates?

Leaf age and species had a significant effect on photosynthetic rates between October and March (Kruskal: χ^2=143.7; d.f.=5; p<0.001, Figure 1), with new leaves from each species showing the significantly lowest rates overall (Kruskalmc: p<0.05). No difference was observed between species within leaf age categories for new and fully expanded leaves, however, mature *B. africana* leaves (9.73 µmol CO_2 $m^2.s^{-1}$) had significantly lower rates than mature *T. sericea* leaves (12.4 µmol CO_2 $m^2.s^{-1}$) (Kruskalmc: p<0.05, Figure 1). No overall differences between fully expanded and mature leaves were observed for either species (Kruskalmc: p<0.05, Figure 1).

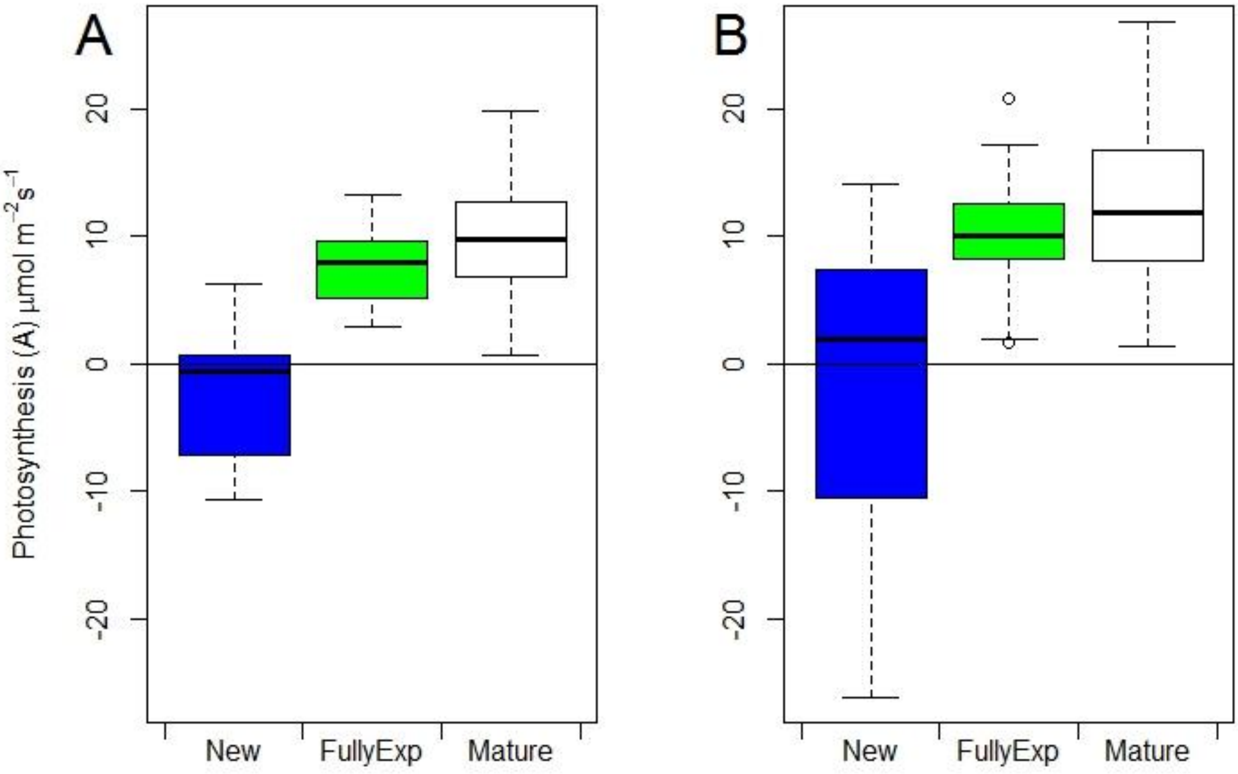

Figure 2. The photosynthetic rates of new, fully expanded and mature leaves from (A) *Burkea africana* and (B) *Terminalia sericea* between October to March. Significant differences are indicated with letters above each age category.

4.2.2.2 Are new leaves functional when they first flush?

Photosynthetic rates only became positive after the first ~14 days in *B. africana* and ~20 days after the start of leaf flush in *T. sericea* (Figure 3). New leaves of both *B. africana* (-2.13±1.0 µmol CO_2 m^2s^{-1}) and *T. sericea* (-0.95±1.6 µmol CO_2 m^2s^{-1}) had a negative carbon balance, indicating that respiration exceeded photosynthesis (Figure 3). Photosynthetic rates increased with time from the start of new leaf growth, but then decreased for fully expanded and mature leaves for both *B. africana* (χ^2=12.8; d.f.=2; p<0.002; AIC=1460.4, Table 2) and *T. sericea* (χ^2=84.6; d.f.=2; p<0.001; AIC=1592.6, Table 2).

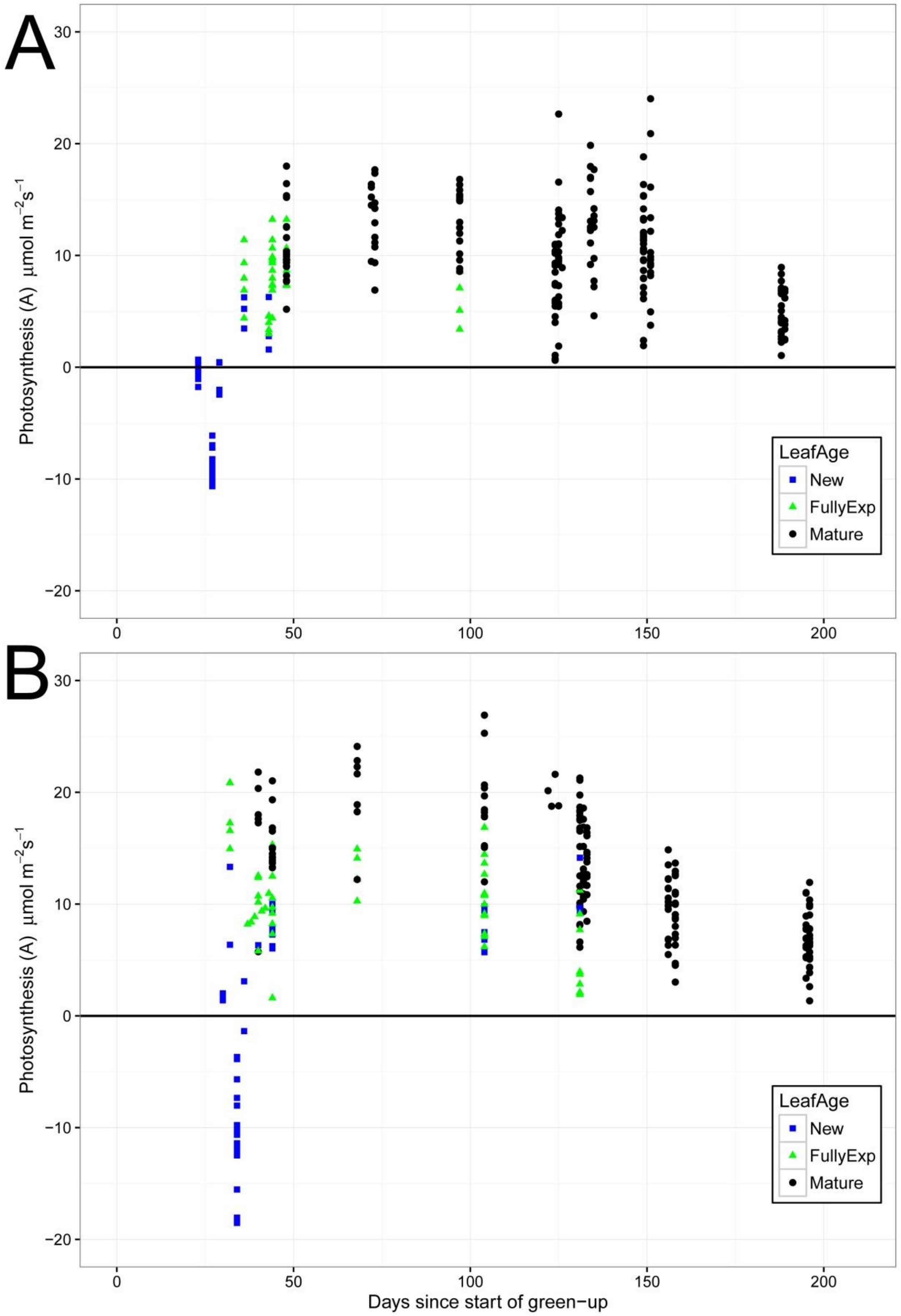

Figure 3. The photosynthetic rates of new, fully expanded and mature leaves of (A) *Burkea africana* and (B) *Terminalia sericea* trees in relation to the number of days after the start of green-up of each species.

Table 2. Summary of the linear mixed effects model outputs: B=beta coefficient (slope), CI=95% confidence intervals, p=p-value, as well as the group counts for the random effects and the Intra-Class-Correlation-Coefficients (ICC) for each set of models relating to the effects that leaf age and time since start of green-up have on photosynthetic rates of *Burkea africana* and *Terminalia sericea*. Akaike Information Criterion (AIC) values for comparisons within species are provided.
Model 1: Photosynthesis~Days After Green-up+(1|TreeID)
Model 2: Photosynthesis~Leaf Age (New, Fully expanded, Mature)+ (1|TreeID)
Model 3: Photosynthesis~Leaf Age (New, Fully expanded, Mature)+Days After Green-up+(1|TreeID)
Model 4: Photosynthesis~Days After Green-up*Leaf Age (New, Fully expanded, Mature)+(1|TreeID)

Burkea africana

	Model 1 (AIC: 1618.4)			**Model 2** (AIC: 1502.9)			**Model 3** (AIC: 1469.2)			**Model 4** (AIC: 1460.4)		
	B	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*
Fixed Parts												
(Intercept)	7.57	5.45 – 9.70	**<.001**	-1.91	-3.74 – -0.08	**0.04**	-0.68	-2.42 – 1.06	0.443	-13.3	-20.31 – -6.20	**<.001**
Days After Green-up	1.12	-0.23 – 2.46	0.104				-4.18	-5.51 – -2.86	**<.001**	39.4	15.67 – 63.13	**0**
Leaf Age (Fully expanded)				9.83	7.70 – 11.95	**<.001**	10.9	8.89 – 12.91	**<.001**	22.98	15.22 – 30.75	**<.001**
Leaf Age (Mature)				11.9	10.02 – 13.70	**<.001**	16	13.85 – 18.17	**<.001**	28.81	21.56 – 36.07	**<.001**
Days After Green-up: Leaf Age (Fully expanded)										-42.7	-67.04 – -18.25	**0**
Days After Green-up: Leaf Age (Mature)										-43.8	-67.54 – -20.00	**0**
Random Parts												
$N_{TreeID2}$		6			6			6			6	
$ICC_{TreeID2}$		0.085			0.027			0.025			0.028	
Observations		258			258			258			258	

Terminalia sericea

	Model 1 (AIC: 1821.4)			**Model 2** (AIC: 1710.7)			**Model 3** (AIC: 1673.2)			**Model 4** (AIC: 1592.6)		
	B	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*
Fixed Parts												
(Intercept)	10	7.72 – 12.36	**<.001**	-0.3	-2.18 – 1.58	0.754	2.41	0.48 – 4.34	**0.01**	-9.11	-11.93 – -6.29	**<.001**
Days After Green-up	0.35	-1.31 – 2.01	0.679				-5.06	-6.57 – -3.54	**<.001**	16.43	11.97 – 20.89	**<.001**
Leaf Age: (Fully expanded)				10.6	8.11 – 13.04	**<.001**	11.8	9.47 – 14.10	**<.001**	22.95	18.91 – 26.99	**<.001**
Leaf Age (Mature)				12.8	10.67 – 14.83	**<.001**	16.8	14.55 – 19.12	**<.001**	31.64	28.13 – 35.16	**<.001**
Days After Green-up: Leaf Age (Fully expanded)										-21	-26.62 – -15.45	**<.001**
Days After Green-up: Leaf Age (Mature)										-23.9	-28.63 – -19.23	**<.001**
Random Parts												
$N_{TreeID2}$		6			6			6			6	
$ICC_{TreeID2}$		0.024			0			0			0	
Observations		265			265			265			265	

Small, but positive transpiration rates were observed in new leaves, but there was either a very weak, or a not significant relationship between stomatal conductance and photosynthesis for these young leaves. Transpiration levels in new *B. africana* leaves were very low and no significant relationship between photosynthesis and transpiration was detected (Figure 4A). However, a weak positive relationship between photosynthesis and conductance was detected for new *B. africana* leaves (Figure 4C). In contrast, significant positive relationships between photosynthesis and both transpiration and conductance were detected for the fully expanded and mature *B. africana* leaves (Figure 4A&C) and the slopes of these relationships were much steeper. Results of an ANCOVA analysis showed that the relationship between photosynthesis and transpiration in new *B. africana* leaves was significantly different from both fully expanded and mature leaves ($F_{2,275}=45.13$, $p<0.001$), however, no difference was detected between the slopes of the latter two age classes (posthoc: $p>0.05$; Figure 4A). An ANCOVA for *B. africana*'s photosynthesis and conductance between leaf age classes showed that new leaves were significantly different from all other age classes ($F_{2,275}=49.14$; $p<0.001$) and fully expanded and mature leaves also showed a difference (posthoc: $p=0.03$, Figure 4C). *Terminalia sericea* leaves showed a stronger positive relationship between photosynthesis and transpiration in the new age class than the new *B. africana* leaves but this difference was not significant ($F_{1,64}=1.67$; $p=0.20$; Figure 4A&B). New *T. sericea* leaves' photosynthesis and transpiration relationship was significantly lower than either of the other age classes ($F_{2,\ 287}=47.55$; $p<0.001$), with no difference between the slopes of fully expanded and mature age classes (posthoc: $p>0.05$; Figure 4B). New *T. sericea* leaves did not show a difference to new *B. africana* leaves for the photosynthesis and conductance relationships either ($F_{1,64}=3.99$; $p=0.05$; Figure 4C&D), however, new *T. sericea* leaves were significantly different to all other age classes

($F_{2,287}=42.54$; $p<0.001$; Figure 4D). Fully expanded and mature *T. sericea* leaves had similar trends between photosynthesis and conductance (posthoc: $p>0.05$; Figure 4D).

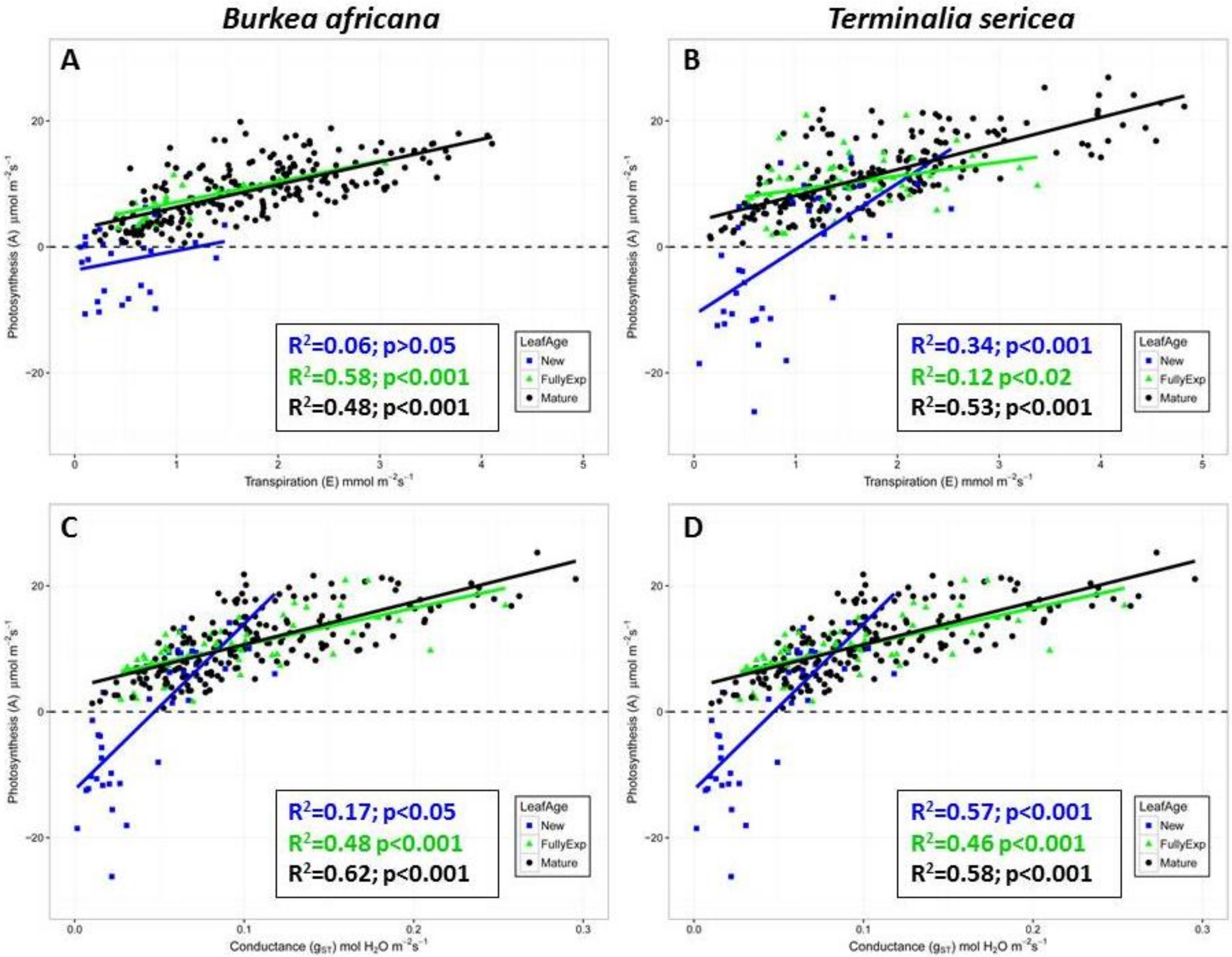

Figure 4. The relationships between photosynthesis and (A & B) transpiration rates, as well as (C & D) stomatal conductance rates for (A & C) *Burkea africana* and (B & D) *Terminalia sericea* leaves of new, fully expanded and mature age classes.

4.2.2.3 Does soil moisture influence photosynthetic rates?

A mixed effects model showed that as soil moisture increased photosynthetic rates decreased (AIC: 3454.6; Table 3 (Model 3)). This is most likely because Model 3 does not address the effect of leaf age where new leaves have low photosynthetic rates during the beginning of the rainfall onset when soil moisture increases rapidly. The model was improved when soil moisture, leaf age and species were included where each variable had a positive effect on photosynthesis (χ^2=231.05; d.f.=3; p<0.001; AIC: 3229.6; Table 3 (Model 2)). The model fit was further improved upon the inclusion of an interaction term between soil moisture and leaf age (χ^2=104.34; d.f.=2; p<0.001; AIC: 3129.2; Table 3 (Model 1)). New leaves showed little response to soil moisture, and appear to require a certain amount of time to become functional (Figure 3, Table 2). However, fully expanded and mature leaf photosynthetic rates responded positively to increased soil moisture. (Table 3 (Model 1)).

Table 3. Summary of linear mixed effects model outputs: B=beta coefficient (slope), CI=95% confidence intervals, p=p-value, as well as the group counts for the random effects and the Intra-Class-Correlation-Coefficients (ICC) for each model.
Model 1: Photosynthesis ~ Soil moisture*Leaf Age(New, Fully expanded, Mature)+Species(Burkea africana, Terminalia sericea)+(1|TreeID);
Model 2: Photosynthesis ~ Soil moisture+Leaf Age(New, Fully expanded, Mature)+Species(Burkea africana, Terminalia sericea)+(1|TreeID);
Model 3: Photosynthesis ~ Soil moisture+(1|TreeID).

	Model 1			**Model 2**			**Model 3**		
	B	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*
Fixed Parts									
(Intercept)	27.08	20.20 – 33.95	**<.001**	-5.08	-7.76 – -2.41	**<.001**	12.46	10.02 – 14.90	**<.001**
Soil Moisture (SM)	-370.27	-454.54 – -285.99	**<.001**	32.66	3.97 – 61.36	**0.026**	-42.52	-75.80 – -9.23	**0.012**
Leaf Age (Fully expanded)	-16.09	-24.27 – -7.91	**<.001**	10.56	8.89 – 12.23	**<.001**			
Leaf Age (Mature)	-23.82	-30.96 – -16.68	**<.001**	13.08	11.60 – 14.55	**<.001**			
Species (*Terminalia sericea*)	2.78	1.93 – 3.64	**<.001**	2.39	1.49 – 3.29	**<.001**			
SM:Leaf Age (Fully expanded)	322.89	218.98 – 426.80	**<.001**						
SM:Leaf Age (Mature)	476.14	386.41 – 565.87	**<.001**						
Random Parts									
N_{TreeID}	12			12			12		
ICC_{TreeID}	0.001			0			0.065		
Observations	523			523			523		

5. Discussion

The strong seasonality associated with savanna climates is mirrored by equally strong seasonal changes in deciduous savanna tree phenology (Huntley and Walker, 1982; Williams et al., 1997; Chidumayo, 2001; Elliott et al., 2006; February and Higgins, 2016; Whitecross et al., 2016). Of the theories explaining the benefits of the utilization of an early-greening strategy, these data support the theory that early-greeners will extend their growing season beyond that of other herbaceous and late-greening vegetation (Dye and Walker, 1987; Scholes and Walker, 1993; Elliot et al., 2006), if the onset of rainfall occurs at least two-three weeks after the first flush. However, from this study it is clear that new leaves are carbon-sinks for *B. africana* or *T. sericea* trees and are not functional during at least the first two weeks of flush prior to leaf expansion. The leaf canopy at the start of the growing season is not actively photosynthesising, but it is hydraulically active (transpiring), thus implying that these leaves can potentially enable the uptake of nutrients (via mass flow) (Scholes and Walker, 1993; February and Higgins, 2016), and that they can keep themselves cool (albeit with the loss of water resources.

5.1. When do new leaves become functional?

The first aim of this study was to assess at what point a new leaf becomes a benefit to the tree as a source of carbon, rather than a carbon sink relying on stored reserves to produce the initial leaf tissues (Turgeon, 1989; February and Higgins, 2016). The data show that gas exchange is low during the first two weeks of development, despite the fact that transpiration was occurring (Figure 5).

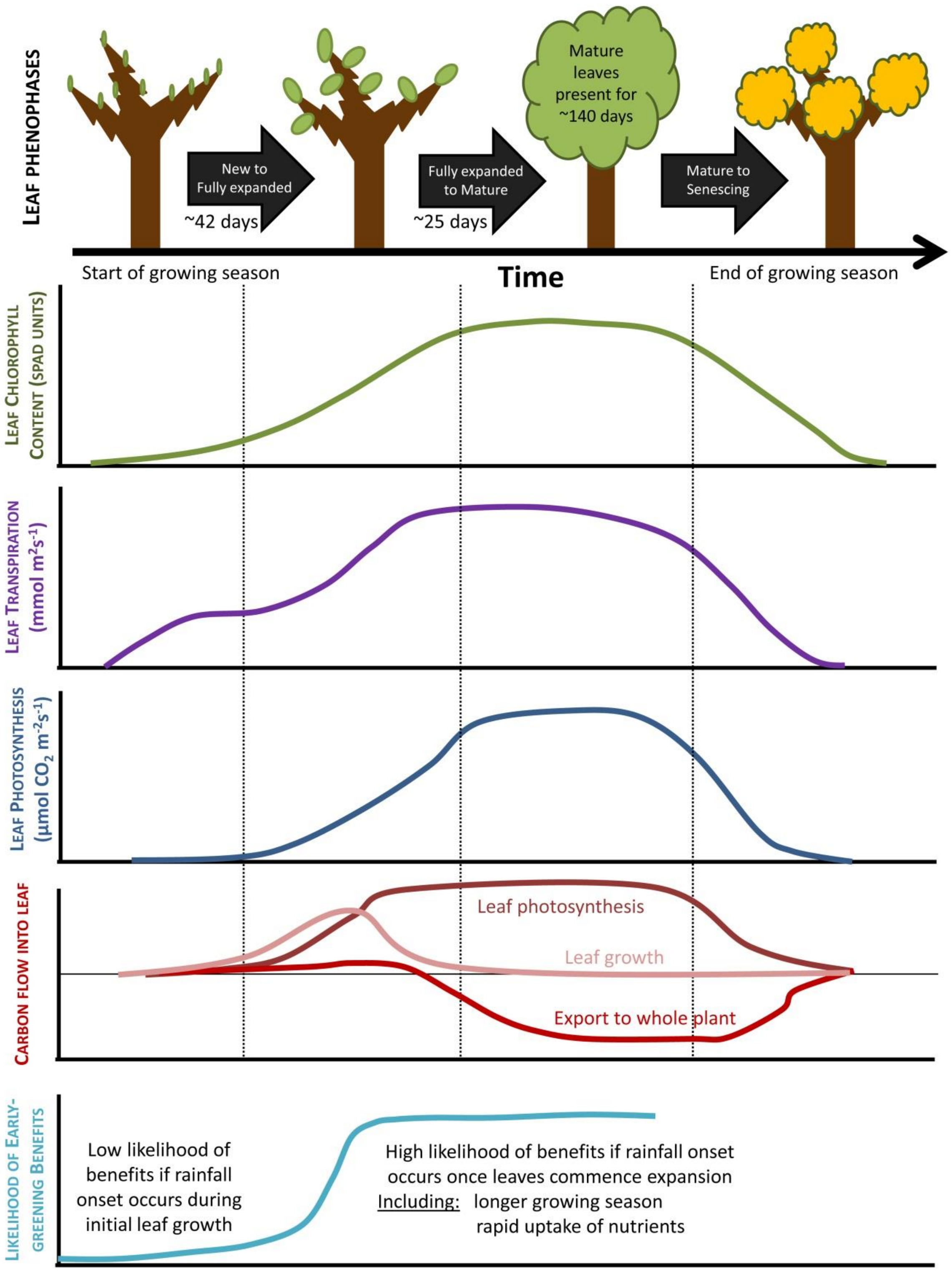

Figure 5. Graphical summary of the major findings of this study in relation to leaf development over the growing season highlighting phenological and physiological changes over time, as well as the likelihood of when early-greening will benefit the trees.

Photosynthetic capacity rapidly increased with leaf expansion, with *T. sericea* leaves photosynthesising higher on average than *B. africana* leaves in the fully expanded and mature age classes (Figures 2 and 3). During the fully-expanded phenophase, leaves are likely to be investing the carbon gained through their low levels of photosynthesis to expand their leaf area and develop the organs required to photosynthesize at maximum capacity (Dale, 1985; Turgeon, 1989). Upon reaching full maturity, the photosynthetic rates of the leaves peaked in December 2012 and 2013 with 13.1±1.0 and 13.0±0.6 µmol CO_2 $m^{-2}s^{-1}$ for *B. africana* respectively and 19.2±1.0 and 18.4±0.6 µmol CO_2 $m^{-2}s^{-1}$ for *T. sericea* respectively. The photosynthetic rates for these two broad-leaved savanna trees were higher than the peak rates measured by Kgope (2005) in the Skukuza broad-leaved savanna where *T. sericea* dominates (8.64±0.54 µmol CO_2 $m^{-2}s^{-1}$) and Stevens et al. (2016) in the Phalaborwa mopane-dominated savanna (~6.5 µmol CO_2 $m^{-2}s^{-1}$), but were similar to the *T. sericea* rates measured by Midgley et al. (2004) in Botswanan savannas (range of 10-22 µmol CO_2 $m^{-2}s^{-1}$).

The trends in the data suggest that the new leaves of both species take at least two weeks to become photosynthetically active. This study’s findings showed similar trends in photosynthesis and transpiration values to those observed in developing leaves of *B. spiciformis* – new leaves were not initially functional despite small levels of transpiration occurring (Choinski and Johnson, 1993). These small levels of transpiration may be linked to water loss through the developing cuticle as observed in *O. pulchra* (Cresswell et al., 1982; Ludlow, 1991).

This study also supports the recent findings of February and Higgins (2016) that deciduous savanna trees in Africa (including *T. sericea*) are able to conserve carbon and limited resources, such as nitrogen, through the reabsorption of these elements from the leaves into the attached branches at the end of the growing season, and that they only deploy nitrogen to their leaves once they are fully expanded. Using these stored reserves the trees are able to rapidly deploy their leaves at the start of the following growing season when available resources are scarce (Scholes and Walker, 1993; February and Higgins, 2016). The chlorophyll content data correspond with February and Higgins’ (2016) findings where senescent leaves show a decrease in chlorophyll content prior to abscission suggesting a reabsorption of nutrients back into the plant at the end of the season (Figure 1).

5.2. Do early-greeners experience a longer growing season?

The second aim of this study was to assess whether leaves of early-greening savanna trees are likely to be functional prior to the onset of seasonal rainfall. Unfortunately, this study was unable to directly test whether new leaves became functional prior to the onset of seasonal rainfall as new leaves produced prior to the onset of rainfall were too small to accurately measure during early-greening seasons (Whitecross et al., 2016 - *see Chapter 2*). However, having assessed these physiological data with respect to the low levels of new leaf photosynthesis, conductance and transpiration during the first two weeks of the growing season, new *B. africana* leaves were functioning at <1% of mature leaves, while fully expanded leaves were functioning at 46% of mature leaves. Thus, considering the canopy is dominated by new leaves for an average of 42 days and fully expanded leaves for 21 days, whole tree carbon gain will only take off after approximately 63 days, despite leaf transpiration taking place during this period which could potentially aid in nutrient uptake should rainfall onset occur (February and Higgins 2016). Therefore, this study postulates that leaf functionality (carbon gain) is likely to be low prior to the onset of seasonal rainfall and then increase rapidly thereafter with the increase in available soil moisture (Cramer et al. 2009; February and Higgins 2016). The first rains of the season also result in a sizeable pulse of nitrogen mineralisation – potentially the largest of the whole season – and it is likely that capturing as much of this available nitrogen as possible is a high priority for plants in a nutrient-poor ecosystem such as the NNR broad-leaf savanna (Scholes and Walker 1993;

Cramer et al. 2009). This study found that soil moisture had an effect on leaf photosynthetic rates during the growing season, which concurs with Whitecross et al.'s (2017 - *see Chapter 3*) finding that an increase in soil moisture decreased the time between phenophases. The increase in carbon gain is likely responsible for driving the faster growth rates. Priyadarshini et al. (2016) have shown that trees (including *T. sericea*) are capable of opportunistically switching from deep soil water during the dry season to surface soil water sources after the onset of seasonal rainfall. By flushing new leaves prior to the onset of seasonal rainfall, this may provide trees with an advantage – over dormant grasses and late-greening trees – to rapidly access plant available nutrients released after the rains (Scholes and Walker, 1993; Elliott et al., 2006; Archibald and Scholes, 2007; Higgins et al., 2011; Whitecross et al., 2016 - *see Chapter 2*; Whitecross et al., 2017 - *see Chapter 3*). This study emphasises the need for modellers of carbon assimilation in savanna systems to be mindful of the variability within and between different species' phenological strategies and how these differences may affect their carbon losses/gains, as well as that of the system as a whole.

In relation to the theorised benefit that early-greening trees have a longer growing season, this would only be likely during a year when seasonal rainfall arrived particularly late (i.e. at least one month after the onset of leaf flush) (Scholes and Walker, 1993; Elliott *et al.*, 2006). If such a scenario were to occur, the physiological costs that these early-greening trees may undergo could potentially be more disadvantageous than the benefit of a longer, competition-free growing season (Figure 4). Some of these risks include damage/loss of vulnerable leaf tissues to late-season fires (Rutherford, 1981), and exposure of new leaves to high heat stress and water loss during the driest period of the growing cycle, which could result in the abscission of the new canopy if rainfall is delayed for too long (Do et al., 2005). Whitecross et al., 2016 *in press – see Chapter 6*) determined that early-greening in the NNR has occurred approximately 20% of the time between 2002 and 2014 and the period of leaf flush prior to rainfall onset is an average of ~15±10 days. Whitecross et al. (2017 – *see Chapter 3*) used historical rainfall data and concluded that between 1980-2014, early-greening has potentially occurred 46% of the time in the NNR. They also demonstrated that grass green-up is closely linked to the onset of seasonal rainfall in the NNR with new grass growth commencing within 4-8 days of the first sizeable precipitation event (Whitecross et al. 2017 – *see Chapter 3*). This minor delay in grass growth affords early-greeners a small window to attain limited nutrients made available by the rainfall in the absence of strong competition from dormant grasses.

5.3. Contrasting phenology and physiology

Burkea africana and *T. sericea* are co-dominant species in the broad-leaved woodland of the NNR; however, their phenological and physiological strategies differ substantially. *Terminalia sericea* leaves measured consistently higher rates of photosynthesis than *B. africana* across fully expanded and mature leaf age classes, despite both species leaves having similar overall chlorophyll content. The specific leaf area (SLA) values for the mature *B. africana* leaves were higher than that of *T. sericea*, with both species falling into the tropical deciduous tree and woodland categories on the leaf mass area (LMA) curve produced by Poorter et al. (2009) (LMA: BA: 121.8±3.4 $g.m^{-2}$; TS: 148.3±3.5 $g.m^{-2}$). Lower SLA values correspond to an increase in investment into structural leaf defences such as surface hairs as observed in the *T. sericea* leaves, as well as a higher level of photosynthetic activity and shorter leaf lifespan (Cornelissen et al., 2003; Wright et al., 2004). Phenologically, both species differ at the mature leaf stage: *B. africana* maintained the same set of leaves produced during the first flush throughout the season, while *T. sericea* continued to produce new leaves throughout the growing season – a strategy called compensatory growth (McNaughton, 1983; *see Chapter 5)*. It can be deduced that *B. africana* places a higher investment of nutrients and chemical defences into its leaves, which are maintained throughout the season with lower levels of photosynthesis on both a daily and seasonal scale (Bryant et al., 1991; Scholes and Walker, 1993) and have greater leaf longevity of 175±2 days compared to *T. sericea* at only 128±5 days. Conversely, *T. sericea* employs higher rates of photosynthesis at both daily and seasonal scales to rapidly assimilate carbon into its newly produced leaves, which allows it to maintain high carbon assimilation rates and enables it to turnover its canopy throughout the season with a lower investment in leaf nitrogen content (Scholes and Walker, 1993). These high photosynthetic rates are also aided by increased light availability within the *T. sericea* canopies due to the lower levels of self-shading in the open-structured canopies (leaf area: ~3.98 m^2m^{-2}, Rutherford, 1979; Yeaton, 1988; Belsky, 1994; Whitecross et al., 2017 – *see Chapter 3*). *Burkea africana* in comparison has high levels of self-shading due to its dense canopy structure (leaf area: ~5.08 m^2m^{-2}, Rutherford, 1979; Yeaton, 1988; Belsky, 1994; Whitecross et al., 2017 – *see Chapter 3*), which may influence the lower levels of photosynthesis observed in this species (Figure 2).

5.4. Conclusion

This study has highlighted the changes in leaf functionality in relation to phenology of two savanna trees in the Nylsvley Nature Reserve. It is important to understand tree functionality across a range of spatial and temporal scales in order to accurately portray carbon gain within this highly variable ecosystem. New leaf functionality was minimal – as was expected; however, it is possible that during years with significantly late onset of rainfall, leaf functionality could increase prior to the start of seasonal rainfall. Whitecross et al. (2017 – *see Chapter 3*) found that 46% of years since 1980 would potentially have resulted in early-greening occurring and that these events occur on average for 15±10 days prior to the onset of rainfall (Whitecross et al. 2016 *in press – see Chapter 6*). Thus it is likely that minimal leaf functionality will occur prior to the onset of seasonal rainfall in the NNR. Early-greening will assist with the rapid deployment and expansion of new leaves when conditions become suitable for growth and soil moisture increases as seasonal rainfall commences, giving trees a window of opportunity for competition-free growth from grasses prior to the flush of new grass about 4-8 days after the start of rainfall (Whitecross et al. 2017 – *see Chapter 3*). Physiologically, early-greening presents trees with an advantage for rapid development of new leaves (carbon sink) into fully-expanded (carbon neutral) at the start of the growing season ahead of other late-greening trees and grasses. Another key finding of this study was the apparent trade-off between high investment in leaf longevity and functional intensity with *B. africana* investing more in longer-lived leaves with high nitrogen content and chemical defences and lower photosynthetic rates. In comparison, *T. sericea* had high photosynthesis and lower leaf lifespans and nitrogen content.

6. Acknowledgements

Thanks to the following institutions for the financial support given to this project: the National Research Foundation (NRF), the Centre for Tree Health Biotechnology (CTHB), and the Centre for Scientific and Industrial Research (CSIR). Thanks to Natasha du Plessis and the staff at the Nylsvley Nature Reserve. Thanks to Ricardo Holdo for the use of his soil moisture probe data. Thanks to Barney Kgope and Nic Venter for their input and guidance during this study. Thanks to the following individuals for their help and support in the field: Caroline Howes, Robert Whitecross, Helenor Whitecross, Wesley Hattingh, Giuseppe Venturi, Kirstin Olsen, Marcelle Johnson, Vidosava Jakovljevic, Isabelle Inder, Ryan Thomas, Nadine Cronk and Roxy.

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8. Appendix

8.1. Tree and leaf characteristics

The mean±SE tree heights were 9.4±0.37 m and 7.09±0.43 m and stem diameters were 30.7±2.29 cm and 18.9±1.13 cm for *B. africana* and *T. sericea* respectively. *Burkea africana* leaves had significantly higher SLA than *T. sericea* leaves (t-test: t=5.35, d.f. =35, p<0.001, Table S1). Moisture content of the leaves indicated a significantly higher moisture content in *B. africana* than *T. sericea* leaves (t-test: t = 9.21, d.f. = 32, p<0.001, Table S1). *Terminalia sericea* leaves were thicker than the *B. africana* leaves (t-test: t=-6.42, d.f. =20, 9<0.001). Scholes and Walker (1993) presented the average nutrient content of *B. africana* and *T. serice*a leaves at the NNR (Table S1).

Table S1. Specific leaf area (SLA) and leaf moisture content measured in this study compared to the nutrient concentrations for *Burkea africana* and *Terminalia sericea* leaves at the Nylsvley Nature Reserve from Scholes and Walker (1993).

Species	**SLA ($m^2.kg^{-1}$)**	**Leaf Moisture Content ($ml.g^{-1}$)**	**Leaf Thickness (mm)**	**Carbon (%)**	**Nitrogen ($mg.g^{-1}$)**	**Phosphorus ($mg. g^{-1}$)**
Burkea africana	8.33±0.23	1.38±0.03	0.05±0.005	45.8	12.1	0.68
Terminalia sericea	6.82±0.17	1.24±0.03	0.10±0.008	45.9	8.41	0.59

8.2 Seasonal photosynthetic rates

The photosynthetic rates of different leaf age classes in relation to the days since the start of green-up for each season (Figure S1).

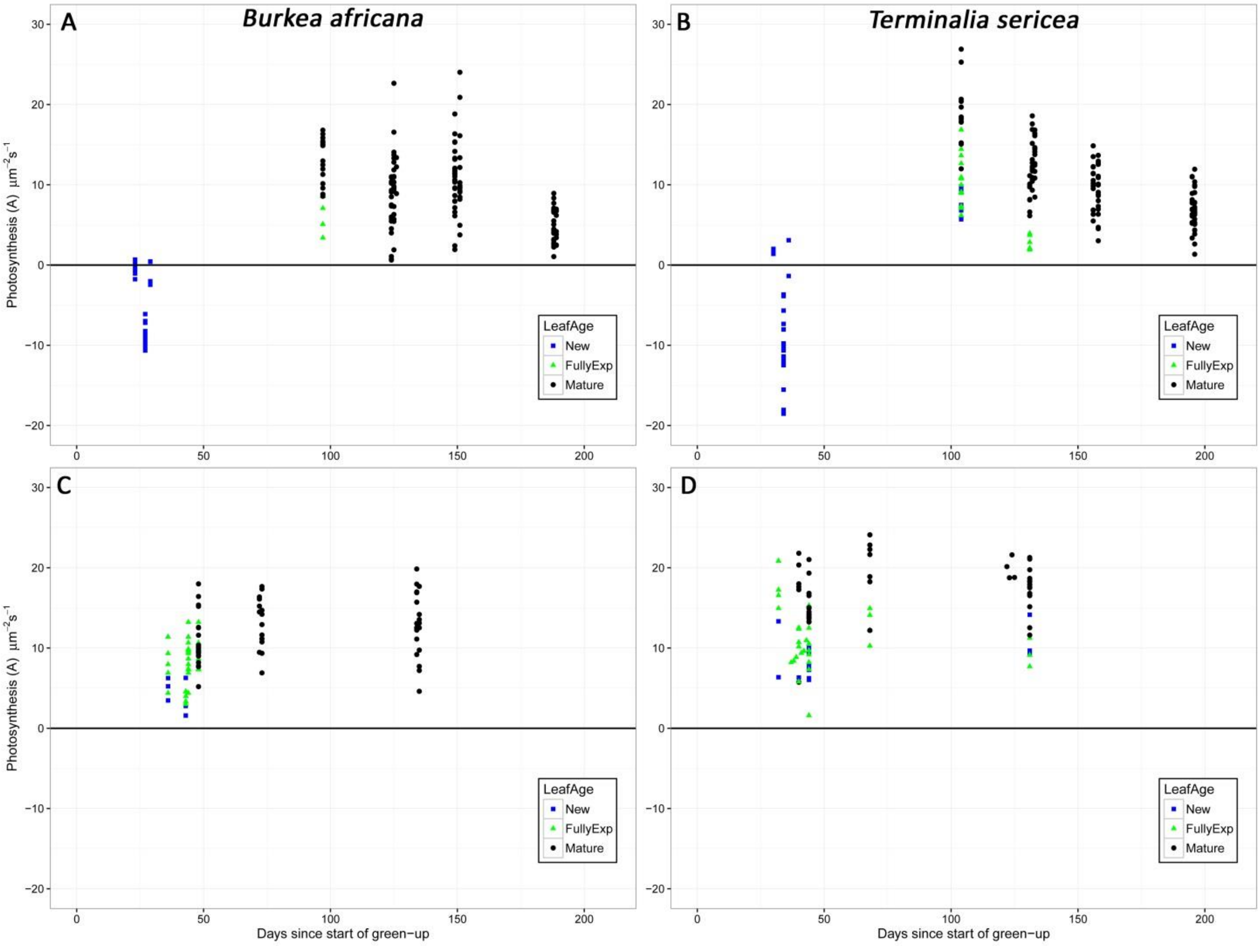

Figure S1. The photosynthetic rates of new, fully expanded and mature leaves of (A & C) *Burkea africana* and (B & D) *Terminalia sericea* trees in relation to the number of days after the start of green-up of each species for the (A & B) 2012-13 and (C & D) 2013-14 seasons.

CHAPTER 5

Phenological defences: Does leaf phenology influence the impacts of invertebrate herbivory in an African savanna?

This chapter has been prepared for submission to the Journal of Vegetation Science

1. Abstract

Two of the green-up strategies observed in deciduous savanna trees are: early-greeners which produce new leaves during the dry season before the onset of seasonal rainfall, and facultative-greeners which commence new growth thereafter. Early-greeners are suggested to benefit from lower herbivory on young leaves, as they flush when invertebrate activity is at its lowest (at the end of the dry season). Herbivory levels were compared on two species over two growing seasons – in the first both species greened-up together due to early rainfall onset. In the second season, the early-greener (*Burkea africana*) began leaf flush two weeks before the onset of rainfall and the first flush of the facultative-greener (*Terminalia sericea*). Lower leaf-level herbivory rates on *B. africana* (20% consumed) after early-greening suggest that this strategy does prevent leaf loss early in the season, but at a canopy scale (important for whole plant photosynthesis) herbivory rates between years reached similar levels by late November - indicating that this benefit was temporary. Although facultative-greening in *T. sericea* appeared to result in much higher leaf-level losses (40% consumed), by the end of the growing season, canopy scale losses were not statistically distinguishable between species. This observation is explained by the different patterns of leaf flush shown by these species - *T. sericea* replaces lost canopy biomass (compensatory growth), while *B. africana* does not - resulting in herbivory losses increasing linearly throughout the season. The results confirm that early-greening can be a successful strategy for avoiding invertebrate herbivory early in the growing season.

Keywords: *Burkea africana*, compensatory growth, early-greener, leaves, *Terminalia sericea*.

2. Introduction

Savannas are strongly seasonal systems where a hot, wet summer season is succeeded by a warm, dry winter season that has little to no rainfall (Huntley and Walker 1985). This seasonality is a key driver of the variety of phenological and life history strategies employed by fauna and flora to tolerate or avoid the stresses brought about by these long, dry periods (Childes 1989; Borchert 1994; Haddad et al. 2001; Jolly et al. 2005). Much of the vegetative structural diversity, a continuous grass layer coupled with a discontinuous tree layer, is maintained through frequent disturbances from fire, frost and herbivory (Huntley and Walker 1985; Childes 1989; Owen-Smith 1994; Holdo 2007; Whitecross et al. 2012). These disturbances themselves show seasonal patterns – with fire and frost occurring in winter, and insect herbivory peaking in summer (Scholes and Walker 1993). Characteristically, patterns of leaf display (leaf phenology) of savanna plants show an intense growth period during the wet summer and a dormant phase during the dry winter (Huntley and Walker 1985; Borchert 1994; Chidumayo 2001). But, within this general phenological cycle there is high variability in the timing of leaf flush and senescence within and between species and growth forms (Rutherford and Panagos 1982; Childes 1989; Chidumayo 2015; Whitecross et al. 2017 – *see Chapter 3*). As the availability of water is a key aspect for savanna invertebrate survival (Gandar 1982; Huntley and Walker 1985; Cumming and Bernard 1997), the emergence of numerous invertebrates generally occurs only after the onset of seasonal rainfall (Sinclair 1978; Denlinger 1986; Childes 1989; Scholes and Walker 1993; Borchert 1994). Temperature and photoperiod also play a role in terminating the diapause (winter dormancy) of invertebrates, with warmer temperatures and longer daylight hours resulting in an earlier termination of diapause (Denlinger 1986).

Green-up or flushing is the emergence of leaves on deciduous tree species after winter or the dry-season, usually signalling the start of spring or the next growing season (Kikuzuwa

1983, 1995; Polgar and Primack 2011). Two common phenotypes of deciduous savanna trees include: the early-greeners and the facultative-greeners (Chidumayo 2001; Jolly and Running 2004; Elliott et al. 2006; Higgins et al. 2009; February and Higgins 2016; Whitecross et al. 2016 – *see Chapter 2*). Early-greeners are species which begin green-up during the dry-season, up to two months prior to any rainfall event occurring, whilst facultative-greeners will only begin green-up after the start of the season's rainfall. Hence, early-greeners are able to grow their canopies at a time when invertebrate herbivore pressure is at its lowest, allowing new leaves to mature with little initial disturbance from invertebrate herbivores (Sarmiento et al. 1985; Aide 1988, 1992). This is one possible explanation for the prevalence of this early-greening strategy in savannas globally (Sarmiento et al. 1985; Murali and Sukumar 1993; Williams et al. 1997; February and Higgins 2016; Whitecross et al. 2016 – *see Chapter 2*), which otherwise is fairly counter-intuitive. There is some evidence for this: early-greeners in a dry deciduous forest in India suffered less herbivore damage in comparison to trees which begin flushing with the onset of seasonal rainfall (Murali and Sukumar 1993) and Aide (1988) demonstrated decreased levels of herbivore damage on early-greening species in Panamanian lowland moist forests.

When taken on an individual scale - invertebrate vs. mammalian herbivores - there is little doubt that even a small browser such as a common duiker (*Sylvicapra grimmia*) is capable of consuming more biomass in a day than an individual caterpillar in an African savanna. However, invertebrate herbivores often occur in high numbers which enables these tiny herbivores to consume an above ground biomass (9-12 g m^{-2} y^{-1}) that is close to double what their larger ungulate competitors (6 g m^{-2} y^{-1}) are able to eat during a year in the Nylsvley Nature Reserve (Scholes and Walker 1993). Given that invertebrate herbivores can account for much more than half the total biomass lost to herbivory (Gandar 1982; Janzen

1988; Anderson and Lonsdale 1990), they have the potential to be a major drain on the fitness of individual trees.

Savanna trees, however, have evolved adaptations to cope with the resource drain posed by both mammalian and invertebrate herbivores including structural adaptations such as hairy leaves or thorns that make it difficult for herbivores to access high amounts of susceptible plant biomass from an individual plant (Milewski et al. 1991; Midgley et al. 2001). Another adaptation is the accumulation of toxic or distasteful chemical compounds into the leaf tissues that make the consumption detrimental to herbivores (Cooper and Owen-Smith 1987; Zangerl and Bazzaz 1992; Owen-Smith 1994; Gowda 1997). A less investigated defence adaptation is that of phenology; where plants alter and/or reduce the timing of their production of vulnerable tissues to decrease the risk of herbivory over the growth period (Aide 1988, 1992; Cooper et al. 1988; Murali and Sukumar 1993; Van Schaik et al. 1993; Dekker and Smit 1996; Scogings et al. 2004). These defensive strategies are usually used in combination with one another, thereby providing the most suitable broad-scale defences for a tree (Scogings et al. 2004). Structural defences are generally more effective against mammals, although hairy leaves have been shown to deter invertebrates (Edwards and Wratton 1980; Coley et al. 1985; Cooper and Owen-Smith 1986; Milewski et al. 1991; Letourneau 1997), and it has long been thought that chemical defences were the main means of avoiding or tolerating invertebrate herbivory (Bryant et al. 1991). However, given the strong seasonality of invertebrate activity in savannas, it is possible that altered patterns of leaf display and leaf production is an effective defensive strategy against invertebrate herbivores for savanna trees.

A noticeable gap exists in the African savanna literature regarding the effects of phenological adaptations on the rates of invertebrate herbivory. The extent of invertebrate damage on the leaves of two deciduous broad-leaved savanna trees was compared to investigate how differing greening strategies could influence the impacts of invertebrate herbivory. Sampling took place over two green-up periods (September to November) – one where the two species greened up together due to early onset of seasonal rainfall (2012/13) and one where early-greening was observed in one species, while the other delayed greening until the rains had commenced (2013/14). An investigation into whether patterns of leaf display could impact on the rates of herbivory experienced by individual trees at both the leaf and whole plant scales took place. The costs and/or losses to the trees through the impacts of invertebrate herbivory across different leaf age classes were explored.

3. Methods and Materials

3.1. Site and species

This study took place in a semi-arid savanna ecosystem at the Nylsvley Nature Reserve (NNR), Mookgophong, Limpopo Province, South Africa (24°39’ S, 28°42’ E; 3975 ha), a well-studied area, ideally suited to test the impacts of invertebrate herbivory in a typical southern African savanna system (Coetzee et al. 1977; Bryant et al. 1991; Scholes and Walker 1993). The climate is strongly seasonal with hot, wet summers that experience an average of 85% of the 623 mm mean annual precipitation (with a coefficient of variation of 24%) between October and March (Huntley and Morris 1982). The mean minimum and maximum temperatures in the summer months are 17°C and 29°C respectively (December/January), whilst in winter they are 4°C and 21°C (June/July) (South African Weather Services 2013). Sampling was focused on the green-up periods between September to November of 2012 and 2013. Monthly measurements were also taken during January to April 2013 and March 2014 to assess the full impact of invertebrate herbivory over the growing season as a whole. The annual average precipitation between September to November is 258 mm, with an average rainfall onset date (storm >15 mm) around the 19th September (sd 28 days; CI 95%: ±10 days) (Huntley and Morris 1982). The high variance in the start date of the rainfall strongly influences the green-up of facultative-greeners. A severe fire occurred during the dry season of 2013 and caused widespread damage to the study site details of which can be found in Whitecross et al. (2016 – *see Chapter 2*). These conditions have allowed us to compare a season of early and abundant rainfall (2012) with a season of disturbed and drier growing conditions immediately following a severe fire (2013).

Two of the dominant tree species were selected based on their phenological patterns and known exposure to herbivory (Coetzee et al. 1977; Gander 1982; Scholtz 1982; Scholes and Walker 1993). *Burkea africana* (Hook.) (Caesalpiniaceae) is an early-greening species that flushes leaves ahead of the start of seasonal rainfall (Childes 1989; Whitecross et al. 2016 – *see Chapter 2*) and is distributed throughout central and southern Africa. It is a medium-sized deciduous, leguminous tree approximately 10-12 m in height. It has an average green-up period of 8 weeks usually commencing in late September in the NNR (Rutherford and Panagos 1982; Whitecross et al. 2016 – *see Chapter 2*). Owen-Smith and Cooper (1987) reported that the available biomass of this species was high during November to June relative to other more palatable deciduous species. *Burkea africana* is not considered a palatable species for mammalian herbivores, but is eaten by insect herbivores (Owen-Smith and Cooper 1987; Bryant *et al.* 1991). This species does experience extreme defoliation events during years in which lepidopteron larval outbreaks occur (Scholtz 1976, 1982). The leaves are hairless, spineless and bipinnately compound, often clustered at the tips of shoots. There are two to three pairs of pinnae with 5 to 12 leaflets per pinna and leaflets are 25-50 mm x 15-25 mm in size.

Terminalia sericea (Burch. ex DC.) (Combretaceae) is a facultative-greener (Childes 1989), however early-greening has been occasionally observed (February and Higgins 2016; Whitecross et al. 2016 – *see Chapter 2*) and is distributed throughout southern Africa. It is a small to medium-sized deciduous tree with an average height of 9 m. This species is not associated with high levels of mammalian herbivory, however, invertebrate herbivory and parasitism is considered high (Owen-Smith and Cooper 1987; Scholes and Walker 1993; Fritz et al. 1996). The leaves of *T. sericea* are clustered at the branch tips and are narrowly obovate-elliptic with an entire margin, measuring 55-120 x 15-45 mm. A dense layer of silver, silky hairs covers the leaves, giving them their characteristic silvery sheen.

3.2. Seasonal Phenological Patterns

Leaves were divided into new (<0.5 the full size of an average leaf, with no waxy cuticle), fully expanded (>0.5 the full size of an average leaf, with no waxy cuticle) and mature (the full sized, sun-drenched adult leaf with a mature cuticle) age classes. A sample of 15 *B. africana* trees and 15 *T. sericea* trees were monitored for weekly phenological patterns over the green-up periods during 2012 and 2013. Each tree was assigned a percentage cover value for each leaf age class on the canopy. Full details on the phenology of the trees during the study period can be found in Whitecross et al. (2016 – *see Chapter 2*). Mammalian herbivory was excluded in this study - a large game fence was erected to exclude all large ungulate herbivores.

3.3. Leaf Mass Area and Chlorophyll Content

Samples of 20 mature leaves per species were collected to calculate the leaf mass area (LMA, $g.m^{-2}$) for each species (Cornelissen et al. 2003). The chlorophyll content of ten randomly selected leaves per age class on each of the 15 trees per species was measured over the green-up period using a SPAD-502 chlorophyll meter (Minolta Camera Co. Ltd., Japan), which measures chlorophyll within a specified area of a leaf based on the absorption of red and infrared spectra.

3.4. Observations of seasonal herbivory – Leaf-scale

Insect herbivory and any damage resulting from invertebrates (i.e. laying eggs on the leaves which deform the mid-vein and leaf centre) on individual leaves was monitored using a herbivory scoring system derived by Pérez-Harguindeguy et al. (2003) to estimate the proportion of leaf area consumed per leaf (0=0%, 1=1-24%, 2=25-49%, 3=50-74%, 4=75-89%, 5=90-100%). Leaves were tagged using zip-ties and monitored at weekly intervals from the start of the green-up period (September 2012) to the end (November 2012), after which leaves were monitored monthly for the rest of the growing season (January 2013 to May 2013). A second season of data was collected for the 2013 green-up period between September to November and in March 2014. The number of leaflets per leaf (*B. africana,* compound leaves) or the number of leaves per cluster (*T. sericea*, simple leaves) were counted during each session and an overall herbivory score was awarded for the leaf or cluster as a whole based on the amount of damage or consumption that had taken place. Nine leaves or clusters were tagged on each of nine trees per species (total leaf biomass per individual: 24.83 kg (*B. africana*); 15.07 kg (*T. sericea*); calculated from Rutherford (1982)).

3.5. Leaf turn-over rates – Terminalia sericea

Leaf turn-over rates were monitored weekly for the month of November 2013 by tagging three leaf clusters on nine individual *T. sericea* trees. Newly produced leaves were numbered consecutively each week using a permanent marker and the number of days each numbered leaf was present was recorded. This allowed us to determine whether the *T. sericea* trees were using a compensatory growth strategy in response to herbivory. *Burkea africana* did not show a compensatory growth response - original leaves were maintained throughout the season without the production of new leaf tissue after the first flush, hence their exclusion from this aspect of the experiment.

3.6. Observations of seasonal herbivory – Canopy-scale

Fifteen individuals from each species were observed for total canopy-scale herbivory damage using an adaptation of the Walker scale (Walker 1976): the proportion of visibly damaged leaves relative to undamaged leaves was scored from 1 to 7 where 0=0%, 1=1-10%, 2=11-25%, 3=26-50%, 4=51-75%, 5=76-90%, 6=91-99%, 7=100%. Over the green-up period, estimates of herbivory and canopy flush of new, fully expanded and mature leaves were taken on a weekly basis from the start of leaf flush.

3.7. Data analysis

Seasonal rainfall totals were calculated on a monthly basis between January 2012 and November 2013 together with corresponding average maximum temperatures. The average time taken for each species to green-up was compared using a Wilcox Test. A t-test was used to compare LMA between species. A Kruskal-Wallis test was used to differentiate between the chlorophyll content of different leaf age classes sampled each week (Norusis 1992). Leaf turn-over rates and average leaf lifespan were calculated for *T. sericea* and the average number of new leaves produced per cluster per week was also calculated. Leaf-scale herbivory was averaged for each species between weeks. A linear regression was used to indicate the relationship between total number of leaves available and percentage leaf area consumed per week for *B. africana*. The mean canopy-scale herbivory and flush scores for 15 trees from each species were converted to median percentages and averaged for each week. The average rates of canopy-scale herbivory during the green-up period were then compared using a Wilcox Test. All data analyses were conducted in Microsoft Excel (2010) and the R Statistics package (v2.12.2).

4. Results

4.1. Seasonal phenological patterns

Early onset of seasonal rainfall was observed with an 80 mm rainfall event on the 7th September 2012 in the first sampling season, together with higher than average total monthly rainfall from September 2012 to April 2013 (Fig. 1). The following season was drier with later commencement of seasonal rainfall on the 7th October 2013 with a 22 mm rainfall event.

Summer average temperatures were warmer during the 2013 summer months with a noticeable difference between the September 2012 and 2013 averages (Fig. 1).

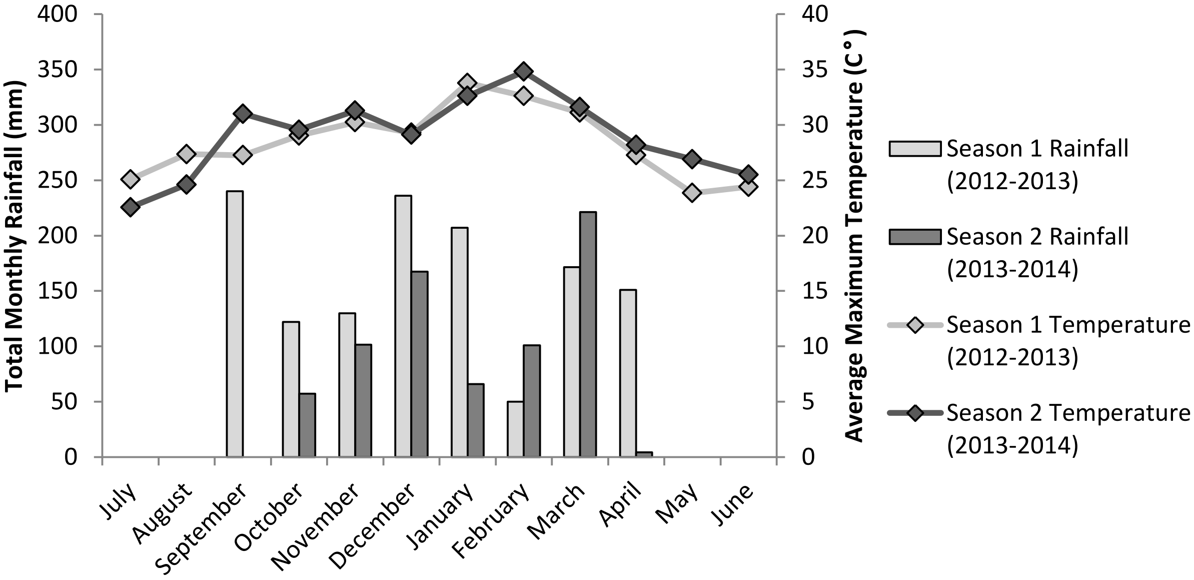

Fig. 1. Total monthly precipitation (mm) and average maximum temperatures (°C) during the study period (July 2012- June 2014).

Rainfall in 2012 started 12 days prior to the commencement of any leaf flush, while rainfall in 2013 commenced two weeks after the start of leaf flush by *B. africana* and a day before the start of *T. sericea* leaf-out. Both species flushed at a similar rate during 2012, however, the turn-over from new to mature leaves occurred one week faster in *T. sericea* (Wilcox Test: W=157, d.f.= 59, p=0.06, Fig. 2). *B. africana* trees took slightly longer to green-up than *T. sericea* (Wilcox Test: W=22.5, d.f. = 59, p<0.001, Fig. 2). *Burkea africana* flushed approximately 2 weeks faster than *T. sericea* in the second season (Fig. 2). New leaf production of both species was stunted in comparison to the previous season – presumably due to the combined effects of the fire and later rains (Fig. 2). *B. africana* recovered faster with higher total canopy coverage towards the end of November 2013 (Fig. 2). By the end of October 2012 both species had 50-70 percent of the canopy covered with fully-expanded leaves, but by the end of October 2013 *T. sericea* had less than 10% and *B. africana* had less than 30% fully expanded leaf coverage.

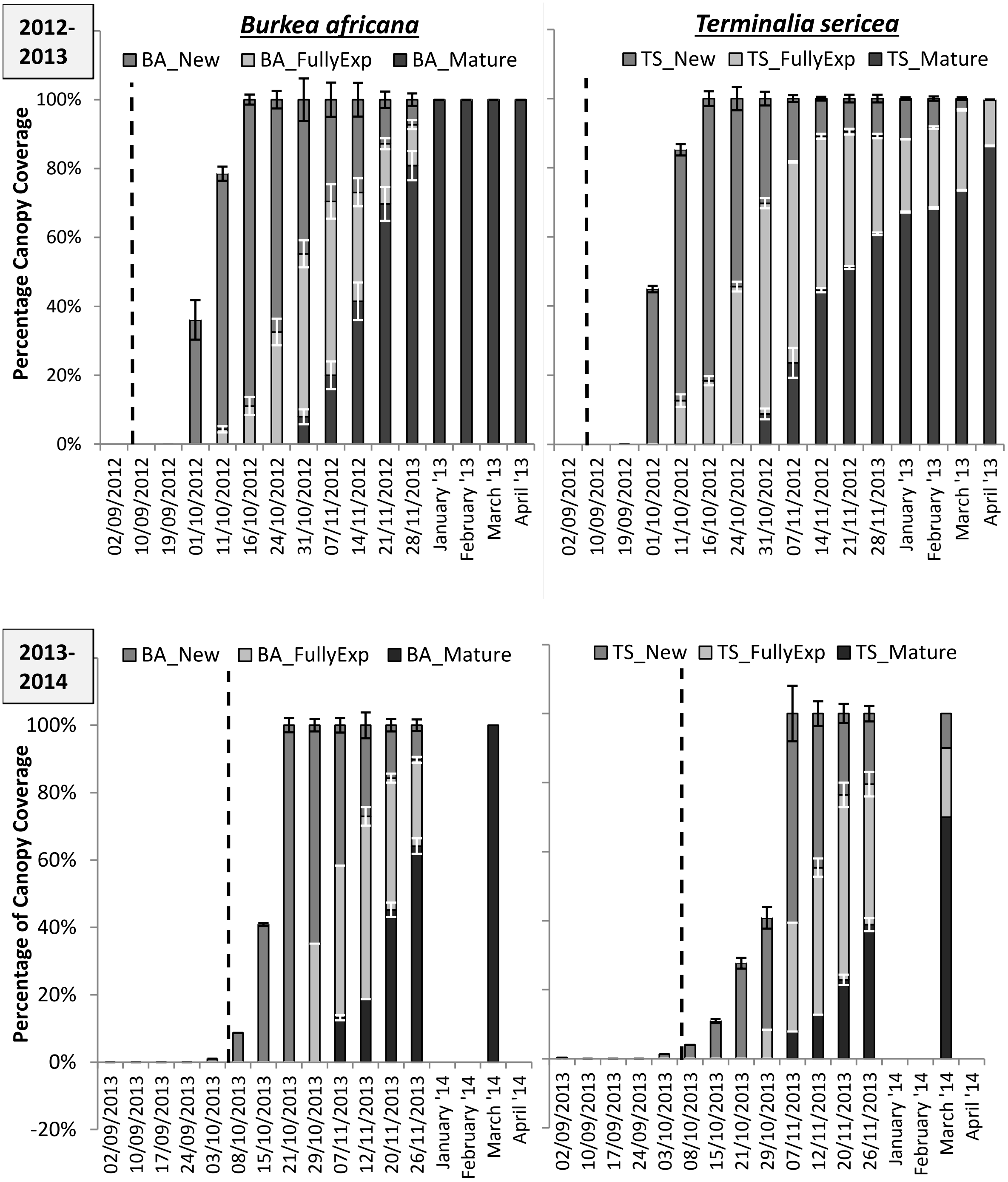

Fig. 2. Canopy level leaf phenology (mean±SE) for *Burkea africana* (BA) and *Terminalia sericea* (TS) between the 2012-2013 and 2013-2014 growing seasons. The dashed line indicates the arrival of the seasonal rainfall and leaf ages are represented by new, fully expanded and mature age classes. Standard error bars are displayed.

4.2. Leaf Mass Area and Chlorophyll Content

The *B. africana* leaves had slightly lower LMA than the *T. sericea* leaves (t=1.69, d.f. =38, p<0.001, Table 1), but both species have fairly low LMA relative to savanna trees in general (Scholes et al. 2004, Scogings *et al.* 2012). Chlorophyll content was low in new leaves of both species but rapidly increased in *T. sericea*. In contrast, *B. africana* leaves increased their chlorophyll more slowly, with mature leaves of this species having the highest values overall at the end of the season (Kruskal-Wallis: $H_{7;\ 271.23}$= 397.10, p<0.001, Table 1).

Table 1. Comparison of traits (mean±S.E.) between *Burkea africana* and *Terminalia sericea* leaves. Bold values indicate a significant difference between species. *Owen-Smith and Cooper (1987)

Leaf Trait	***Burkea africana***	***Terminalia sericea***	***p-values***
Leaf Mass Area (g.m^{-2})	122±3	148±4	**<0.001**
Chlorophyll Content (SPAD Units)			
New Leaf	19±1.1	21±0.7	0.64
Fully Expanded Leaf	41±0.7	45±0.8	**0.04**
Mature Leaf (November)	48±0.7	47±0.7	0.99
Mature Leaf (March)	51±0.6	46±0.7	**<0.001**
Herbivore defence mechanisms*	Chemical (condensed tannins)	Physical (leaf hairs) & Chemical (alkaloids)	

4.3. Leaf turnover rates

Leaf production differed for both species with *B. africana* producing only one set of leaves per season, whilst *T. sericea* continued to produce new leaves throughout both seasons (Fig. 3). Monitoring individual *T. sericea* leaf clusters over a month during the 2013 green-up period indicated that new leaf production was continuous (Fig. 3). On average, 4.4±0.3 new leaves were produced per cluster per week: i.e. approximately 20% of leaves in the cluster have a lifespan of only 11.7±0.7 days (Fig. 3). The high production of new leaves within each cluster compensates for constant loss to herbivory (see below), fungal damage and aging.

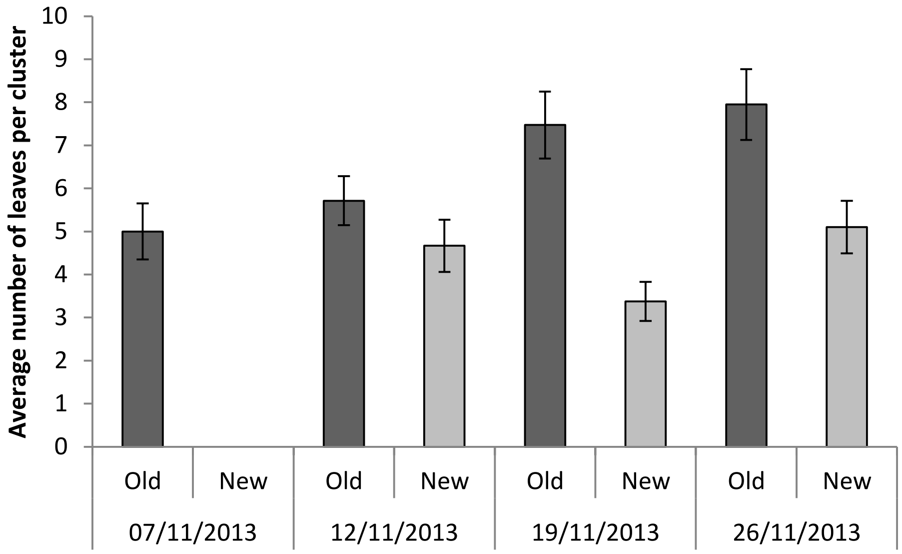

Fig. 3. Number of old leaves per *Terminalia sericea* leaf cluster per week in November 2013 in comparison to the number of new leaves produced per cluster each week (mean±SE).

4.4. Leaf-scale herbivory

When comparing between years, *B. africana* had higher initial herbivory rates in the year when early-rains coincided with the start of its leaf flush (2012) and by the end of November the average percentage of leaves consumed was still 30% more in 2012 than 2013 where only 20% of leaves were consumed after early-greening had occurred (Fig. 4). In contrast, *T. sericea* had lower herbivory rates when it flushed at the same time as *B. africana* (2012). The average percentage of *T. sericea* leaves consumed had reached 40% by the end of October 2013, and it took until the end of November 2012 to reach these levels when it flushed together with the early-greening *B. africana* in 2012 when the first rains fell very early (Fig. 4). Overall, *T. sericea* always had higher levels of herbivory than *B. africana* – even when the new leaves were exposed to the same level of invertebrate herbivory (2012). Early flush does therefore appear to reduce leaf-level invertebrate herbivory rates, however, it is possible that effects of the 2013 fire could have contributed to the lower herbivory during that period.

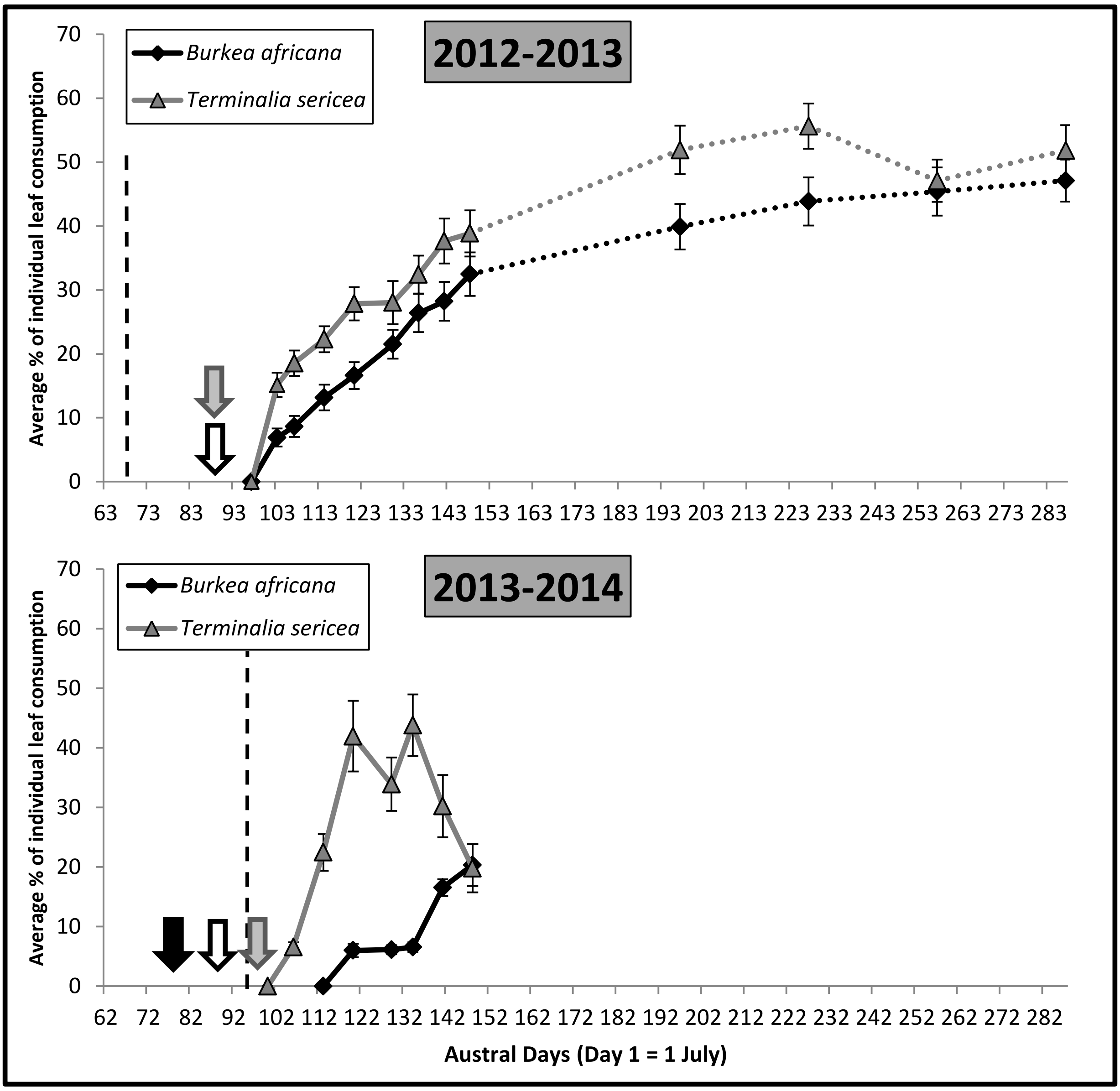

Fig. 4. Weekly to monthly leaf-scale assessment of herbivory on two species of semi-arid savanna trees. The 2012-2013 and 2013-2014 growing seasons showed different trends of leaf-scale consumption for the facultative-greener *Terminalia sericea.* Monthly herbivory estimates are shown for January to April 2012 as separate markers. Standard errors are displayed. The dashed line represents the arrival of seasonal rainfall. The white and grey arrows respresent the approximate dates of first flush for *B. africana* and *T. sericea* respectively (see Fig. 1 for specific details). The black arrow represents the date of the fire.

Owing to differences in leaf production above, the two species showed very different patterns of leaf-scale herbivory. The percent leaf area consumed increased for both species over time (Fig. 5), which resulted in a significant decline in total leaf number for *B. africana* (R^2= 0.997, Fig. 5). There was, however, much higher variability for *T. sericea* – whose leaf number was constantly being supplemented by the production of new leaves.

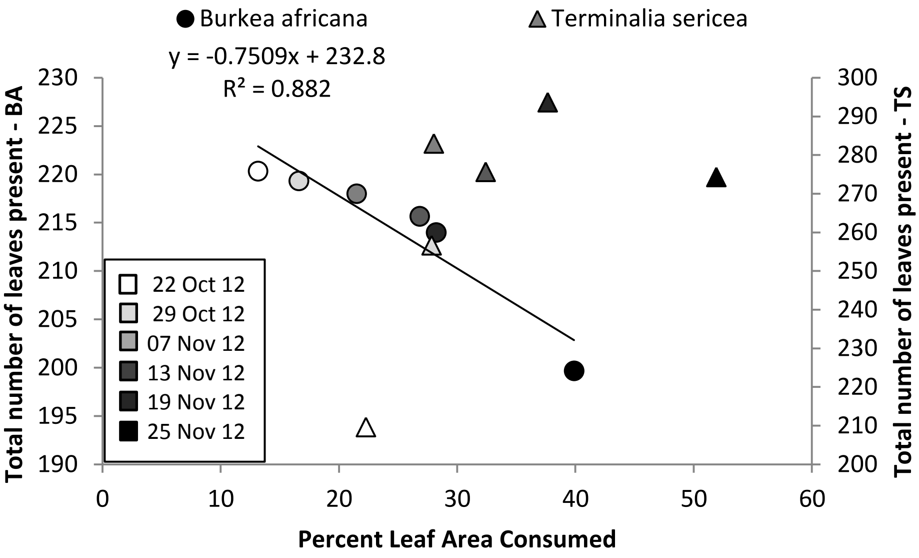

Fig. 5: Relationship between the mean weekly percentage of leaf area consumed and the total number of leaves counted each week for 81 leaf clusters on each species. While the number of available leaves of *B. africana* is driven by the amount of herbivory it experiences, *T. sericea*'s leaf number shows no clear trend as a result of the trade-off between herbivory losses and the production of new leaves each week. A linear trend for *B. africana* and the corresponding equation and R^2 value are displayed. The grey-scale values correspond to the date at which the sample was calculated.

4.5. Consequences for canopy-scale herbivory

When these results are accumulated to a canopy scale the impact of *T. sericea*'s continuous leaf display become clear: although *T. sericea* experienced higher overall rates of herbivory than *B. africana* over the green-up periods, canopy-scale damage remained constant in *T. sericea* through much of the season, and even decreased (Fig. 6). In contrast, canopy damage to *B. africana* accumulates throughout the growing season – reaching levels as high as *T. sericea* by the end of the season (Wilcox Test: April 2013: W=76.5, p=0.099; March 2014: W=79.5, p=0.134, Fig. 6). This pattern occurred in both years, and both species reached similar levels by the end of the season each year (about 40-50% of the canopy) although it is also clear that there are several spikes in invertebrate herbivory throughout the season (e.g. October 2012, February 2013, November 2013) (Fig. 6).

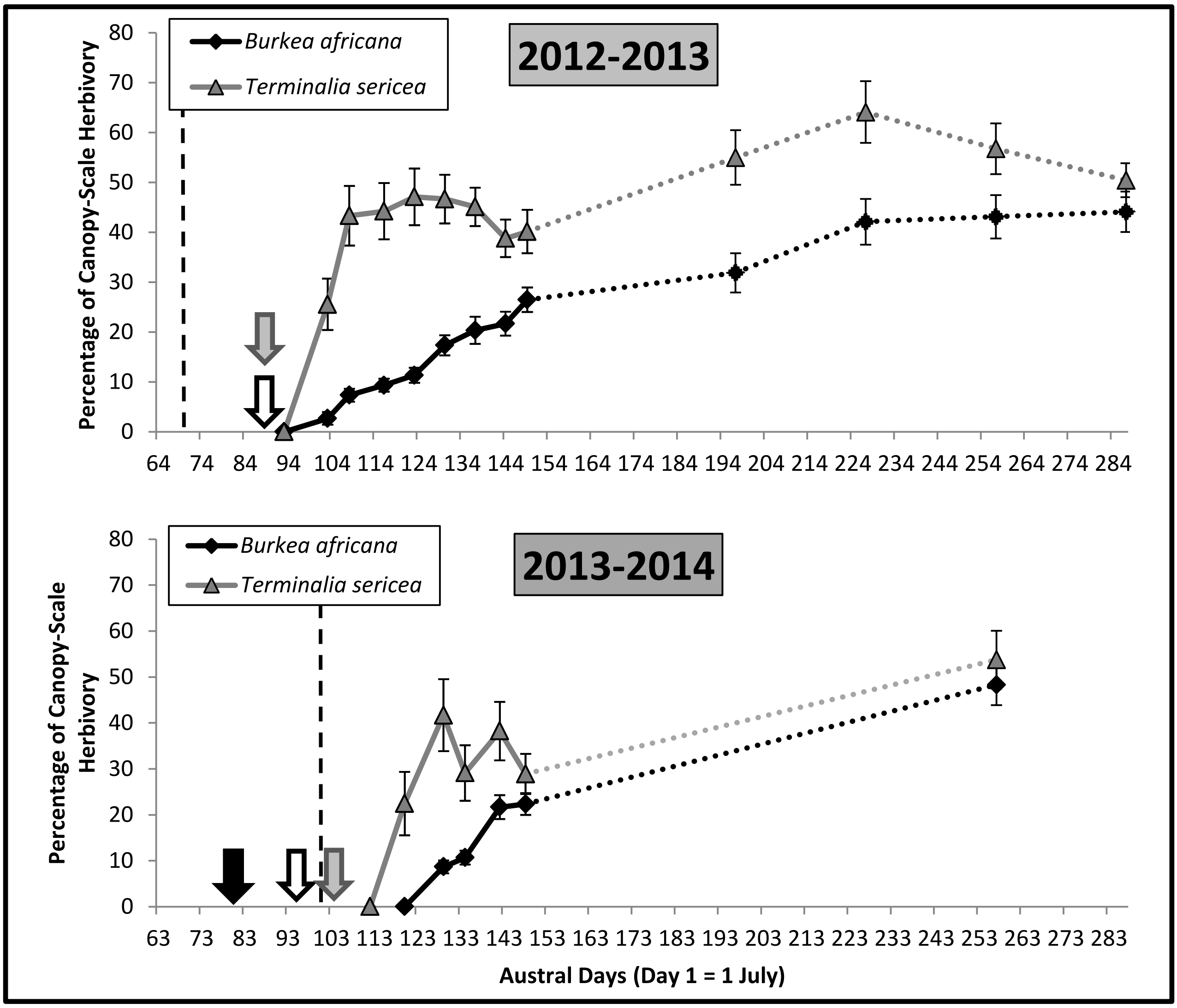

Fig. 6. Weekly to monthly canopy-scale assessments of herbivory over the green-up period of the 2012-2013 and 2013-2014 growing seasons on two semi-arid savanna trees. The herbivory on *Burkea africana* shows a cumulative trend over both seasons, whilst the herbivory on *Terminalia sericea* maintains a stable level. Monthly estimates are shown for January-April 2013 as separate markers. Standard error bars are displayed. The dashed line indicates the start of seasonal rainfall each season. The white and grey arrows respresent the approximate dates of first flush for *B. africana* and *T. sericea* respectively (see Fig. 1 for specific details). The black arrow represents the date of the fire.

The rate of total canopy consumption by invertebrate herbivores was calculated for the 2012 and 2013 green-up periods. *Burkea africana* showed no differences in herbivory consumption rates for 2012 and 2013 (3.3±0.4% versus 3.4±0.4% consumption/week during green-up, respectively) (p=0.99), whilst *T. sericea* had a 30% decrease from the 2012 to 2013 season (6.3±0.5% versus 4.5±0.6% consumption/week during green-up, respectively) (Kruskal-Wallis: H=19.20, d.f. =3, p<0.001).

5. Discussion

5.1.Are phenological defences effective against savanna invertebrate herbivory?

Phenological defences have been suggested as a means for trees to escape threats, such as fire or herbivory, through the alteration of the timing of the growth of vulnerable tissues (Aide 1988, 1992; Van Schaik et al. 1993; Scogings et al. 2004). Few studies have shown evidence for early-greening as a phenological defence strategy in southern African savannas, but this study showed that herbivory levels are consistently lower for an early-greener compared to a facultative-greener at the start of the growing season. At a canopy scale, the facultative-greener (*T. sericea*) experienced higher overall levels of invertebrate herbivory during both growing seasons. The trends of herbivore damage were distinctly different for each species; *B. africana* gradually increased in damage as the seasons progressed, while *T. sericea* experienced a sharp increase in herbivore damage two weeks into each season. The high damage levels on *T. sericea* reached a plateau during both seasons, which was likely a result of the trees employing a compensatory growth strategy in response to the high levels of herbivory (McNaughton 1983). By adding new leaves to the canopy, *T. sericea* maintained a fuller canopy for longer despite experiencing higher average herbivory overall. The frequent onset of new leaves enables the tree to discard the older, damaged leaves and proceed through the season with a fuller, more functional canopy (McNaughton 1983). This strategy could be viewed as expensive in comparison to *B.*

africana which produced and maintained a single set of leaves throughout each season. However, *T. sericea* has significantly higher photosynthetic rates across the growing season compared to *B. africana* which enables it to absorb more carbon into the continuous production of new leaves (*see Chapter 4*).

At a leaf-scale, differences between the early- and facultative-greeners were smaller throughout the 2012/13 growing season when conditions were wetter and more favourable for growth. This may be related to the simultaneous green-up of both species together with the immediate emergence of invertebrates after the early onset of seasonal rainfall (Sinclair 1978; Cumming and Bernard 1997). Both species were exposed to the same levels of herbivory pressure during this season with *B. africana* experiencing higher levels of herbivory than those observed during the 2013/14 season when early-greening occurred. This indicates that early-greening does provide *B. africana* with an advantage against invertebrate herbivory, as its new leaves are present during a time of low invertebrate activity (Sinclair 1978; Scholtz 1982; Sarmiento et al. 1985). Despite *T. sericea* experiencing lower initial herbivory damage when it flushed simultaneously with *B. africana* (2012), it is unclear as to whether this was a direct response by herbivores to the availability of preferable new *B. africana* leaves, or whether there may have been a change in the suite of invertebrate herbivores present at the start of each of the green-up periods between years that may have preferentially fed on *B. africana* during 2013 (Scholtz 1982). It is also possible that the green-up of *T. sericea* trees were affected by the late season fire which occurred on the 19th September 2013 (Whitecross et al. 2016 – *see Chapter 2*). *Burkea africana* trees which are larger than 400 mm in diameter are considered fire tolerant (Wilson and Witkowski 2003), and all of the *B. africana* individuals fall into this fire tolerant category. The *T. sericea* trees, however, have a thinner stem and from the slower green-up rate observed after the fire, it can be assumed that the fire had a negative impact on these individuals. The fire combined with the later arrival of rainfall in the 2013/14 green-up may have influenced the abundance of invertebrate herbivores as well; as it is clear that there was a reduced level of herbivory in both the early-greening species (2012: 30%, 2013: 20%) and the facultative-greening species (2012: 45%, 2013: 30%). Increases in herbivory rates were only observed from late-October to mid-November during both seasons, when leaves were already fully expanded or mature. Mature leaves are better able to tolerate the impacts of invertebrate herbivory as opposed to younger, less developed leaves, but are still vulnerable to physiological losses caused by the resulting damage to leaf tissues (Nabity et al. 2009).

5.2.What are the costs associated with invertebrate herbivory?

Recent studies on oak saplings and wild parsnips have indicated that even a 5% loss of leaf tissue can result in up to 20% loss of photosynthetic productivity of the remaining tissues (Zangerl et al. 2002; Nabity et al. 2009). In this context, the apparently costly strategy of *T. sericea* constantly replacing lost leaf biomass makes more sense. Whether net plant carbon gain is maximised by having high herbivory losses and constantly replacing lost biomass (as *T. sericea* does) or by having lower herbivory losses but not investing in compensatory growth (as *B. africana* does) is not clear. It has been suggested that early-greening species have higher leaf nitrogen content, and more costly leaves in general (Prior et al. 2003; Franco et al. 2005), and this is certainly the case with the two species studied here. Scholes and Walker (1993) found that *B. africana* leaf nitrogen concentration (12.1 $mg.g^{-1}$) was 25% higher than in *T. sericea* leaves (8.41 $mg.g^{-1}$), while the phosphorus in *B. africana* (0.68 $mg.g^{-1}$) was also higher in comparison to *T. sericea* (0.59 $mg.g^{-1}$). However, having only one species to represent each phenological strategy limits the conclusions that can be drawn and it is not clear whether all facultative-greeners display compensatory growth, or whether a correlation exists between employing an early-greening strategy and only producing one set of leaves during the season. Further investigates of this strategy across a range of savanna trees in the future will need to be conducted.

Consider must also be given to how expensive it is for a species to maintain its canopy during the growing season. Decreases in mature leaf chlorophyll content of *T. sericea* leaves at the end of the growing season suggest that some physiological trade-off is occurring, possibly as a result of compensatory growth costs, as well as the reabsorption of limited nitrogen from the leaves back into the branches (February and Higgins 2016; *see Chapter 4*), but this will need to be further investigated to better quantify those costs. It is also important to note that the decrease in leaf chlorophyll may impact on the tree's ability gain carbon at the middle and late stages of the growing season (Cornelissen *et al.* 2003; Nabity *et al.* 2009). Both of these species had LMA values that were on the higher end of the range found in tropical deciduous tree species (50–150 gm^{-2} - Poorter et al. 2009), which implies higher investments in structural leaf defences and longer leaf lifespans (Cornelissen et al. 2003). *Terminalia sericea* is exceptional here as it has particularly short leaf lifespans, but a higher LMA value than that of *B. africana*. This higher LMA value corresponds with the investment in leaf hairs as structural defences by *T. sericea* trees, and the lower nitrogen content of its leaves, but this does not explain why invertebrate herbivores are preferentially consuming *T. sericea* leaves over *B. africana* leaves. Bryant et al. (1991) measured high levels of phenolic compounds and tannins in *B. africana* leaves which reiterates the theory that *B. africana* is investing in maintaining its leaves and using chemical defences to protect them from herbivory (*see Chapter 3*).

At a species level, *B. africana* has been observed to suffer large losses through canopy defoliation during years of invertebrate outbreaks, which are mainly caused by the larvae of the emperor moth, *Cirina forda* (Westwood, 1849) (Scholtz 1982; Scholes and Walker 1993). According to Scholes and Walker (1993), these outbreaks occur irregularly approximately every 4 years, but it has not yet been determined whether these outbreaks are predictable. In terms of its phenology, *B. africana* is able to tolerate invertebrate herbivory during non-outbreak years with less overall damage than *T. sericea*; however, it suffers near total losses in years when outbreaks do occur. In comparison, *T. sericea* tolerates higher levels of invertebrate herbivory during all years, but has not been targeted as a species which suffers major defoliation events (Scholtz 1982). This leads one to suggest that in the longer term the overall losses experienced by these species are closer than initially thought.

5.3. Savanna invertebrate herbivores: the forgotten disturbance

Owen-Smith and Cooper (1987) indicated that certain species of deciduous savanna trees are more likely to be eaten by large ungulate herbivores, whilst others will only be eaten if the preferred species are no longer available. However, they did not consider the preference of invertebrate herbivores on the species which they deemed as unpalatable for mammals (Gander 1982; Scholtz 1982). The impacts of large ungulate herbivores on vegetation in savannas have been extensively studied (Cooper and Owen-Smith 1986; McNaughton and Georgiadis 1986; Cooper et al. 1988; Agrawal and Fishbein 2006) and indicate mammalian herbivory as a large, destructive agent which occurs continuously throughout the year. Invertebrate herbivory is largely under-played as a destructive agent and driver of plant adaptation in tropical savanna ecosystems, possibly because mammalian herbivores tend to affect the structure, as well as the leaf area, of affected plants and can directly impact the demographics of tree populations (Cooper and Owen-Smith 1986; Smallie and O’Connor 2001; Helm and Witkowski 2012).

If savanna trees have evolved unusual seasonal patterns of leaf display to avoid invertebrate herbivory, this together with the large quantity of biomass consumed by invertebrates, would be evidence of the importance of invertebrates as evolutionary forces in these systems. Early-greening is risky because it requires the plants to use stored water, carbohydrate and nutrient reserves to produce leaves that will be lost if there is a drought (Do et al. 2005; February and Higgins 2016), and because there are often fires in the hot dry season before the rains that can scorch new leaf canopies (Archibald 2009; Whitecross et al. 2017 - *see Chapter 3*). The benefits of early-greening through avoided invertebrate herbivory would have to be greater than these risks for it to have evolved, which is potentially the case considering that more than 50% of a canopy can be affected by these herbivores.

Unfortunately the phenology or identification of the invertebrates responsible for this damage was not determined; however, Scholtz (1982) conducted a thorough investigation into the trophic ecology of woody-associated lepidopterous larvae in the NNR. Both of the study species are important host plants for numerous species of lepidopterous larvae: *Burkea africana* has been recorded as host to nine lepidopteron larvae including *Cirina forda*, *Gonimbrasia belina* and *Sphingomorpha chlorea* (Gandar 1982; Scholtz 1982; Picker et al. 2004). *Terminalia sericea* has been reported to host up to twelve species of lepidopteron larvae within a season including *Euproctis fasciata*, *Maurilia arcuata* and *Acryophora carniola* (Scholtz 1982; Picker et al. 2004). Many of these lepidopteron larvae have two generations during the growing season (November-December and March), which corresponds with this study's observations of the two spikes in herbivory levels (Scholtz 1982). *Euproctis fasciata* larvae are laminar feeders and have been observed to feed on *T. sericea* during the first generation's emergence and *B. africana* during the second generation's emergence (Scholtz 1982). Larval development takes 30 days to complete on *T. sericea*, but 55 days on *B. africana*; Scholtz (1982) was unsure why this difference may exist. During the second generation, *E. fasciata* larvae are still observed on *T. sericea* and complete their development faster than on *B. africana*, indicating that both tree species are still nutritious and palatable enough at this stage in the growing season (Scholtz 1982). This suggests that the more costly *B. africana* leaves are better defended through secondary compounds, which lowers the growth efficiency (conversion of ingested food to biomass) these consumers are able to achieve (Scholtz 1982; Bryant *et al.* 1991; *see Chapter 4*).

5.4. Conclusion

The data show that invertebrate herbivory has a significant role to play – both in the amount of biomass consumed, and as a possible selective force on leaf phenology: early-greening can reduce invertebrate herbivory, and compensatory regrowth can minimise the reduction in photosynthetic capacity caused by invertebrate herbivory. It is not clear whether these two phenological strategies have evolved specifically in response to invertebrate herbivory, but it is likely that it has played an important role. It is clear that phenological defences are a viable defence mechanism against invertebrate herbivores in the savanna system, being more or less effective in any one year depending on the onset of the first rains, as shown in this study.

6. Acknowledgements

I would like to acknowledge funding from the National Research Foundation of South Africa (NRF), CSIR Parliamentary Grant and the DST/NRF Forestry and Biotechnology Institute’s (FABI) Centre for Tree Health Biotechnology (CTHB), as well as Limpopo Parks and Tourism Board for granting access to Nylsvley Nature Reserve. Thanks to Natasha Du Plessis and the staff at the Reserve for their support and assistance. Thanks Robert Whitecross, Helenor Whitecross, Caroline Howes, Wesley Hattingh, Ryan Thomas, Isabelle Inder, Marcelle Johnson, Nadine Cronk, Kirstin Olsen, Giuseppe Venturi, Vidosava Jakovljevic and Roxy.

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CHAPTER 6

Assessing the frequency and drivers of early-greening in broad-leaved woodlands along a latitudinal gradient in southern Africa

This chapter has been accepted for publication in Austral Ecology

Whitecross, M.A., Witkowski, E.T.F., and Archibald, S. (2016) Assessing the frequency and drivers of early-greening in broad-leaved woodlands along a latitudinal gradient in southern Africa. *Austral Ecology, in press*.

Melissa Whitecross was responsible for the design of the study, acqusition and analyses of the data. She also wrote up the paper which was reviewed by her supervisors Prof. Ed Witkowski and Prof Sally Archibald prior to submission.

1. Abstract

Savannas are the only deciduous system where new leaf flush pre-empts the onset of suitable conditions for growth, a phenological phenomenon known as early-greening. Limited understanding of the frequency and drivers of the occurrence of early-greening in southern African savanna trees exists. This study aimed to estimate the frequency of early-greening events across southern Africa and investigated potential environmental drivers of green-up. Seven broad-leaved woodland sites where *Burkea africana* was a dominant species were selected and compared using remotely-sensed data along a latitudinal gradient from South Africa to Zambia. Normalized difference vegetation index (NDVI) values were extracted from the MODIS satellite imagery at each site from January 2002 to June 2014. Using an austral year (July 1st – June 30th), early-greening was recorded if the green-up start date occurred prior to the onset date of seasonal rainfall. A latitudinal gradient of early-green-up was detected across southern Africa (R^2= 0.74) with the two most northerly (Zambian) sites showing the earliest and most consistent green-up start dates (3 Oct ± 5.34 days). A strong latitudinal gradient was observed between the variability in the amount of rainfall in the first six months of green-up and the green-up start dates across southern Africa (R^2=0.92). Photoperiod appeared to play a role in areas where the onset of rainfall commenced late into the austral year. Mean maximum temperatures recorded 10 days prior to green-up start dates suggested a potential threshold of about 35°C, which could drive early-greening in the absence of rainfall. Correlations between the proportion of early-greening years and the above mentioned environmental factors indicated that rainfall variability had the strongest influence over the observed phenological gradient (R^2=0.96). Understanding early-greening in complex savanna systems is a vital step in furthering predictive phenological models under changing climatic conditions.

Keywords: broad-leaved woodland, early-greening, NDVI, phenology, rainfall variability

2. Introduction

No other deciduous system, besides savannas, is known to experience patterns of leaf display were trees pre-empt the arrival of suitable conditions for growth. This phenological phenomenon is called early-greening, however, not all deciduous savanna trees display this strategy and its prevalence varies both temporally and spatially (Williams *et al.* 1997; de Bie *et al.* 1998; Chidumayo 2001; Whitecross *et al.* 2016). Early-greening has been recorded in savannas in Africa (Childes 1989; de Bie *et al.* 1998; Chidumayo 2001; February & Higgins 2016; Whitecross *et al.* 2016 *in review*), Central and South America (Sarmiento *et al.* 1985; Borchert & Rivera 2001), Asia (Elliott *et al.* 2006), as well as Australia (Williams *et al.* 1997; Prior *et al.* 2003). Southern hemisphere savannas have highly seasonal climates with hot, wet summers surpassed by warm, dry winters during which little to no precipitation occurs (Huntley & Walker 1982). The high temporal variability associated with the onset of rainfall each year has led to a variety of phenological strategies within these ecosystems (Williams *et al*. 1997; Elliott *et al*. 2006). Tree phenology can be categorized into several phenological types based on the length and timing of leaf retention, termed the growing season (Sarmiento *et al.* 1985; Williams *et al.* 1997; de Bie *et al.* 1998). The early-greening species in this study best align with the description of the spring flushers provided by Elliott *et al.* (2006), where new flush is produced at the end of the dry season, prior to the onset of seasonal precipitation.

The savanna biome occupies approximately 50% of southern Africa's land mass, hence it is vital that a quantifiable and robust understanding of the biome's variable phenology be developed (Scholes & Walker 1993; Fitchett *et al.* 2015; Whitecross *et al.* 2016), however, to date phenological research in Africa's savannas is limited (Chambers *et al.* 2013). In African savannas, the majority of vegetation phenology is closely linked to the cyclical rhythms of the wet and dry seasons (Chidumayo 2001; Zhang *et al.* 2005; Higgins *et al.* 2011; Whitecross *et al*. 2016 *in review*), but a full understanding of the frequency and influence of early-greening in these complex systems is still needed. Relatively little is known about the drivers of green-up in African savannas (Fuller & Prince 1996; Chidumayo 2001; Do *et al*. 2005; Chambers *et al*. 2013), in comparison to the vast body of literature which shows the influence of temperature and photoperiod on green-up in other savannas (Reich & Borchert 1984; Borchert & Rivera 2001; Rivera *et al.* 2002) and northern hemisphere temperate systems (Heide 1974; Cannell & Smith 1983; Polgar & Primack 2011; Richardson *et al.* 2013). In Africa, the range of mean annual precipitation across savannas is approximately 150-1600 mm (Lehmann *et al.* 2011). This wide range of mean annual precipitation is accompanied by an equally large variance in annual rainfall amounts and seasonal starting dates (Sankaran *et al.* 2004; Lehmann *et al.* 2011). Poulter *et al.* (2011) highlighted the difficulty experienced by earth system modellers when incorporating savanna type vegetation into their dynamic global vegetation models. The uncertainty produced in the dynamic global vegetation models is closely linked to the diversity of plant functional types and the mixture of phenology displayed by the vegetation of semi-arid savannas (Chidumayo 2001; Zhang *et al.* 2005; Archibald & Scholes 2007; Higgins *et al.* 2011; Poulter *et al.* 2011; Whitecross *et al.* 2016 *in review*). A better understanding of the phenology within the different savanna vegetation types will enable modellers to reduce the uncertainty pertaining to this biome under predicted future climate conditions.

Understanding how different vegetative components within a system green-up in relation to these system drivers is important, as the timing of green-up often influences the growth and reproduction of biota, forage availability, carbon and water uptake, as well as energy exchanges (Scholes & Walker 1993; Owen-Smith 1994; Keeling *et al.* 1996; Myneni *et al.* 1997; Borchert 1999). Unlike the temperate systems, water-availability is often the limiting factor in semi-arid savanna growth (Scholes & Walker 1993; Fuller & Prince 1996; Chidumayo 2001), yet early-greeners will commence green-up prior to the onset of seasonal rainfall, suggesting that flushing in these trees is not cued by water availability (Sarmiento *et al.* 1985; de Bie *et al.* 1998). The growth of late-greeners and grasses, in comparison, is restricted to the wet-season after the first sizeable precipitation event (Dye & Walker 1987; Childes 1989; Scholes & Archer 1997; Chidumayo 2001; Higgins *et al.* 2011; Whitecross *et al.* 2016 *in review*). Do *et al.* (2005) have suggested that atmospheric conditions such as relative humidity or vapour pressure deficit have a stronger influence over canopy phenology in northern Africa than ground water availability. This statement is important to consider, given that early-greeners are producing leaves when upper soil moisture is at its lowest (Reich & Borchert 1984). Accessing reliable long-term records for environmental variables across southern Africa can be challenging; hence it is advantageous to use the easily accessible remotely-sensed datasets for variables such as rainfall and temperature. In southern Africa, variability of rainfall is high across both temporal and spatial scales (Higgins *et al.* 2000; Lehmann *et al*. 2011), yet this is unlikely to be the sole driver of leaf flush in spring. Campo-Bescós *et al.* (2013) mention that temperature plays an underrated role in driving NDVI in areas of southern Africa with a mean annual precipitation greater than approximately 950 mm. Photoperiod has also been shown to play a role in influencing green-up in several tropical trees in central America and Africa (Borchert & Rivera, 2001). Rainfall, photoperiod and temperature were investigated in this study due to the easily accessible datasets and the high likelihood that these drivers are potentially involved in driving savanna phenology. This study does, however, acknowledge that these are not the only potential drivers in this system and that many environmental/climatic variables are highly inter-correlated, thus when assessing the influence of these variables on phenology one should be careful to distinguish between correlation/association and potential causality. In dealing with the problem of auto-correlation, attempts were made to test the potential environmental drivers of green-up individually to determine which had the strongest influence over each area's phenology.

Proposed benefits of early greening include longer growing seasons, earlier access to nutrients, decreased threats from invertebrate herbivory and lower competition from late-greeners (Sarmiento *et al.* 1985; Dye & Walker 1987; Scholes & Walker 1993; Elliot *et al.* 2006). Potential risks include losing leaves in late-dry season fires (Rutherford 1981) and physiological water and heat stress (Do *et al.* 2005). Few studies have quantified either the benefits or the risks of early greening, meaning that predictions of which environments are likely to select for trees showing this strategy cannot yet be made. This study explores the prevalence of early-greening in similar vegetation communities across an environmental gradient. The first aim of this study was to assess the frequency of early-greening events along a latitudinal gradient across southern Africa using remote-sensing. Assessments of whether broad-leaved woodlands situated closer to the equator showed a greater number of early-greening years than those at higher (more southern) latitudes were made. Thereafter, investigations were made into whether this phenological trend showed any relationship to temporal and spatial rainfall variability. The second objective was to investigate the influence of potential environmental drivers responsible for the commencement of tree greening in the selected deciduous savannas including rainfall seasonality and mean annual precipitation, cumulative photoperiod and cumulative temperature prior to green-up.

3. Methods

3.1. Study sites and species

Burkea africana (Caesalpiniaceae) is a widely-distributed, deciduous, leguminous, broad-leaved savanna tree often occurring in woodlands on dry, nutrient-poor soils such as the Kalahari sands of southern Africa (Coetzee *et al.* 1977; Brummitt *et al.* 2007). Early-greening of this species has been observed in several phenological studies (Rutherford &

Panagos 1982; Childes 1989; de Bie *et al.* 1998). In Nylsvley Nature Reserve (South Africa), it has an average green-up period of 8 weeks, usually commencing in late September (Rutherford & Panagos 1982).

Seven sites were sourced from the literature to test the spatio-temporal dynamics of the early-greening strategy across a range of broad-leaved woodlands where *B. africana* is known to occur in southern Africa (Figure 1, Table 1). Only sites where *B. africana* was listed as one of the top three dominant species and all co-occurring species were also broad-leaved were selected. These sites form a latitudinal gradient from Zambia to South Africa enabling us to detect phenological patterns in green-up (Table 1). Sites were assessed using Google Earth imagery and while this study acknowledges that not all sites are identical in species composition, the utmost care was taken in selecting comparable sites that closely resembled the Nylsvley Nature Reserve site in both species and structural composition across southern Africa.

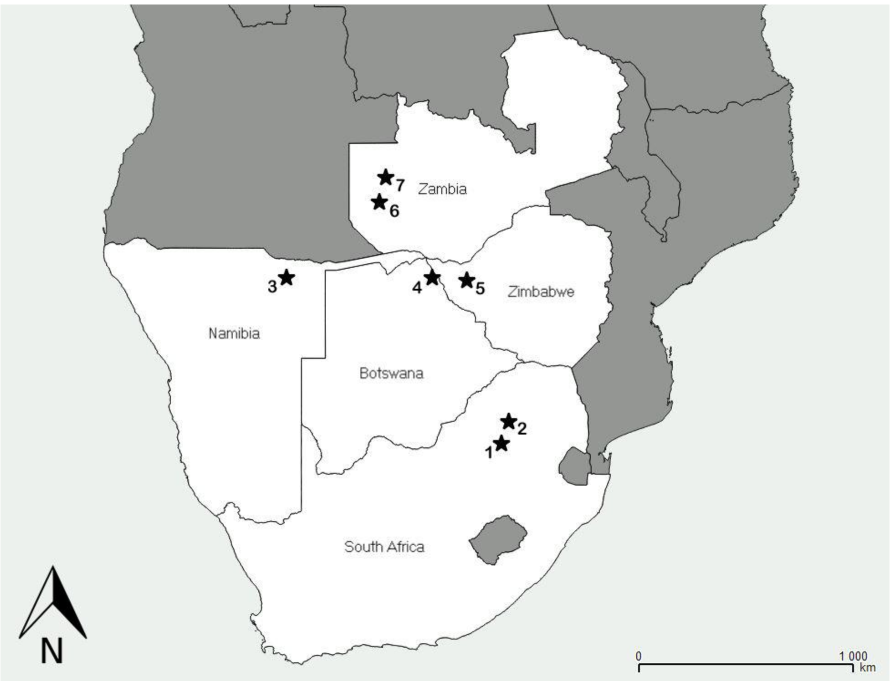

Figure 1. The locations of the seven broad-leaved woodland sites: 1. Seringveld Conservancy, 2. Nylsvley Nature Reserve (NNR), 3. Kavango region, 4. Pandamatenga, 5. Hwange National Park, 6. Mongu Namushakende, 7. Lukulu.

Table 1. The seven broad-leaved woodland sites and their dominant species chosen from the available literature to form a latitudinal gradient of similar structured broad-leaved savannas across southern Africa.

Co-ordinates	**Site name and dominant occurring species**	**Country**	**Reference**
S14.420; E23.520	East of Lukulu, Kanoti (*Burkea africana* and *Cryptosepalum exfoliatum*)	Zambia (ZAM)	Hansen *et al.* 2002
S15.450; E23.250	South of Mongu Namushakende (*Brachystegia spiciformis* and *Burkea africana*)	Zambia (ZAM)	Hansen *et al.* 2002
S18.640; E19.359	Kavango region (*Burkea africana* and *Terminalia sericea*)	Namibia (NAM)	De Sousa Correira & Bredenkamp 1986
S18.733; E26.950	Hwange National Park (*Burkea africana* and *Terminalia sericea*)	Zimbabwe (ZIM)	Childes & Walker 1987
S18.655; E25.500	Pandamatenga (*Schinzophyton rautanenii, Baikiaea plurijuga* and *Burkea africana*)	Botswana (BTW)	Privette *et al.* 2002
S24.650; E28.700	Nylsvley Nature Reserve (*Burkea africana* and *Terminalia sericea*)	South Africa (RSA)	Rutherford 1982
S25.601; E28.395	Seringveld Conservancy (*Burkea africana* and *Terminalia sericea*)	South Africa (RSA)	La Grange 2010

3.2. Experimental design, protocol and data analyses

3.2.1. Measuring green-up phenology using remote-sensing techniques

Several studies have shown the value of using remote sensing as a means for monitoring phenological changes in large-scale systems (Fuller & Prince 1996; Chidumayo 2001; Privette *et al.* 2002; Zhang *et al.* 2005). Most of these studies used the normalized difference vegetation index (NDVI) to assess the "greenness" within an area and infer changes in phenological states. Others have attempted to look at more specific changes in classifying which vegetative components (i.e. tree or grass) are responsible for changes observed in NDVI signals (Archibald & Scholes 2007; Higgins *et al.* 2011; Whitecross *et al.* 2016 *in review*). Remote sensing offers scientists access to long-term, large-scale data sets which include not only vegetation indices, but system drivers such as precipitation, photoperiod and temperature as well (Chidumayo 2001; Archibald & Scholes 2007; Higgins *et al.* 2011; Buitenwerf *et al.* 2015). Many automated methods have been used to identify the date of greening from remotely sensed NDVI data (Chidumayo 2001; Jolly & Running 2004; Archibald & Scholes 2007; Higgins *et al.* 2011). However, few studies have looked at the specific green-up period in detail, rather focusing on the growing seasons as a whole. Moderate Resolution Imaging Spectroradiometer (MODIS) MOD13Q1 version 5 images were downloaded (www.reverb.echo.nasa.gov (accessed: 30 October 2014)), providing a 16 day, 250 m resolution NDVI dataset corrected for water, clouds, aerosols and cloud shadows. This study chose to use NDVI over the enhanced vegetation index (EVI), as an assessment into the relative changes through time and not the absolute values of greenness at each site was the main priority, so differences in soils were deemed not important. This study acknowledges that soil bio-geochemistry can influence water infiltration among other factors (Scholes and Walker 1993), however, all of the sites selected were found on deep, sandy soils with a similar texture and composition throughout. Annual MODIS images between 1 August and 31 April (austral green-up period and peak growing season) were downloaded for the Nylsvley Nature Reserve and the six other broad-leaved woodland sites between 2002 to 2014 (NASA LP DAAC 2000). The first two years (2000-2001) of MODIS imagery were not analysed due to missing data during the period of interest for some of the sites, thus for consistency only the data from 2002 onwards was analysed.

One of the challenges of using remotely sensed data for phenological studies is to develop ecologically-meaningful measures. Whitecross *et al.* (2016 *in review*) showed that NDVI is sensitive enough to detect early-greening in *B. africana* woodlands at the Nylsvley Nature Reserve prior to the onset of seasonal rainfall. Using field data from the Nylsvley Nature Reserve (Whitecross *et al.* 2016 *in review*) a comparison of the observed date of greening against the measured NDVI values and determined that the tree green-up start dates corresponded to a threshold of 20% of the total NDVI range. This threshold was used as a robust index to assess the green-up start dates across the seven sites, due to its independence of total NDVI at each of the sites. If the 20% threshold was attained prior to the onset of seasonal rainfall at the start of the growing season, it was assumed that early-greening had occurred. Austral start dates, where the 1st July is assigned as day 1, were used for all phenological analyses as suggested by Whitecross *et al.* (2016 *in review*).

3.2.2. Spatio-temporal dynamics of broad-leaved woodland phenology across southern Africa

This study compared three environmental conditions to the observed phenological trends.

3.2.2.1. Rainfall

The amount of daily rainfall was calculated from the 3-hourly estimates of precipitation rate produced by the 3B42 Tropical Rainfall Measuring Mission (TRMM) satellite (NASA Earth Science Enterprise). Data were downloaded for the corresponding 250 m pixels at the seven selected sites from January 2002 to 2014. To compare the accuracy of TRMM rainfall estimates with ground-based rainfall collection data (South African Weather Service's Bela Bela station), a t-test was conducted comparing the estimates of each from and found no overall difference (paired t-test: t=-0.09; d.f.=21; p=0.92). This study thus accepted that TRMM data were sufficient for analysing the onset of rainfall across the study region. The TRMM data product was presented as a 3-hourly rainfall rate and this rate was converted to an amount in millimetres by multiplying the rainfall rate by 3 hours. To acquire an estimate of the daily amount of rainfall, the rainfall amounts within a 24 hour period were summed. The start of seasonal rainfall was calculated as the first precipitation event to produce 15 mm or more rainfall within a 24 hour period after the dry season. This estimate is 5 mm lower than the typically defined rainfall onset proposed for agricultural crops in a semi-arid area of southern Africa (Hachigonta *et al.* 2008). The reason is that savanna grasses are able to commence growth as soon as soil moisture increases sufficiently for grass roots to take up available soil moisture and commence growth, and 15 mm is sufficient to trigger the onset of grass growth (Bate *et al.* 1982). In order to detect the early-greening signal by only the trees, without any grass green-up influence on the NDVI values, the 15 mm precipitation event was used as the onset value for seasonal rainfall to exclude any signal from grass growth that occurs once this threshold has been reached.

At each of the seven sites across southern Africa the date of greenup (identified from MODIS NDVI) was compared with the date of first rainfall (TRMM daily rainfall) from 2002-2014. If the start date for the green-up was earlier than that of rainfall onset, it was assumed that early-greening had occurred. Grasses are restricted to green-up during the wet season and should have little bearing on the NDVI signal prior to the start of seasonal rainfall (Archibald & Scholes 2007; Higgins *et al.* 2011). The start dates of green-up and rainfall for each year were compared for each site using paired t-tests. In order to establish how pronounced the early-greening signal was at each site, the average number of days of early-greening prior to the arrival of seasonal rainfall was compared to the percentage of early-greening years over the 14 years for the seven sites using a regression. The longer the early-greening period prior to the arrival of rainfall, the longer trees have to potentially handle physiological risks such as evaporative water loss and heat stress during the driest time of the season (Do *et al.* 2005; Archibald & Scholes 2007). It was hypothesised that systems with higher temporal rainfall variability at the beginning of the growing season may be less likely to have early-greening, as this would represent an increased risk for the trees in these systems in terms of sustaining a canopy when conditions may be dry for an extended period. The average Coefficient of Variation (CV) for early-season rainfall for each site was calculated using the first six months (July to December) rainfall for each season. The CV for the mean annual precipitation of the austral year was also calculated and regressions were fitted to both datasets. The mean annual precipitation was calculated from 2002 to 2014 by summing the monthly rainfall at each site within each austral year (1^{st} July- 30^{th} June). A Kruskal-Wallis test with a Kruskalmc post-hoc analysis from the package ‘pgirmess’ (Giraudoux, 2014) was used to compare the mean annual rainfall estimates between sites in R (R Core Team, 2014; version 3.1.0).

3.2.2.2. Temperature

Temperature estimates were downloaded from the MOD11A1 Land Surface Temperature (LST) and Emissivity dataset with daily temporal resolution and 1 km spatial resolution (NASA LP DAAC 2000). Day maximum and night minimum temperatures were extracted for each site. The mean cumulative temperature from one month after the winter solstice (21 July) to the start of green-up was calculated from 2001 to 2013 and compared between sites using an ANOVA and Tukey HSD in R (R Core Team, 2014; version 3.1.0). The average maximum and minimum temperatures during the 10 days prior to the start of green-up at each site were also calculated and compared using a Kruskal-Wallis and Kruskalmc posthoc test (Giraudoux, 2014).

3.2.2.3. Photoperiod

The photoperiod that corresponded to the green-up date at each site was calculated using the ‘geosphere’ package in R (Hijmans, 2014). The mean cumulative photoperiod and corresponding standard errors between one month after the winter solstice (21 July) and the start of green-up were compared to the mean start dates of green-up across the seven sites. An ANOVA and Tukey HSD were used to compare the differences in photoperiods among the seven sites during 2002-2013 (R Core Team, 2014; version 3.1.0).

3.2.3. Investigating the key environmental drivers

Regressions were run between the correlated rainfall variables: CV of the first six months of rainfall, the CV of the annual rainfall and the mean annual precipitation, to determine which variable had the strongest correlation with early-greening. This variable was then included with temperature and photoperiod in a multiple regression model to predict the proportion of early-greening years. The ‘dredge’ function from the package “MuMIn” was used to test which model was the strongest for both analyses (Barton, 2013). The models used site data averaged over the period 2002-2014 to model the proportion of early-greening years.

4. Results

4.1. Frequency of early-greening across southern Africa

Greening dates across southern Africa showed a clear latitudinal gradient with an increased period of early-greening prior to rainfall onset and more frequent early-greening years occurring at sites situated closer to the equator (R^2=0.74, p=0.01, Figure 2). Lukulu (Zambia), the most northerly site, showed the highest number of early-greening years, with the longest average number of days prior to the onset of rainfall (Figure 2). Temporal variability in green-up start dates was highest for Mongu (Zambia) and lowest for Hwange (Zimbabwe) (Figure 2).

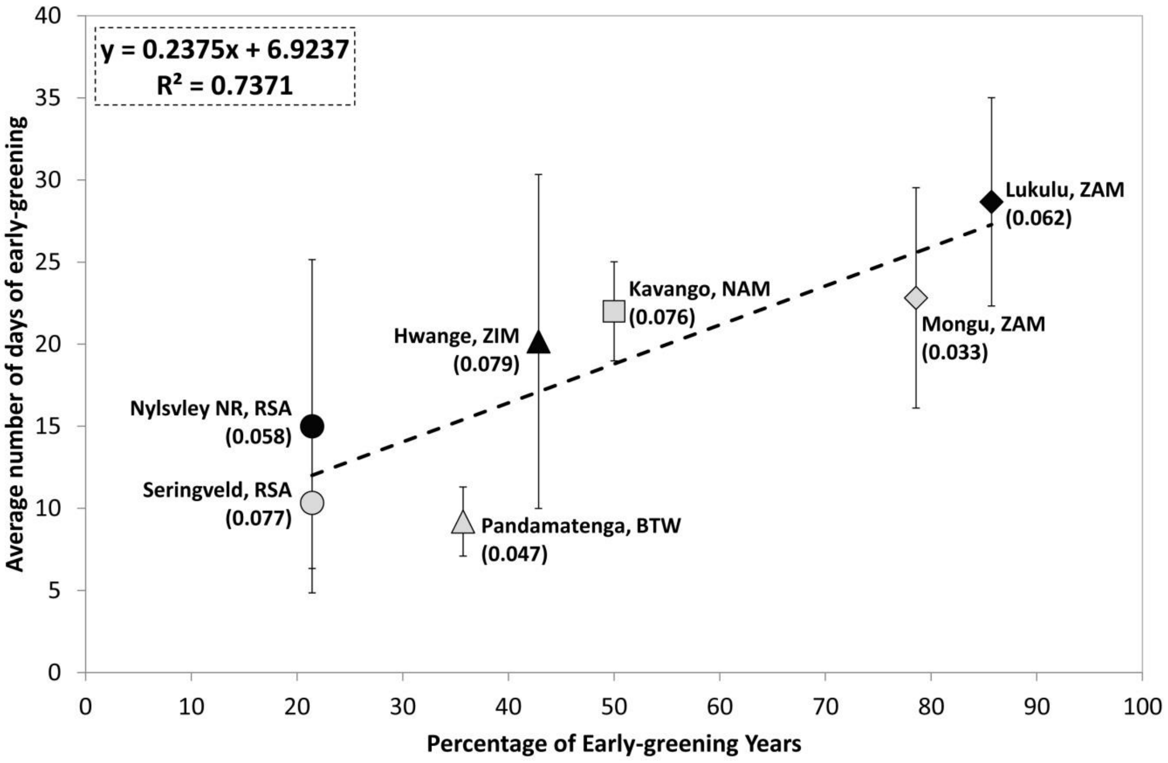

Figure 2. The relationship between the percentage of early-greening years between 2002-2014 and the mean±SE number of days of early-greening prior to the start of seasonal rainfall across seven broad-leaved woodland sites in southern Africa. Names, country codes and coefficient of variation for the green-up start dates (in parentheses) displayed next to each site.

4.2. Environmental correlates of the early-greening strategy

On average, only the Zambian sites showed significant positive differences between the green-up start dates and the onset of rainfall dates (Mongu: t=3.18, d.f.=13, p=0.007; Lukulu: t=3.37, d.f.=13, p=0.005, Figure 3). The green-up of the southernmost site, Seringveld, occurred predictably after the onset of rainfall (t=-2.50, d.f.=13, p=0.02, Figure 3). Site specific start dates of green-up and rainfall are presented in the Appendix (Figure S1).

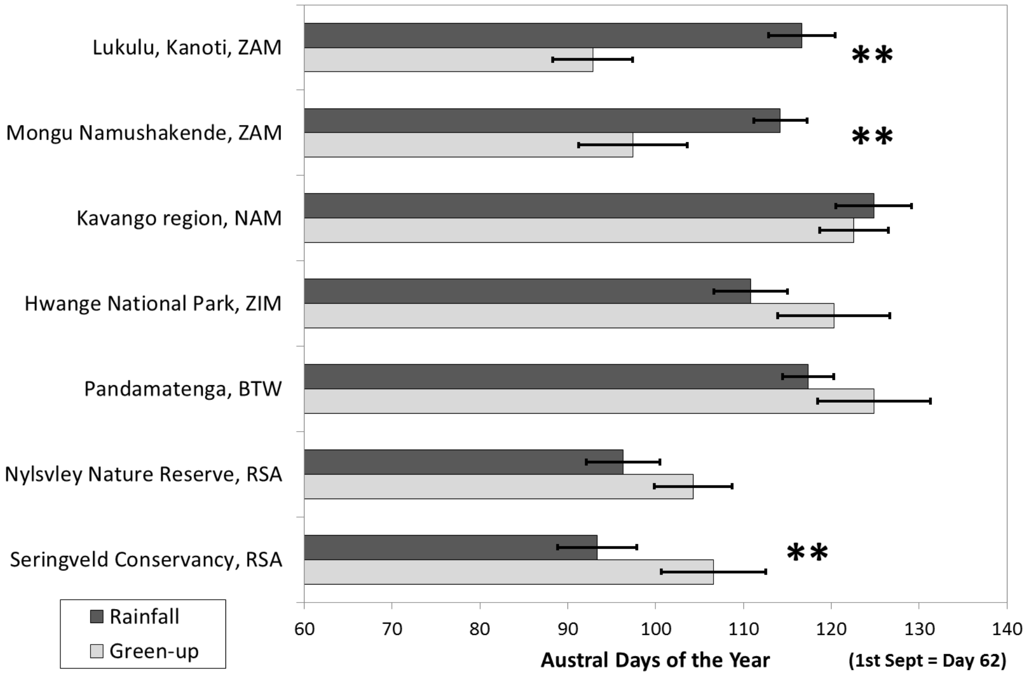

Figure 3. The mean±S.E. austral start dates of rainfall (TRMM) and green-up (MODIS NDVI) in seven broad-leaved savanna woodlands along a latitudinal gradient in southern Africa between 2002-2014. Significant differences (paired t-tests) for each site are illustrated by stars. Sites ordered by latitude, from the northernmost going southwards. Day 1 = 1st July.

The mean annual precipitation recorded at the seven sites showed a clear difference between the Zambian sites and the more southern sites (H=38.83, d.f.=6, p<0.001, Figure 4). None of the more southern sites showed any overall differences in mean annual precipitation (Kruskalmc: p>0.05, Figure 4).

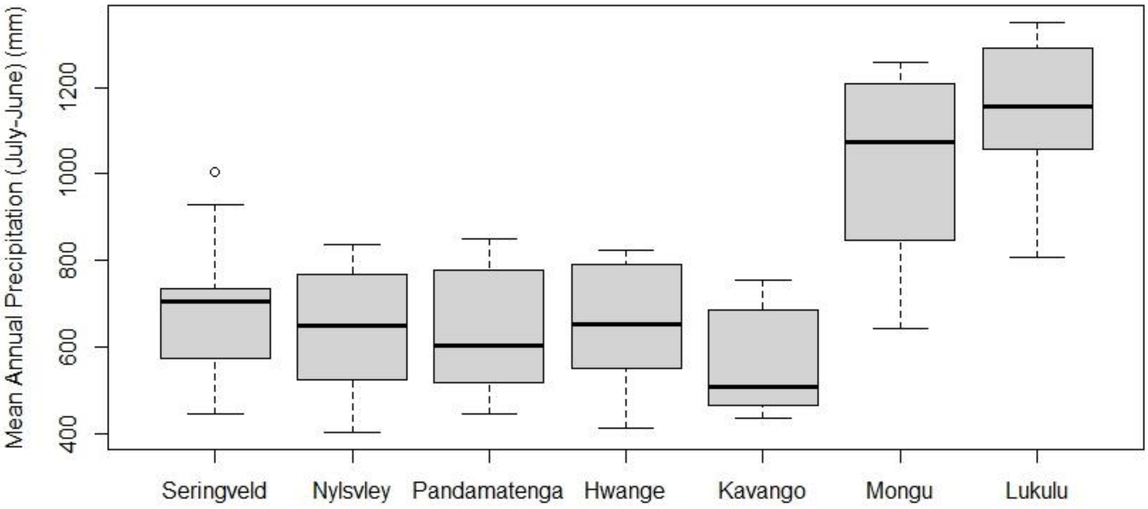

Figure 4. Box and whisker plots of mean annual precipitation for austral years between 2002-2014 at seven broad-leaved woodland sites along a latitudinal gradient in southern Africa.

Variability in the amounts of early-season rainfall (July to December) showed a strong latitudinal trend with the early-greening Zambian sites displaying the most consistent levels of rainfall during the early months of the austral season (Figure 5A). The southern sites all showed high variability in rainfall amounts in the early months of the season (Figure 5A). The CV in mean annual precipitation maintained a similar trend to that of the first six months of rainfall (Figure 5B). The high variability in the amounts of both the early- and full-season rainfall in the southern sites may have contributed to the lower proportions of early-greening years.

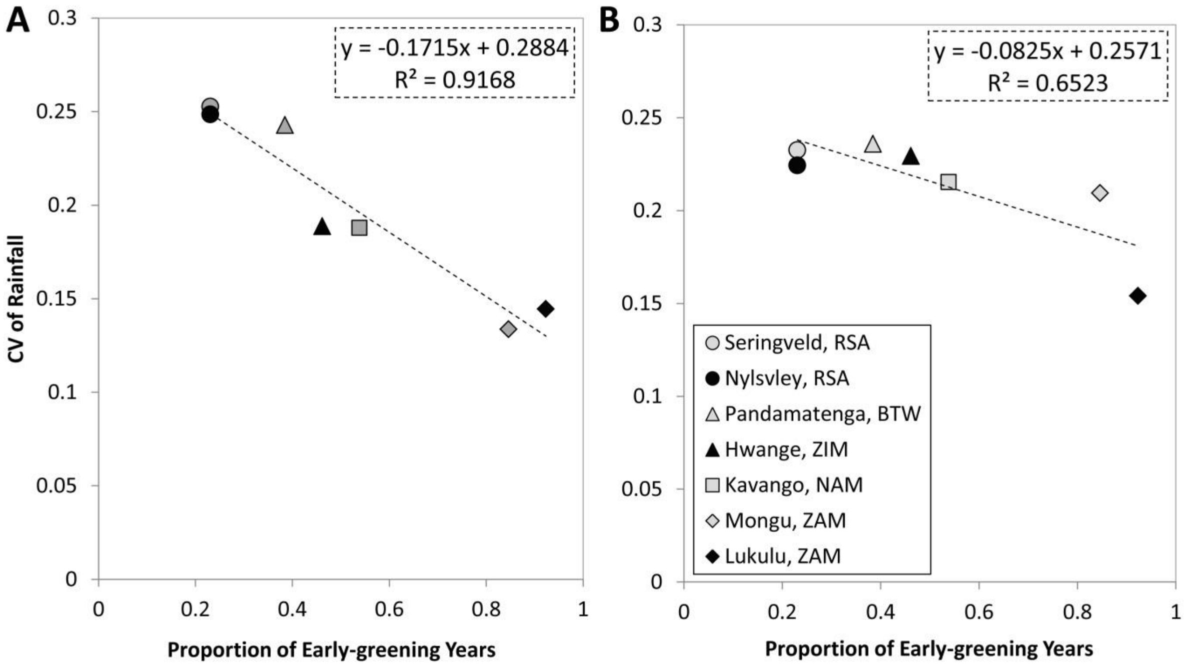

Figure 5. The Coefficient of Variation (CV) of (A) first six months of rainfall in the austral year (July to December) and (B) austral mean annual precipitation in relation to the proportion of early-greening years at seven broad-leaved woodlands along a latitudinal gradient in southern Africa.

4.3. Potential environmental cues for early-greening

The mean accumulated temperature was highest in the more central sites ($F_{6,84}$=8.12, p<0.001, Figure 6A). The Zambian and South African sites had the lowest mean values at the time of green-up with no differences observed (Tukey HSD: p<0.05, Figure 6A).

The means±S.E. for the 10 day average maximum land surface temperatures prior to green-up had a range between 36.4±1.0°C (Seringveld Conservancy, RSA) and 44.1±1.5°C (Pandamatenga, BTW) (Figure 6B). Pandamatenga showed differences to Lukulu, Nylsvley and Seringveld (H=30.58, d.f.=6, p<0.001, Figure 6B). All other sites showed no differences in temperatures prior to green-up suggesting that a threshold temperature may exist at about 35°C, which could be a driving factor behind early-green-up in the broad-leaved woodlands in southern Africa (Kruskalmc: p>0.05, Figure 6B).

The 10 day average minimum temperatures showed no overall differences between Mongu and Pandamatenga (Kruskalmc: $p>0.05$), however, Pandamatenga had higher average minimum temperatures than all other sites ($H=50.21$, d.f.=6, $p<0.001$, Figure 6B). The Zambian sites (Lukulu and Mongu) showed no difference in average minimum temperatures (Kruskalmc: $p>0.05$, Figure 6B).

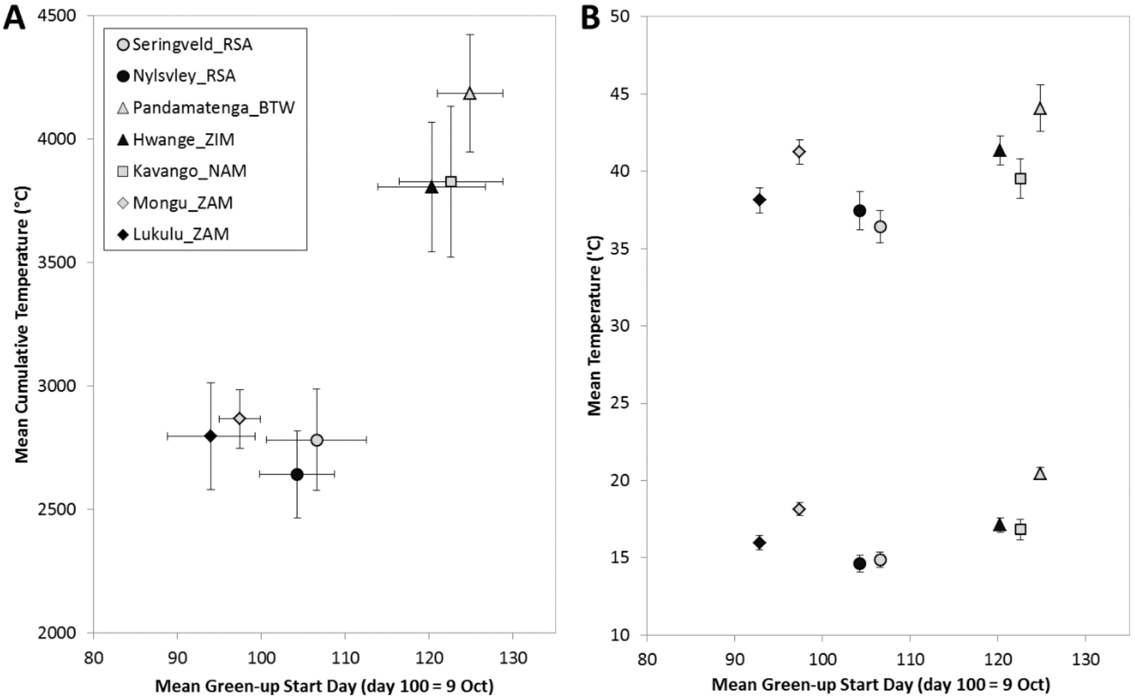

Figure 6. (A) The mean± S.E. accumulated temperatures from one month after the winter solstice (July 21st) to the start of austral green-up and (B) mean± S.E. 10-day average maximum and corresponding minimum temperatures prior to green-up at seven broad-leaved woodlands along a latitudinal gradient in southern Africa between 2002 – 2014 using MODIS land surface temperature estimates.

The Zambian sites had the lowest cumulative photoperiod prior to green-up suggesting that, for these early-greening sites, it is unlikely that photoperiod is having the biggest influence on whether early-greening occurs or not (Figure 7A). When comparing the average photoperiod at the start of green-up, again the Zambian sites show the lowest number of hours of photoperiod in comparison to the sites further south ($F_{6,39}=12.95$, $p<0.001$, Figure

7B). The southern sites showed no overall difference (Tukey HSD: $p>0.05$). This would suggest that photoperiod is not playing a key role in driving early-greening, however, a possible compound effect of photoperiod with other environmental drivers in influencing green-up in the southern latitudes cannot be ignored.

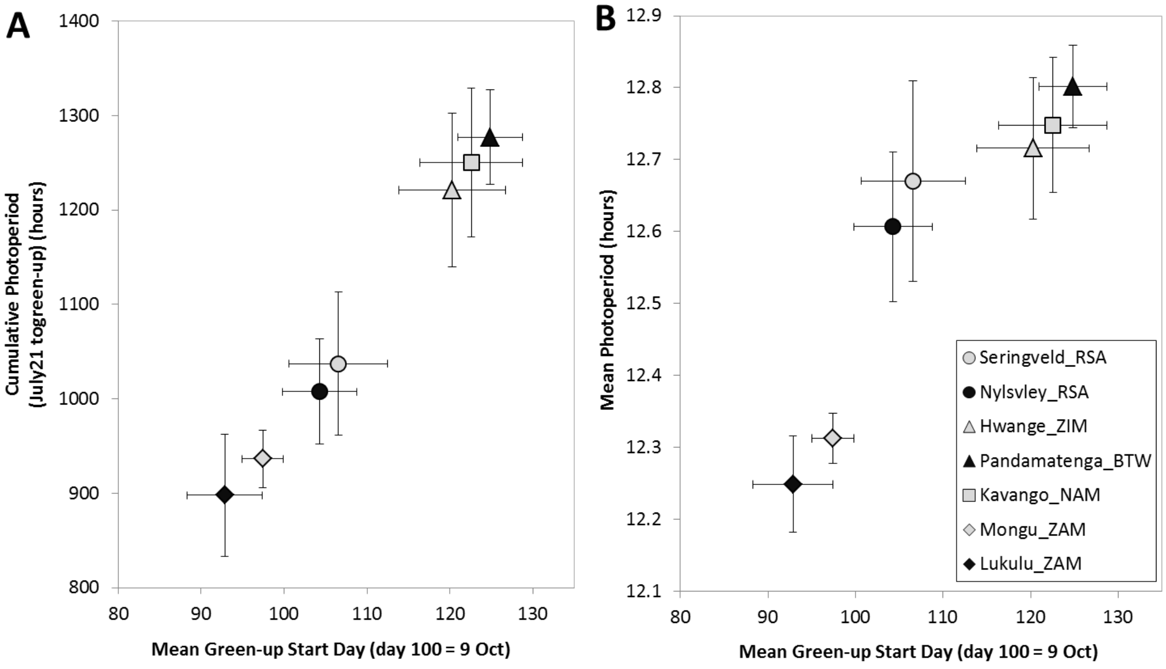

Figure 7. The mean±SE (A) cumulative photoperiod and (B) photoperiod at the start of green-up in relation to austral start dates of green-up in seven broad-leaved woodland sites along a latitudinal gradient in southern Africa.

4.4. Investigating the key environmental drivers

The CV of the first six months of rainfall was the most highly correlated variable to the proportion of early-greening years ($R^2=0.92$; $p=0.01$, Table 2 – model 1). A second model showed that the CV of the first six months of rainfall better explained the proportion of early-greening years than that of accumulated temperature or photoperiod ($R^2=0.96$, $p=0.005$, Table 2 – model 2).

Table 2. Model selection to determine the explanatory power of environmental variables on the proportion of early-greening years across seven broad-leaved savannas in southern Africa. The top two models are presented (CV=coefficient of variation).

Model	Factors Included	Degrees of Freedom	AICc	Delta	Weight
Model 1: Proportion of early-greening years ~ CV of annual rainfall + CV of first six months rainfall + mean annual rainfall					
A	CV of first six months rainfall.	3	-2.6	0	0.959
B	Mean annual rainfall.	3	5.7	8.36	0.015
C	CV annual rainfall & CV of first six months rainfall.	3	7.1	9.72	0.007
Model 2: Proportion of early-greening years ~ CV of first six months rainfall + accumulated temperature + accumulated photoperiod					
A	CV of first six months rainfall.	3	-2.6	0	0.976
B	Accumulated temperature & Accumulated Photoperiod.	3	6.7	9.32	0.009
C	Accumulated photoperiod.	3	7.6	10.26	0.006

5. Discussion

5.1. Variability in seasonal rainfall in relation to early-greening

The data confirm a strong latitudinal gradient of early-greening phenology observed across southern Africa's broad-leaved savannas (Zhang *et al.* 2005). The study indicated that variability in the amount of rainfall during the first six months of the austral year is strongly correlated with this trend in green-up across southern Africa – a model with CV of early-season rainfall alone has an $R^2=0.92$. The more predictable early-season rainfall is, the less risky early-greening becomes for a tree (Do *et al.* 2005), and it is at these sites where the benefits presumably outweigh the risks. To demonstrate this conclusively, however, would require better quantification of the risks and the benefits of early-greening across these systems. For example, in Kruger National Park, South Africa, high-rainfall savannas (>600 mm mean annual precipitation) produce the highest grassy fuels, so competition with grass would be highest at these sites (benefiting early-greeners), but so too would the risks due to increased fire intensity (detrimental to early-greeners) (Govender *et al*., 2006; Archibald *et al.* 2010). Nonetheless, these results represent the first predictive model thatthis study knows of for the conditions which might promote early-greening as a phenological strategy in seasonally arid systems.

Lehmann *et al.* (2011) found that the seasonality of rainfall was an important predictor in determining the distribution of savannas across Africa. Given that southern Africa's savannas are semi-arid systems and water availability is a fundamental component in driving the growth of plants, it is not surprising that this environmental factor would also have such a strong influence on savanna phenology (Scholes & Archer 1997; Borchert 1999). Globally, a decrease in rainfall variability is observed with an increase in mean annual rainfall (Nicholls & Wong, 1990), as seen in this study. Continentally, Africa follows this trend in spatial and temporal variability of rainfall (Lehmann *et al*. 2011), as do other tropical environments, such as Australia (Stafford Smith 2008; van Etten 2009). The rainfall regimes in southern Africa are influenced by interactions between the westerly flow across the Congo Basin driven by currents in the South Atlantic Ocean, the south-east trade winds, which originate over the Indian Ocean, as well as the dry north-easterly monsoonal flow originating from the Indian subcontinent (McHugh & Rogers 2001). In Australia and South America, for example, high rainfall areas show high temporal variability in the rainfall onset date between years (Bowman 2002), however, Cook & Heerdegen (2001) found high rainfall onset variability in lower rainfall areas of Australia too. Early-greening is common in both of these savanna ecosystems (Reich & Borchert 1984; Prior *et al*., 2003). A clear prediction from the results is that early-greening on these continents could show different regional patterns – being less common in lower rainfall areas of Africa.

5.2. Potential drivers of early-greening

In systems where trees show early-greening, upper soil moisture cannot be the environmental driver of leaf flush, hence there must be another seasonal trigger to cue leaf-out (Do *et al.* 2005). This study's findings in the high rainfall Zambian sites where the onset of green-up occurs consistently without the arrival of rainfall concur with the findings of Campo-Bescós *et al.* (2013), that increasing temperature is potentially driving early-greening, but further investigation is needed regarding this claim. The maximum temperature data (Figure 6) suggest the possible existence of a threshold value (about 35°C) which needs to be achieved before any greening will take place. Given the strong role that temperature plays in the phenology of northern hemisphere species, the role of this environmental factor on phenology deserves more attention in southern African systems (Menzel & Fabian 1999; Zhang *et al.* 2005; Polgar & Primack 2011).

When attempting to predict green-up in South African savannas, previous studies have suggested that an increase in photoperiod is the most reliable predictor of green-up in South African savanna trees (Archibald & Scholes 2007; Higgins *et al.* 2011). Given that photoperiod is much less variable at sites close to the equator, these data gave us an opportunity to test these ideas. The data show a clear delineation between the predominantly early-greening and late-greening sites when comparing the cumulative and average photoperiods at the start of green-up (Figure 7). The cumulative photoperiod is lowest in the Zambian sites followed closely by the South African sites. From this it can be inferred that photoperiod does potentially have an influence on the onset of green-up, particularly in systems where seasonal rainfall commences late into the austral summer. Whitecross *et al.* (2016 *in review*) found that *B. africana* woodlands in the Nylsvley Nature Reserve greened-up during mid-October if no seasonal rainfall had commenced, suggesting another potential back-up environmental driver that is responsible for cueing green-up when rainfall onset is late. Do *et al.* (2005) also suggest atmospheric vapour pressure deficit as a potential environmental cue for driving early-greening; however, remotely sensed data on this parameter are not available, so more research is needed to investigate this in relation to the above mentioned potential cues.

Despite slight variation in the species present in each of the seven sites, care was taken to choose sites which held the closest structural and species composition to the Nylsvley Nature Reserve *B. africana* woodlands (Whitecross *et al.* 2016). By assessing the relative changes in NDVI, it was hoped that a decrease in the effects of any species variability between the sites would occur. For example, in Lukulu (Zambia) the codominant species was *Cryptosepalum exfoliatum* (Hansen *et al.* 2002), an evergreen species whose NDVI values showed low change in comparison to the deciduous *B. africana*, thus it can be assumed that the increase in NDVI observed in the dry season at this site is related to the deciduous early-greener rather than its co-occurring evergreen. Conversely, in Mongu (Zambia), *Brachystegia spiciformis* is also an early-greener (Trouet et al. 2012), thus this study acknowledges that separating the early-greening signals between this species and its common codominant *B. africana* (Chidumayo 1987) will be more challenging, but nevertheless, this study was still able to detect the early-greening of these species in the site. The contrasting results point to local adaptation – either to different environmental cues, interactions between cues, or to different levels of the same cue – at each site. This has been suggested by Higgins *et al.* (2011) and demonstrated by Chmura & Rożkowski (2002) in temperate systems. Demonstrating this would require detailed transplant or lab experiments on the same species.

The ground-based study in Nylsvley Nature Reserve allowed us to link remotely sensed information to the observed patterns of greening in the field, and extrapolate this across the region. This study showed that linking field-gathered information on leafing patterns to large-scale remotely sensed datasets could greatly enhance the information that could be utilized from these large-scale resources. By targeting known broad-leaved woodlands with a similar species composition, this study was able to assess the ecological factors that influence the green-up in these woodlands with a smaller species effect not adding additional complexity to the analyses. It is interesting to find such a variable range of green-up strategies within such similar systems, with the extreme early-greeners in the Zambian sites contrasting strongly with the more frequent late-greeners in South Africa. Studies in Europe have shown that both altitudinal and latitudinal effects can influence the timing of leaf flush within populations of the same species (Menzel & Fabian 1999; Chmura & Rożkowski 2002; Zhang *et al.* 2005; Vitasse *et al.* 2009). The majority of these studies, however, are linked to changes in temperature relating to shifts in latitude and elevation and do not take rainfall impacts into consideration.

5.3. Broad-leaved woodland phenology under changing climates

Whether photoperiod or temperature cues early-greening in savanna broad-leaved woodland species is a question that will become more important under changing climates. The broadly accepted prediction for Africa’s future climatic conditions is an increase in both temperature and a shift in rainfall patterns to less frequent, but more intense precipitation events (IPCC 2007; Kusangaya *et al.* 2014). This could place broad-leaved woodlands at risk if their early-greening commences earlier in the year, possibly resulting in longer exposure to dry conditions. Do *et al.* (2005) recorded early-greening *Acacia tortilis* trees in North Senegal dropping their new leaf flush during seasons when rainfall onset was substantially later than average. The same loss could happen to early-greening broad-leaved species in Zambia if mean annual rainfall decreases and rainfall arrival dates shift to later in the year (Kusangaya *et al.* 2014). This could disadvantage them in the longer-term as they would no longer be able to take advantage of their early growing season and earlier access to nutrients (Dye & Walker 1987; Scholes & Walker 1993; Elliot *et al.* 2006).

Senior *et al.* (2002) predicted that rainfall over eastern southern Africa will become more intense, but the number of rain days is not likely to increase. This could potentially expose trees to longer periods of desiccation and may have a long-term, negative effect on their physiological well-being (Zhou *et al.* 2014). However, the South African trees show a more frequent late-greening strategy which suggests that the risk of greening too early may be less in this area. Savanna trees are also renowned for their resilience when faced with disturbances or environmental change (Huntley & Walker 1982; McNicol *et al.* 2015). Setting up long-term phenological assessments is vital if changes in these systems are to be addressed together with their environmental drivers as the world proceeds into a future with uncertain climatic conditions (Fitchett *et al.* 2015; Lindenmayer *et al.* 2015). By understanding the nature of phenology in southern Africa's broad-leaved savannas, this study hopes to further strengthen global vegetation modellers' ability to accurately represent the phenology of these complex systems.

6. Acknowledgements

We acknowledge funding from the National Research Foundation of South Africa (NRF), CSIR Parliamentary Grant and the DST/NRF Centre for Tree Health Biotechnology (CTHB). Thanks to NASA LP DAAC for allowing free access to and use of the MODIS data sets. Thanks to the Giovanni online data system, developed and maintained by the NASA GES DISC for the use of the TRMM data sets. Thanks to Caroline Howes, Penny Mograbi and Jolene Fisher for their support and assistance.

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8. Appendix

The site specific austral green-up start dates using MODIS NDVI data in relation to the rainfall start dates for precipitation events >15 mm using the TRMM dataset for seven broad-leaved woodland sites along a latitudinal gradient in southern Africa (Figure S1).

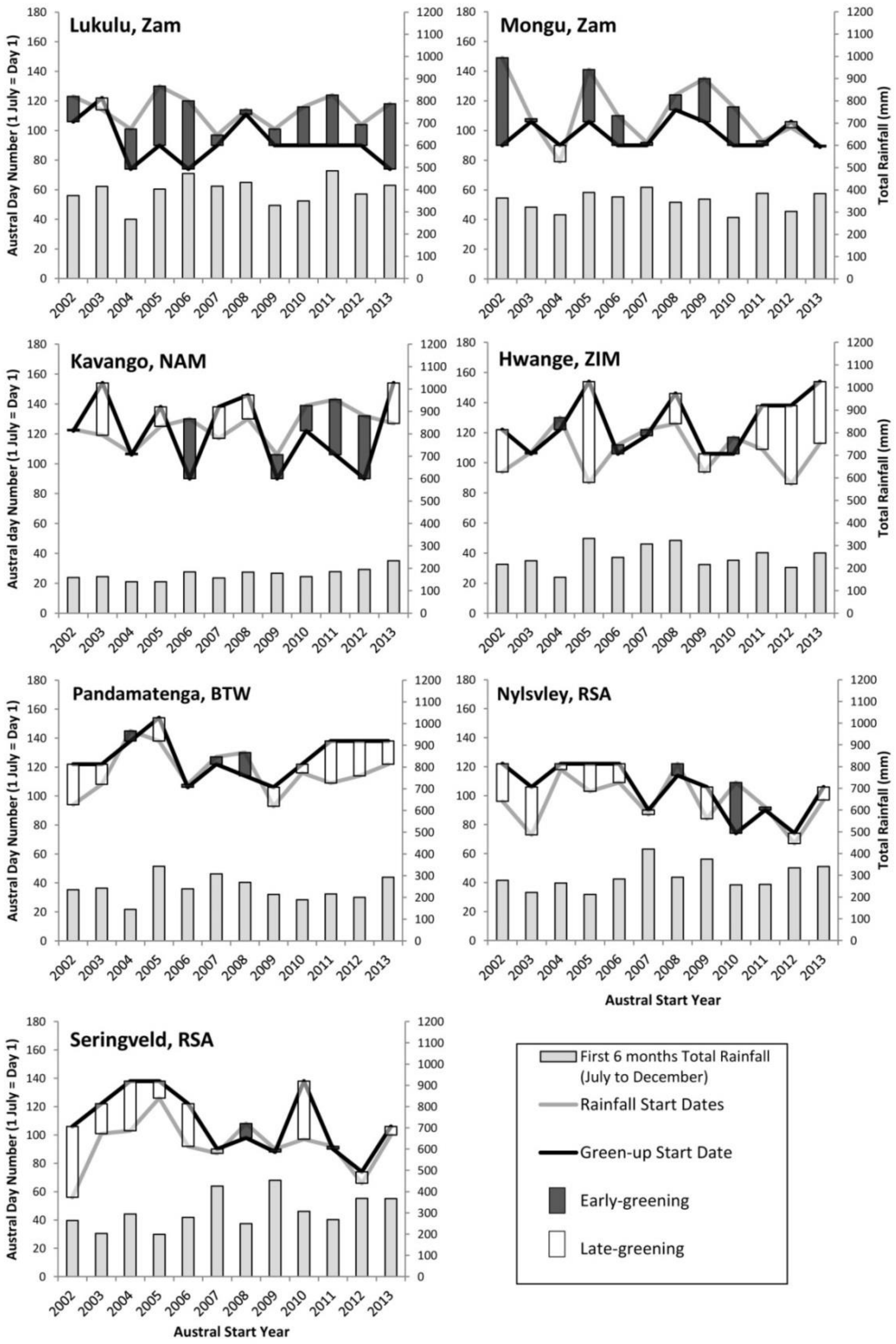

Figure S1. The austral green-up start date in relation to the corresponding rainfall start date for the years 2002-2013 at seven broad-leaved woodland sites along a latitudinal gradient in southern Africa. The total rainfall between July and December of each year is represented at the bottom of each figure and the vertical bars illustrate whether early- or late-greening occurred.

CHAPTER 7

GENERAL DISCUSSION AND SYNTHESIS

1. Introduction

Phenological studies have gained popularity in the environmental research community in the past decade, with numerous calls for improved monitoring of global vegetation phenology in order to understand and assess the impacts that changing climates are having on the planet (Schwartz, 1998; Menzel, 2002; Chambers et al., 2013; Buitenwerf et al., 2015; Fitchett et al., 2015; Lindenmayer et al., 2015; Adole et al., 2016). Plant phenology in particular, is extremely sensitive to variations in weather conditions, making it a valuable indicator in assessing the impacts of changing climates on Earth’s fauna and flora (Richardson et al., 2013). Unfortunately, the vast majority of phenological studies have been conducted in northern hemisphere systems with large gaps in the current southern hemisphere knowledge base (Chambers et al., 2013; Adole et al., 2016). A large proportion of the terrestrial land surface in the southern hemisphere is dominated by savannas and despite numerous studies taking place within these complex systems, only a handful of recent studies have focused on phenological themes (Huntley and Walker, 1982; Chidumayo, 2001; Archibald and Scholes, 2007; Higgins et al., 2011; February and Higgins, 2016). The complexity associated with the structure of savanna vegetation is mirrored by a high level of variability in phenological strategies over both temporal and spatial scales (*see Chapters 3 & 6*). This spatio-temporal variability has made it difficult for ecologists and environmental modellers alike to develop accurate models or predictions for long-term savanna phenology under changing climates (Chidumayo, 2001; Archibald and Scholes, 2007; Chambers et al., 2013; Buitenwerf et al., 2015). The fact that early-greening is only known to occur in savanna systems, makes it an important phenological phenomenon to investigate and understand further. This study has offered some insight into confirming whether early-greening savanna trees benefit from utilizing this strategy. This study has also quantified the frequency of the early-greening phenomenon across southern Africa’s broad-leaved savannas. The major findings relating to the investigation of two of the hypothesized benefits relating to early-greening are discussed below.

2. Summary of the major findings

A graphical summary of the major findings of this study is provided in Figure 1, followed by detailed discussions of the outcomes from each of the major themes of this study.

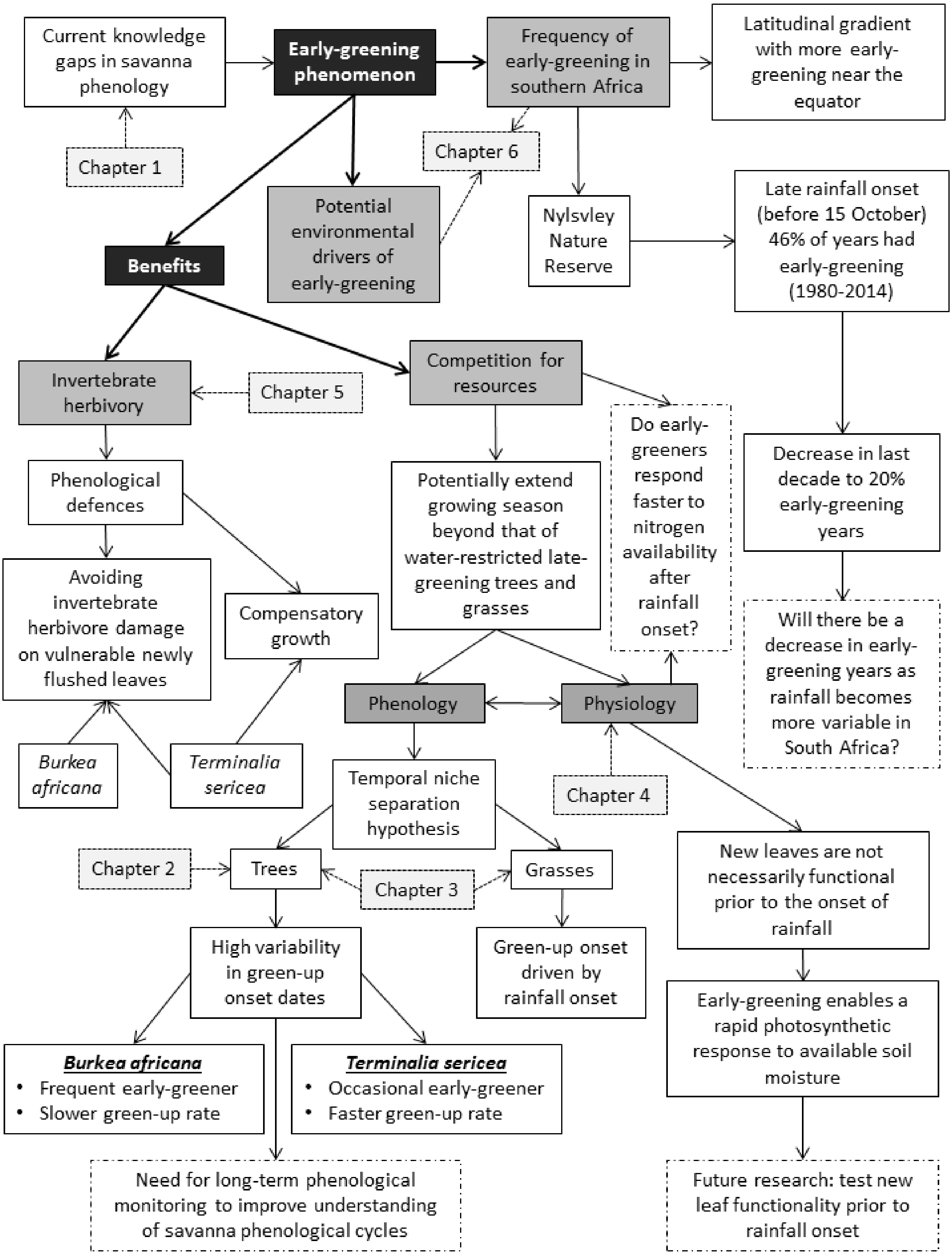

Figure 1. Graphical summary of the major findings from this study (full line boxes) together with the corresponding chapter numbers in the dashed-line boxes, and future recommendations in the dot-dashed lined boxes. Major study themes are highlighted in the grey boxes. The black boxes represent the starting point of the study.

2.1. What are the benefits of early-greening?

Several hypothesised benefits have been proposed for trees utilizing an early-greening strategy including faster access to plant available nutrients after the first rains (Scholes and Walker, 1993), avoidance of nutrient leaching of new leaves which lack a developed waxy cuticle (Sarmiento et al., 1985), an extended growing season beyond that of water-limited grasses and late-greening trees (Dye and Walker, 1987), as well as better use of available soil moisture for growth with an already maturing canopy (Elliott et al., 2006) and finally avoidance of invertebrate herbivory on vulnerable new leaves at the start of the growing season (Sarmiento et al., 1985). This study set out to test two of these benefits:

2.1.1. Do early-greeners experience a longer growing season?

This aspect of the study was investigated on both a temporal (*Chapters 2 & 3*) and physiological scale (*Chapter 4*) to determine whether early-greening trees were expanding the period during which they have leaves present on their canopies, as well as whether or not those leaves were functional prior to the onset of seasonal rainfall – thereby confirming if the growing season had actually been extended as hypothesised by Dye and Walker (1987) and Elliott et al. (2006). The temporal niche separation hypothesis proposed by Scholes and Walker (1993) to explain the early-greening phenomenon observed in certain savanna trees, suggests that trees are able to deploy their leaves earlier in the growing season than grasses, using stored carbon reserves, thus affording trees a period of growth with reduced competition from their herbaceous neighbours (Higgins et al., 2011; February and Higgins et al., 2016). This study has shown evidence to support the temporal niche separation hypothesis where trees with early green-up benefit from this period of low competition from herbaceous neighbours; however, this study has also shown that not all seasons experience conditions conducive to early-greening by trees. In Chapters 2 and 3 the phenology of *B. africana* and *T. sericea* were monitored over three seasons with high variability in green-up start dates and growth rates between seasons. A small early-greening event was observed for the *B. africana* trees during the 2013 green-up period; however, in the 2014 green-up period substantial early-greening was recorded possibly due to the late onset of seasonal rainfall (11th October 2014). These results align with Dye and Walker's (1987), Scholes and Walker’s (1993) and Elliott et al.'s (2006) hypotheses of a longer growing season; however, the physiological data presented in Chapter 4 suggest a significant extension of the growing season does not occur as frequently as one might expect in the Nylsvley Nature Reserve (NNR) broad-leaved savanna. The lack of functionality of new leaves in both species during the first two weeks of flush would suggest that only early-greeners which are able to flush at least two weeks prior to the onset of rainfall would benefit from an extended growing season. In the NNR where early-greening is potentially occurring during approximately 46% of all seasons (Figure 2), and the average period of new leaves present on a canopy prior to rainfall onset is 15±10 days (*Chapter 6*), the potential for some growth during periods when grasses and late-greening trees are still dormant cannot be ruled out entirely. February and Higgins (2016) showed that broad-leaved savanna trees (including *T. sericea*) are able to rapidly deploy their new leaves at the start of the growing season when grasses are still dormant. Given the brief period post-rainfall in which mineralization occurs and nitrogen and other important nutrients are made available to the plants, having new leaves already present (i.e. *B. africana*) or being able to rapidly commence new leaf flush using stored carbon reserves (*T. sericea*) is likely to give trees a growth advantage over grasses at the start of the growing season (*Chapters 2, 3 and 4*).

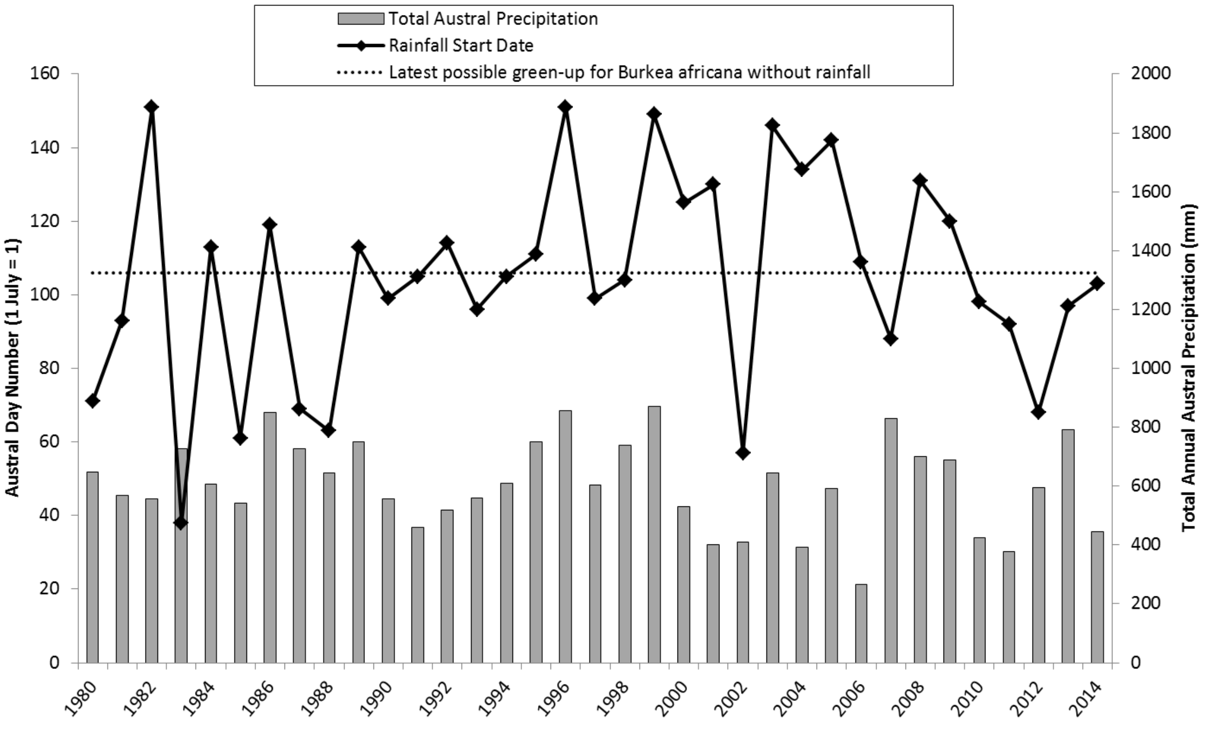

Figure 2. The annual austral rainfall start dates and total austral precipitation in relation to the latest possible green-up date (15th October) prior to the onset of rainfall for *Burkea africana* at the Nylsvley Nature Reserve between 1980 and 2014 (South African Weather Service).

In Chapter 6, the frequency of early-greening years was assessed across southern Africa's *B. africana* dominated savannas – those located in areas with a high amount of annual rainfall, and a low variability in rainfall onset dates had an over 80% occurrence of early-greening years across the 2002 to 2014 study period. It is thus likely that in these high precipitation systems the growing season is almost certainly extended beyond that of neighbouring vegetation, particularly the grasses. Therefore, it can be concluded that early-greening has the potential to provide trees (under the right environmental conditions) with an extended growing season over their grassy and late-greening neighbours. Comparing the 46% early-greening frequency from the historical rainfall records (1980-2014) to the 20% frequency more recently as shown in Chapter 6 (2002-2014), highlights a potential decrease in the frequencies of early-greening events in the NNR. Future forecasts for South Africa's climate suggest a decrease in the number of rainfall events with a corresponding increase in the variability associated with the timing of these precipitation events (Midgley and Bond 2015; van Wilgen et al., 2015). Given the trend of fewer early-greening events in South Africa where rainfall variability is already considered to be high (*Chapter 6*), is the country more likely to see a further decrease in the number of early-greening events in the future as rainfall variability and unpredictability increases?

Using the NNR's current average seasonal rainfall start date of 14 October (austral day 105) and the standard deviation of 28.5 days to represent the variability associated with the onset of seasonal rainfall (*Chapter 3*), a simple rainfall start date model was created for a simulated 1000 years in RStudio (version 0.99.896) (Figure 3). Early-greening has been shown to have an average green-up of 15±5 days at the NNR (*Chapter 6*), so in order to conservatively simulate the average green-up start date; the 4th October (austral day 95) was used as the green-up start date (Figure 3). By calculating the proportion of years within the 1000 year model where rainfall start dates commenced after the start of green-up, a comparison could be made in relation to a simulation where the variability (SD) associated with the rainfall onset date had been increased to 38.5 days. This model indicated that the proportion of early-greening years would likely decrease from 0.65 to 0.58 under higher rainfall variability conditions (Figure 3). This model does not illustrate absolute values of early-greening for the next 1000 years; however, it does successfully illustrate that when rainfall onset date variability is increased, the probability of early-greening occurring decreases.

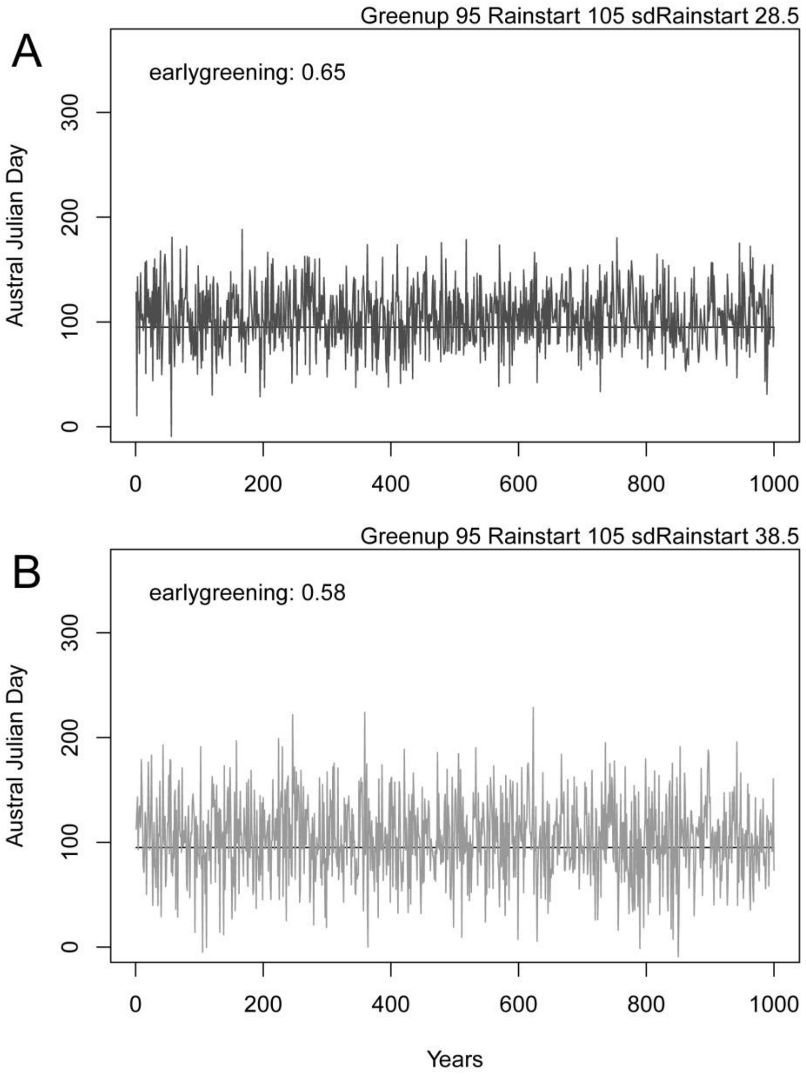

Figure 3: Modelled simulations of rainfall start dates in relation to a hypothetical mean green-up start date of 4th October (austral day 95) for the (A) current variability (standard deviation) of 14th October ± 28 days and (B) a potential increase in variability to SD=38.5 days for the Nylsvley Nature Reserve. (SD = standard deviation)

2.1.2. Do early-greeners avoid invertebrate herbivory?

In Chapter 5, this study tested Sarmiento et al.'s (1985) hypothesis that early-greening trees avoid herbivory from invertebrates that emerge with the onset of seasonal rainfall. This study found that during the first weeks of the green-up period, early-greeners did experience lower levels of herbivory from invertebrate herbivores, thus supporting Sarmiento et al.'s (1985) theory. Murali and Sukumar (1993) observed the same effects in India where early-greening species experienced significantly lower herbivore damage from invertebrates than the late-greening species which flushed at the same time as the emergence of invertebrates after the onset of seasonal rainfall. Charles-Dominique et al. (2016) suggest that mammalian herbivory pressure is the likely influence behind the evolution of spinescence in nutrient-rich, fine-leaved African *Acacia* savanna trees (Hempson et al. 2015). In contrast, it is possible that invertebrate herbivory pressure has played a role in influencing the evolution of early-greening phenology within the nutrient-poor, broad-leaved savannas; where mammalian herbivory pressure is considerably less than in nutrient-rich savannas (Owen-Smith 1994; Hempson et al. 2015; Charles-Dominique et al. 2016). Nutrient-poor savannas occur in higher rainfall areas and their structural composition is often maintained through fires rather than driven by mammalian herbivory (Charles-Dominique et al. 2016). However, as observed in the NNR, many of the plants considered unpalatable for mammalian herbivores are extensively eaten by invertebrate herbivores (Scholtz 1982; Cooper et al. 1988; Bryant et al. 1991; *Chapter 5*). The interaction between fire and invertebrate herbivory pressure in influencing the evolution of early-greening – particularly within the broad-leaved savannas of Africa – is an area of research that requires more attention. Chapter 5 also addressed how savanna trees may be using phenological defences against disturbances, such as herbivory. For example, *T. sericea* experienced the highest levels of herbivory during the early period of the growing season, but it utilises a compensatory growth strategy to replace herbivory damaged leaves throughout the growing season (*Chapter 5*). This would appear to be a physiologically costly strategy for the tree, however, in Chapter 4 this study showed that *T. sericea* is investing less nutrients (such as nitrogen and phosphorus (Scholes and Walker, 1993)) into its leaves and is photosynthesising 5-6 µmol CO_2 $m^{-2}s^{-1}$ higher, with a lower average mature leaf chlorophyll content (±4 SPAD Units) than *B. africana.* Conversely, *B. africana* invests more into maintaining the same leaves throughout the growing season, with an increase in chemical defences (Bryant et al., 1991) and leaf nutrients (Scholes and Walker, 1993). This would suggest that these broad-leaved savanna trees have evolved equally successful strategies to cope with the ever-present threat of invertebrate herbivory through the adaptation of their phenology, leaf turnover and physiology.

2.2. Is an early-greening strategy beneficial to semi-arid savanna trees?

Through assessing this study as a whole, it would appear that the early-greening strategy does provide trees with an advantage over other late-greening species and grasses when certain environmental conditions are met. For example, in a system such as the NNR where early-greening is occurring approximately 46% of the time (Figure 2) and rainfall onset variability is high in comparison to other similar systems within southern Africa (*Chapters 3 & 6*); early-greening trees will only be able to commence early flush if the rainfall onset dates are late (after mid-October) and no major disturbances such as late-season fires occur. During the 2012 green-up period when rainfall onset was early, the early-greening *B. africana* showed slower green-up rates in comparison to the neighbouring grasses and later-greening *T. sericea* (*Chapters 2 & 3*). In the 2014 season when the *B. africana* did show clear early-greening, their green-up rate was still slower than *T. sericea* trees whose leaf deployment responded rapidly to the onset of rainfall (*Chapter 2*). However, the early-greening *B. africana* reached canopy maturity three weeks prior to *T. sericea* indicating that their early-greening provided them with a growth advantage during the late-onset rainfall season. This suggests that the growth of *B. africana* is potentially more internally controlled than that of *T. sericea* which appears to be more responsive to rainfall onset, as is seen in the neighbouring grasses. The broad-leaved savanna in the NNR is a nutrient-poor environment; the slower response by *B. africana* to different environmental conditions, such as rainfall onset, is mirrored by other species in similarly nutrient-poor environments (Coley et al. 1985; Skarpe 1992; Barbosa et al. 2014).

Another area highlighted by this study is the poor understanding behind the potential cues for the commencement of leaf flush in early-greening broad-leaved savanna trees. In Chapter 6, some of the potential cues were explored, however, no conclusive evidence for one specific cue was determined. There is some indication that early-greening in *B. africana* may be linked to temperature in the absence of an increase in soil moisture post-rainfall, however, Do et al. (2005) indicate that changes in atmospheric vapour pressure deficit could also act as a cue.

3. Synthesising the findings of this study

To better understand the complex dynamics associated with the early-greening strategy in savannas, a few simple models have been developed using the data collected from the different elements of this study to illustrate the interactions between leaf phenology and functionality, as well as the impacts of herbivory on the amount of carbon gained by the different tree species over the course of a growing season.

A model with three leaf age classes was created and each leaf age class was assigned a mean photosynthetic rate as recorded in the field data (Table 1, *Chapter 4*).

Table 1. The mean photosynthetic rates assigned to each leaf age class for each species.

Species	New ($g\ C\ m^{-2}\ hr^{-1}$)	Fully expanded ($g\ C\ m^{-2}\ hr^{-1}$)	Mature ($g\ C\ m^{-2}\ hr^{-1}$)
Burkea africana	0	1.22	1.58
Terminalia sericea	0	1.63	1.97

A mature canopy leaf area index (LAI) was assigned to each species to represent the area of canopy covered with leaves during the peak growing season. The daily leaf area of each age class was calculated using the following equation (eqn 1):

$$\boldsymbol{LeafArea_{ijk} = LAI_i * Fullness_{ijk}}$$

Where LAI_i is the species specific LAI calculated from Rutherford (1979) and *Fullness* is the species specific daily (*k*) proportion of canopy fullness of each (*j*) leaf age class of each (*i*) species. This was extrapolated from average weekly and monthly measurements of phenology taken at the site for each species over three seasons (*Chapter 2*).

Daily photosynthesis in micromole/m^2/day was calculated using equation 2 (eqn 2):

$$\boldsymbol{DailyPhotos_{ijk} = LeafArea_{ijk} * PhotosRate_{ij} * 3 * 60 * 60}$$

Where *PhotosRate* is the mean photosynthetic rate measured for each leaf age class (*j*) for each species (*i*) in micromole/m^2/s. I estimated that photosynthesis only occurs for approximately three hours a day (based on diurnal data taken at the site where positive carbon gain occurred from ~7am to ~10am) , while 60 * 60 is to account for the number of seconds in a minute and minutes in an hour.

To estimate full season carbon gain, the sum of daily photosynthesis was calculated for each species; *B. africana* had an estimated total of 1630 g C m^{-2} and *T. sericea* had an estimated total of 1870 g C m^{-2} (Figure 4). To take this model forward, losses due to leaf respiration will need to be incorporated; however, at the time of submission these data had not yet been included.

Respiration losses are not the only factor that affects carbon gain; in order to account for the damaged tissue caused by invertebrate herbivory, an herbivory losses term was incorporated into the carbon gain equation as follows (eqn 3):

$$\boldsymbol{CarbonGain_{ijk} = DailyPhotos_{ijk} - (LeafArea_{ijk} * Herbivory_i * \frac{PhotosMax_i}{2})}$$

Where $Herbivory_i$ is the proportion of total canopy that was damaged by invertebrate herbivory for each (*i*) species and *PhotosMax* is the rate of mature leaf photosynthesis for each (*i*) species (Table 1). The reason behind the halving of the maximum photosynthetic rates is to account for the decreased photosynthetic capacity of the damaged leaves as explained by Nabity et al. (2009).

After including the herbivory losses into the model, total carbon gain was 1270 g C m^{-2} and 1220 g C m^{-2} for *B. africana* and *T. sericea* respectively (Figure 4).

It is important to highlight that the number of days taken by each species to commence a positive carbon balance was slower than the rise in canopy fullness estimates. The majority of global carbon balance models currently rely on increases in greenness or leaf area indices to infer positive carbon gain, however, these models fail to account for the lag in canopy functionality as demonstrated in the above model (Figure 4).

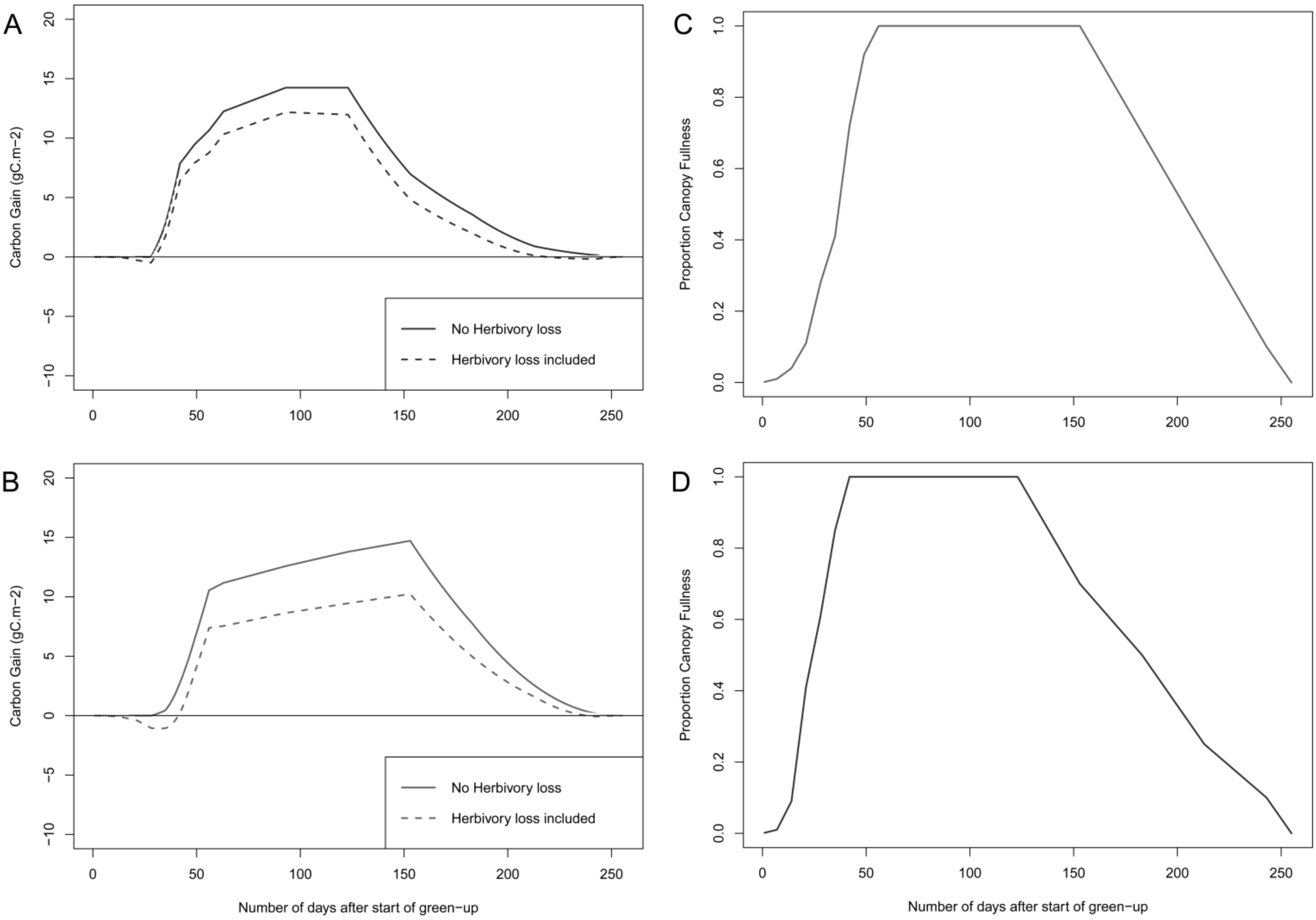

Figure 4. The daily carbon gained by (A) *Burkea africana* and (B) *Terminalia sericea* over the course of an average growing season in relation to the presence or absence of invertebrate herbivory damage, as well as the proportion of canopy fullness in relation to the number of days after the start of green-up for (C) *B. africana* and (D) *T. sericea*.

The next aspect of the carbon modelling was to assess whether early-greening *B. africana* experienced an advantageous period of growth ahead of the later-greening *T. sericea*. Using the findings made in Chapters 2, 3, 4 and 6, outputs from the carbon gain formulas above where used to investigate whether a two week early-greening response by *B.*

africana (as observed in Chapter 6) would be a sufficient period to enable carbon gain to take place without competition from neighbouring *T. sericea*. Based on two rainfall start date scenarios: (A) a late-onset rainfall year and (B) an early-onset rainfall year, *B. africana* green-up response was made to start (A) 14 days prior to the onset of rainfall and (B) at the same time as the onset of rainfall (Figure 5). During the late-onset rainfall scenario, the early green-up of *B. africana* results in several days of competition free carbon gain over *T. sericea* whose green-up was made to start after the onset of rainfall, however, in the second scenario where rainfall arrives early, both species are competing during the same period (Figure 5).

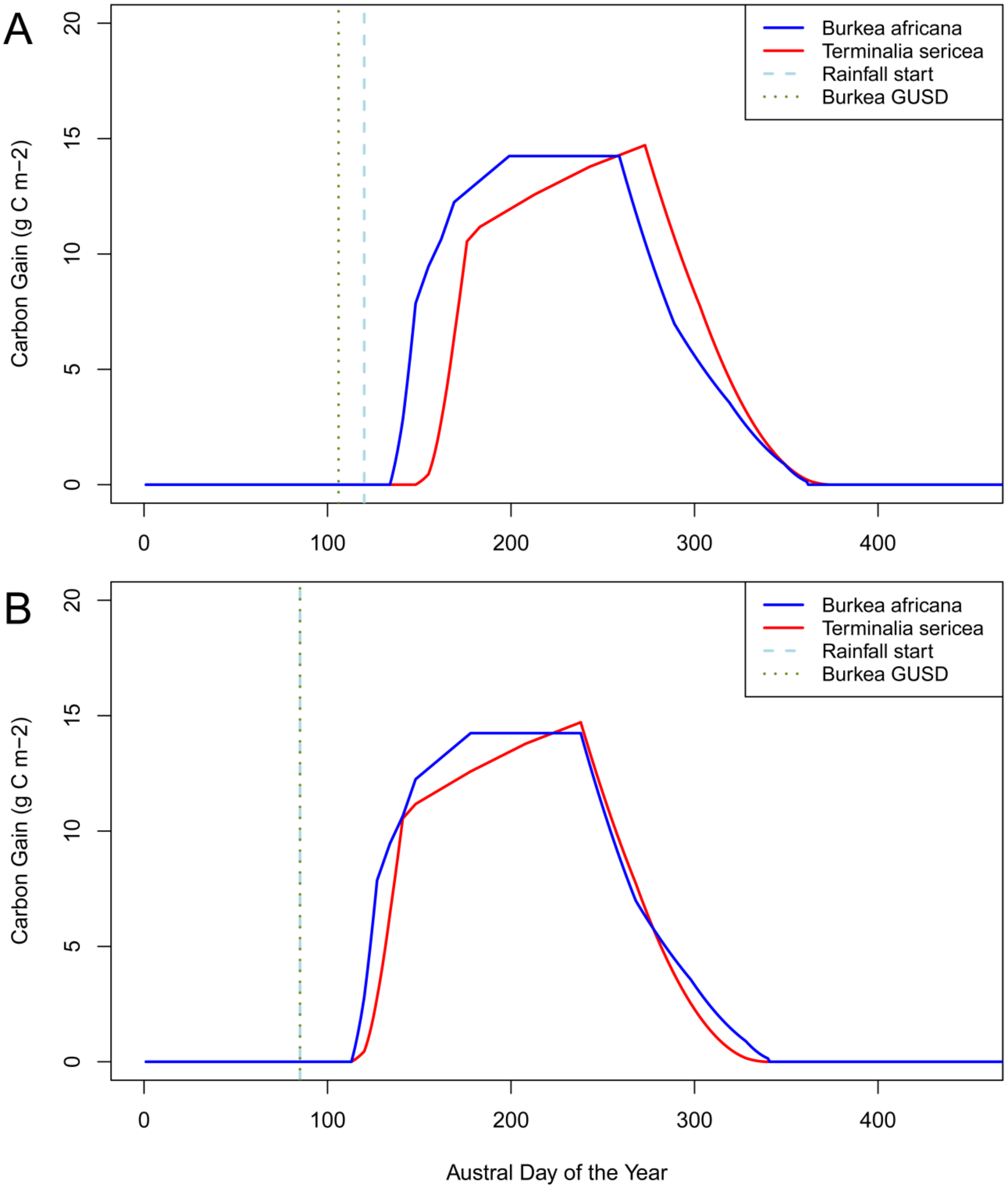

Figure 5. The carbon gain response of *Burkea africana* and *Terminalia sericea* in relation to a (A) late-onset rainfall season and an (B) early-onset rainfall season in the Nylsvley Nature Reserve. Austral days are used where the 1st July is day 1. The dotted green line indicates the green-up start date (GUSD) of *B. africana* which overlaps with the rainfall start date in scenario (B). The GUSD for *T. sericea* occurs at the start of the rainfall in both scenarios.

4. Integration of ecological findings and additional insights

Prior to this study, no previous studies that I know of had investigated or quantified the frequency of early-greening events in any of southern Africa's savannas. Chapter 6 has addressed this knowledge gap by using remote sensing to detect early-greening events in a range of broad-leaved woodlands where *B. africana* dominates. The work conducted in Chapter 6 was made possible by the incorporation of phenological field surveys of the trees and grasses together with MODIS NDVI imagery over the same period (*Chapter 3*). Building on the foundations established by this work, assessing the occurrence of early-greening events in other savanna systems across southern Africa would improve the understanding of just how frequently this phenological phenomenon is occurring. Early-greening has been observed in savannas across a range of continents including Australia (Williams et al., 1997; Prior et al., 2004), Asia (Murali and Sukumar, 1993; Elliott et al., 2006), South America (Borchert, 1994; Rivera et al., 2002) and Africa (Chidumayo, 2001; Archibald and Scholes, 2007; February and Higgins, 2016). The prevalence of this phenological phenomenon would suggest that species employing this strategy are gaining some advantage(s) over their neighbouring flora (Childes, 1989; Reich and Borchert, 1984; Borchert, 1994; Prior et al., 2004; Hoffman et al. 2005; Kushwaha and Singh 2005; Elliott et al., 2006). This study has confirmed some of the potential advantages which early-greeners are gaining, including the potential for an extended growing season (*Chapters 2, 3, & 4*) and the avoidance of invertebrate herbivory on new leaves (*Chapter 5*).

One of the major outcomes from this study was the quantification of variability in both environmental conditions (such as rainfall onset dates between seasons) and variability in the responding phenology because of the unpredictable environmental conditions on both temporal and spatial scales (*Chapters 2, 3 & 5*). The fine-scale, weekly assessments of phenology offered clarity into the varying green-up start dates and green-up rates of individual trees and between the different species (*Chapters 2 and 3*). Incorporating this level of variance into effective and representative models will undoubtedly be a challenge but one that could be overcome through well designed long-term phenological monitoring schemes across a range of individuals, from different species, in different savannas around southern Africa. As stated by Chambers et al. (2013), there is a notable lack in phenological data for Africa and this gap needs to be filled in order for environmental managers, conservationists and policy-makers to undertake better informed decisions about the environment in the future.

Another important indirect outcome from this study is the importance of obtaining multiple seasons' worth of phenological data when trying to determine pheno-types for different tree species within African savannas. If I consider the first season of this study (2012-13), the two tree species would have both been considered late-greeners, only producing their leaves after the early onset of rainfall; whereas in the third season (2014-15) the late onset of rainfall resulted in a clear early-greening response by *Burkea africana* and a smaller early-greening response from *Terminalia sericea*. The high variability in environmental conditions between years resulted in high variability in the tree phenology observed, thus in order to achieve an accurate assessment of which pheno-type a species fits into, numerous environmental scenarios and seasons will need to be observed. Classifications of phenotypes have been conducted in Australia (Williams et al., 1997) and Asia (Elliott et al., 2006), however, a conclusive quantification of Africa's savanna tree phenotypes is yet to be conducted.

February and Higgins (2016) recently investigated the mechanisms behind savanna trees' (including *T. sericea*) ability to rapidly deploy their leaves at the start of the growing season. Their study indicates that stored reserves of nitrogen and carbon are being utilized to produce new leaves at the start of the growing season when conditions for growth become suitable, i.e. just before or with the first precipitation event (February and Higgins, 2016). In Chapter 4 the lack of leaf functionality in the first two weeks of growth complements the theory of stored carbon, as the new leaves are considered a carbon sink, rather than a source during this initial period of growth. February and Higgins (2016) provide a clear mechanism for the use of stored carbon reserves to commence the expansion and subsequently carbon gain of new leaves during the period when access to carbon is limited because of the lack of a functional canopy.

As discussed in Chapter 1, little is known about the risks of early-greening for savanna trees, however, the impacts of the 2013 fire on the phenology fo the irrigated *T. sericea* illustrated the potential impacts of fire on early-greening trees. It would be benefitial to explore the impacts of fire on early-greeners in a more controlled experiment in the future to quantify these impacts in a less descriptive manner.

5. Achievements and uniqueness of this study

This study has advanced the understanding of broad-leaved savanna tree phenology as follows:

- The weekly-scale monitoring of broad-leaved savanna tree and grass phenology over three consecutive growing seasons provides the most thorough assessment of savanna leaf phenology published to date in an African savanna.
- This was the first study to address the impact that a late season fire in conjunction with an irrigation experiment may have on tree and grass phenology.
- This study was the first to use ground-based phenological measures in conjunction with remote-sensing to develop a protocol for the detection of early-greening events in broad-leaved savannas across southern Africa.
- This is the first study to quantify the frequency of early-greening years across some of southern Africa's broad-leaved savannas.
- This study was the first to assess leaf functionality from the start of flush throughout the growing season in *B. africana* and *T. sericea*.
- This was the first study to compare the rates of leaf- to canopy-scale herbivory damage in relation to tree phenology in an African savanna.

6. Future research recommendations

This study has laid the groundwork and highlighted the need for more phenologically related research to be conducted in southern Africa's savannas. Only two of the hypothesised benefits were thoroughly tested during this study and there is room to investigate the other theorised benefits in the future. I have been a co-supervisor on an Honours project during 2016 (likely to be expanded into an MSc in 2017) which is addressing the question of whether early-greening trees have new leaves which are more susceptible to nutrient leaching by rainfall prior to the development of their waxy cuticle (Sarmiento et al., 1985). Further investigations into the functionality of new leaves which are produced prior to the onset of seasonal rainfall would assist in clarifying this study's claims that new leaves lack functionality during the first two weeks of growth (*Chapter 4*). This thesis has largely ignored the costs associated with the early-greening strategy, despite discussing them in Chapter 1 and this area of research will certainly require more attention in the future. Some of the important costs to assess is whether new leaves that flushed prior to the onset of rainfall are experiencing increased levels of heat stress and water loss, as well as whether these leaves are exposed to significant herbivory losses through mammalian herbivory.

This study focused on two of the dominant broad-leaved savanna trees in the Nylsvley Nature Reserve, however, a broader assessment of other savanna species across a range of both broad- and fine-leaved trees would assist in better quantifying the pheno-types which exist within southern Africa's savannas. This assessment would need to be conducted over multiple seasons in order to understand the dominant strategy used by each species under different seasonal conditions. The development of a 'Southern African Phenology Network' (SAPheN) which could be deployed across a range of ecosystems, including savannas, throughout southern Africa using phenocams and citizen scientist contributions would assist in better understanding the changes in phenology within southern Africa in the present and the future. These data can be fed back into vegetation models to improve the accuracy of these models in these highly complex African ecosystems. Kosmala et al. (2016) have indicated how successful the Season Spotter program has been in the United States of America, where citizen scientists classify near-surface remotely sensed images to determine phenophases across a range of scales from individual plants to landscapes. A similar program could easily be developed for South Africa where the engagement of citizen scientists in other areas such as bird monitoring has already proved to be hugely successful (Underhill and Brooks, 2016).

Understanding phenological strategies is also important for the development of accurate carbon cycling models. If one considers the rate at which *T. sericea* is turning over its canopy, the amount of carbon stored within the leaves that it is constantly dropping is much higher in comparison to *B. africana* which maintains the same set of leaves over the season. The carbon gain through *T. sericea*'s higher levels of photosynthesis will also influence the estimates in these models. Greater collaboration with field scientists', whose observations will be vital in further developing the usefulness and accuracy of these models in the future, needs to be supported.

Chapter 6 provided a basic assessment of some of the potential environmental cues that could be responsible for the onset of leaf flush of early-greening trees; however, this area of research requires much more investigation. The roles of temperature and relative humidity in cueing leaf flush needs to be further explored through field, laboratory and modelling techniques. It is my personal theory that either temperature or relative humidity (or potentially an interaction between them) is responsible for driving the onset of savanna tree leaf flush in the absence of rainfall.

7. Conclusion

The major aim of this study was to investigate whether early-greening was beneficial for broad-leaved savanna trees with regard to (1) an extension of the growing season beyond that of herbaceous and late-greening neighbours and (2) an avoidance of invertebrate herbivory on new leaves prior to the onset of seasonal rainfall. This study has shown that trees which are able to utilise an early-greening strategy are able to disconnect the onset of leaf flush from water availability and gain beneficial physiological advantages, while avoiding the threat of herbivory on new leaves. This study has also highlighted that the early-greening strategy cannot be utilised during every growing season; however, under certain environmental conditions (i.e. the late onset of seasonal rainfall); the benefits to the trees are notable. This study has also shown that the high variability in savanna phenology is largely driven by the high temporal variability associated with the unpredictable seasonal rainfall in southern Africa. One of the major outcomes from this research is the highlighting of the importance of and need for long-term, fine-scale phenological research in savannas across southern Africa and the potential to link this research into citizen science initiatives and global modelling scenarios.

8. References

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