Effects of warming on the early establishment of an African savanna tree

By

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Declaration

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Abstract

This study investigates the interactive effects of atmospheric warming and the presence of a C4 grass sward on the growth and mortality of establishing savanna tree seedlings (*Senegalia nigrescens (Oliv.)* P. J. H. Hurter). Atmospheric warming is one of the major drivers of global vegetation change, but has been little studied in tropical African systems. In cool temperature systems of the mid- to high – latitudes, where plant growth is predominantly controlled by climatic factors, warming has been shown to drive an increase in plant growth and establishment. In systems of the mid – to low – latitudes, such as sub-tropical savannas, the potential role of warming is not well known, and the vegetation structure and functioning of these systems is controlled by the interacting impacts of fire, herbivory and climate. Wildfire and herbivory limit the seedling and sapling demographic stages of savanna trees. This stage is expected to be most vulnerable to warming because seedlings are sensitive to fluctuations in temperature and are highly dependent on water availability, a resource that is competed for strongly by C4 grasses. Furthermore, C4 grasses are unlikely to be as adversely affected by warming as C3 seedlings, due to differences in their photosynthetic pathways. Based on these arguments, this study tests the main hypothesis that there is an adverse interactive effect of warming and grass competition on tree seedling establishment in savannas.

In order to test this hypothesis, I carried out a field experiment at Wits Rural Facility in Limpopo Province, South Africa during the 2017/18 growing season. Using passive open-topped, polycarbonate warming chambers, seedlings were warmed on average, by 1-2°C. Soil and plant water content were unaffected by warming but the presence of grass significantly reduced the relative water content of the leaves of establishing seedlings, suggesting competition for water between the different growth forms. Seedling growth rate was unaffected by warming when grown without C4 grasses, but a significant decline was shown by those grown with grasses above a daytime temperature threshold of 30°C. Likewise, seedlings grown with grasses suffered a 65% reduction in survivorship when warmed but those grown in the absence of grass suffered only a 15% reduction in survivorship.

The results of this study therefore show that warming and the presence of grasses had an adverse additive effect on seedling survivorship, through which warming enhanced the dehydrating effect of competing grass on establishing seedlings, thus confirming the primary hypothesis. I propose that the cumulative stresses of carbon imbalance due to warming and grass competition for soil water drove this decline in tree seedling growth, resulting in higher seedling mortality with the implication of reduced successful establishment events under warmer conditions.

This study makes an important contribution to understanding the impact of warming on African savanna species, in that it suggests a future decline in tree establishment under warmer conditions.

To extrapolate these findings, a greater focus on understanding the impacts of warming on a range of savanna plant functional groups across the rainfall gradient, with other global change drivers, is required.

Opsomming

Hierdie studie het die effek van verhoogde temperatuur in die atmosfeer en die teenwoordigheid van 'n C4-gras op die groei en mortaliteit van 'n savanna-boomsaailing (*Senegalia nigrescens (Oliv.)* P. J. H. Hurter) ondersoek. Die temperatuur van die atmosfeer is een van die belangrikste drywers van plantegroei, maar die effek daarvan is selde in tropiese Afrika stelsels ondersoek. Binne klimaat stelses gevind in die middel- tot hoë breedtegrade, waar plantegroei hoofsaaklik deur die klimaat beheer word, is daar 'n direkte vehouding tussen verhoogte temperature en plantegroei sowel as die vestiging van nuwe saailinge. In klimaat stelsels gevind in die middel-tot lae breedtegrade, waar sub-tropiese savanna gevind word, is die effek van temperatuur nog nie duidelik nie. Dit word vermoed dat daar binne hierdie stelses 'n wisselwerking tussen vuur, oorbeweiding en klimaatsverandering die grootste drywer is vir plantegroei. Vorige studies het gevind dat veld brande en oorbeweiding die saadvorming en vestinging van savanna bome grootliks beinvloed. Hierdie stadium in die plant siklus is dus die mees kwesbaarste teen veranderinge in temperature. Saailinge is baie sensitief vir veranderinge in temperature en is baie afhanklik van die teenwoordigheid van water. Water is 'n natuurlike bron waar strek mededinging tussen saailinge en C4-grasse plaasvind. As gevolg van verskille in hulle fotosintetiese fisiologie, sal C4-grasse nie so negatief beïnvloed word deur herhoogde atmosferiese temperature soos C3-plante nie. Hierdie studie het dus die hipotese getoets dat daar 'n nadelige interaksie is tussen verhoogde atmosferiese temperature en kompetiese van gras op die vestiging van saailinge.

Alle veldwerk was gedoen tydens die 2017 en 2018 groei seisoen by die Wits Rural Facility in die Limpopo Provinsie, Suid-Afrika. Saailinge was in die veld tussen 1-2° C passief verhit, deur polycarbonate strukture rondom plante in te stel. Die grond- en plant-water inhoud was nie deur die verhitting beïnvloed nie, alhoewel die teenwoordigheid van gras wel die relatiewe water inhoud van saailinge se blare aansienlik verminder het. Die vestiging van saailinge was darom aansienlik verlaag, wat 'n goeie indikasie is van die kompitiese tussen die verskillende groeivorme. Die groeitempo van saailinge was nie deur verhitting beïnvloed nie, indien die saailing in die afwesigheid van C4-grasse gevestig was. Daar was 'n groot afname in die groeitempo van saailinge wat moes kompeteer teen grasse bo daaglikse temperature van 30° C. Saailinge wat gevestig het in hoë temperature het 'n 60% afname in oorlewing getoon, terwyl saailinge in die afwesigheid van grasses slegs 'n 15% afname gewys het. Die resultate van die studie het dus aangedui dat verhitting en die teenwoordigheid van grasse 'n additiewe nadelige uitwerking op die oorlewing van saailinge gehad het. Dit kan dus afgelei word dat die druk van 'n koolstof wanbalans as gevolg van verhitting, en die kompetisie tussen groeivorme vir groundwater, altesaam 'n afname in die groei van saailinge gehad het. Dit sal moontlik veroorsaak dat hoër saailing sterftes met voorspelde verhitting sal lui tot verlaagde saailing vestiging

in warmer toestande. Hierdie studie help om die impak van voorspelde verhitting op Afrika-savanna spesies te verstaan. Om die breër gevolge van aardsverwarming te verstaan, moet toekomstige werk fokus op hoe verskeie savanna funksionele plant groepe deur die komponente van globale verandering, insluitend reënval en temperatuur, beinvloed word.

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Abbreviations

Abbreviations are ordered in which they appear in the thesis.

IPCC	Intergovernmental Panel on Climate Change
POTC	Passive Open-Topped Chamber
MAP	Mean Annual Precipitation
ITEX	International Tundra Experiment
RWC	Relative Water Content
WG	Warming treatment with grass
WNG	Warming treatment without grass
CG	Control treatment with grass
CNG	Control treatment without grass
RH	Relative Humidity
GLMM	General Linear Mixed Model
PQL	Penalized Quasi-likelihood
AIC	Akaike's Information Criterion
Temp.	Temperature
ΔT	Difference in temperature
RGR_L	Relative Growth Rate (of length)

1. Chapter 1: Relevance of the study, study species, site description and warming method

1.1 General Introduction

Global climatic conditions are changing. Atmospheric carbon dioxide has risen rapidly over the past century, causing terrestrial and oceanic warming and changing precipitation patterns (IPCC, 2014). A warming climate is a prominent feature of global change, with reports of increased temperatures documented worldwide. Between 2030 and 2052, global warming is expected to reach 1.5°C above pre-industrial levels (IPCC, 2018), increasing climate-related risks to both human and natural systems. The global mean surface temperature change for the period 2016-2035 is likely to be in the range of 0.3°C and 0.7°C (IPCC, 2014) yet the subtropics of Africa are warming at twice this rate (Engelbrecht et al., 2015).

A warming climate will have significant ecological effects on terrestrial vegetation (Yin *et al*., 2008) as plant function is largely dependent on water and temperature (Beier *et al*., 2004), with both the extremes of high and low temperatures limiting plant photosynthetic ability. Plants in ecosystems in the temperature – limited mid- to high – latitudes (such as the tundra) have experienced an increase in growth rate as a consequence of warming (Usami *et al.,* 2001; Bronson *et al*., 2009; Zhao and Lui, 2009; Munier *et al.,* 2010; Henry *et al*., 2012). Increased growth rates coupled with longer growing periods (Beier *et al.,* 2004; Wilson and Nilsson, 2009), is driving an increase in woody biomass in these systems (Rustad *et al.,* 2001; Walker *et al*., 2005; Milbau *et al*., 2009; Lin *et al.,* 2010; Myers-Smith *et al*., 2011). However, some warming studies conducted in the Northern Hemisphere (Wu *et al.,* 2011) have recorded a decline in plant growth (Wada *et al*., 2002) and species diversity (Klein *et al*., 2004) where reductions in biomass have been recorded in both above- (Olszyk *et al.,* 2003; Skre *et al.,* 2008) and belowground growth (Way and Sage, 2008). Different responses of woody plants to warming can be accounted for by variation in growth forms (tree/shrub), warming magnitudes and site-specific environmental conditions (Lin *et al.,* 2010; Elmendorf *et al.,* 2012), consequently influencing community structure, vegetation dynamics and ecosystem functioning in different ways.

Although only a few, warming studies conducted in the mid- and low – latitudes of the Southern Hemisphere have also shown variation in the response of woody plants to warming. In Tasmania, Australia warming increased plant growth and allocation of biomass to leaf tissue (Hovenden, 2001), yet it also increased mortality of plants by reducing soil water (Hovenden *et al.,* 2008). In addition, an increase in drier conditions resulting from warming, is predicted to increase the severity and frequency of wildfires (Westerling *et al.,* 2006; Williams *et al.,* 2013), driving a reduction in seedling regeneration (Tercero-Bucardo *et al.,* 2007). It is widely suggested that plants in these warmer

systems are more susceptible to desiccation by warming, predominantly as a result of drier conditions (Shaver *et al.,* 2000; Penuelas *et al.,* 2004), leading to predictions that warming may cause extinctions (Lloret *et al.,* 2004), resulting from range retractions and migrations of plants in these systems (Kelly and Goulden, 2008). However, more research is required within these warm systems to better understand how plants respond to warming, and how their response varies between and within ecosystems.

Although numerous experimental warming studies have been conducted in the tundra (Walker *et al.,* 2006; Rustad *et al.,* 2001), temperate forests and grasslands (Hovenden *et al.,* 2008) and alpine systems (Yin *et al.,* 2008; Wilson and Nilsson, 2009), little experimentation has been done in the (sub) tropical grassy ecosystems of the southern hemisphere, specifically African savannas. These systems are not purely climate – limited, but are structured by interactions between fire, climate and herbivory (Bond *et al.,* 2005; Sankaran *et al.,* 2005; Lehmann *et al.,* 2014). Additionally, water availability, not temperature is the primary limiting climatic variable (Sankaran *et al*., 2005 Scheiter and Higgins, 2009; Vadigi and Ward, 2013) and consequently most climate studies on these systems are centered on the role of water on plant growth and demography. Savannas in southern Africa fall within a climate change hotspot (Diffenbaugh and Giorgi, 2012), where warming is happening at double the global rate (Engelbrecht *et al.,* 2015) with this trend projected to continue. The future trends in warming are likely to be characterized by an increase in the number of extreme hot days (Engelbrecht, 2015). Given this, we are faced with a lack of experimental research investigating how savanna plant communities will respond to these changes into the future.

Savanna systems cover ~ 13.5 million km^2 of sub Saharan Africa (Riggio *et al*., 2013). They consist of two co-dominant plant forms, C4 grasses and woody plants, a coexistence that is determined by the interaction between disturbance regimes and water availability (Scholes and Archer, 1997; Bond, 2008; Lehmann *et al.,* 2014). They exist along a continuum of mean annual rainfall (MAP) from 300mm to 1800mm (Higgins *et al*., 2000; Lehmann *et al.,* 2011). Much of the structure and function of savannas acts through different plant demographic bottlenecks. Tree seedling recruitment acts as a key demographic bottleneck where seedling germination and early establishment are dependent on rainfall, which is highly variable in savannas (Higgins *et al.,* 2000; Jeltsch *et al.,* 2000; Midgley *et al.,* 2010; Stevens *et al.,* 2014a). The transition of woody plants from the juvenile stage into mature trees is a major bottleneck because smaller plants are top-killed by frequent fires (Scholes and Archer 1997; Bond *et al.,* 2003; Hanan *et al.,* 2008) and browsed by herbivores (Midgely *et al.,* 2010; Prior *et al.,* 2010; Bond *et al.,* 2012). Another limiting demographic stage for woody plants is old trees, which are sensitive to fire and disease (Liedloff and Cook, 2007). It is likely that the demographic stage most vulnerable to warming will be the seed germination and early establishment phase (Lloret

et al., 2004). This is because temperature and water (especially precipitation) are key drivers of seed germination (Walck *et al.,* 2011), and seedling establishment is sensitive to diurnal fluctuations in temperature (Kos and Poschlod, 2007) and is highly dependent on water availability (February and Higgins, 2010). Warming – induced declines in soil moisture availability also limits successful seedling establishment (Hovenden *et al*., 2008; Wan *et al*., 2002). Consequently, the focus of this study is on the tree seedling establishment stage where their vulnerability to both direct and indirect effects of warming is most likely to act as a major bottleneck to successful seedling establishment.

Warming can directly increase seedling growth (Stevens *et al.,* 2014a) by increasing the activity of enzymes involved in photosynthesis, which increases both root, and shoot growth rates. However, it can also increase seedling mortality by increasing the rate of dark respiration, where carbon imbalance is induced by less carbon being taken up than released and creating temperatures above their critical limit for photosynthesis and directly damaging cellular components (Sage and Kubien, 2007). Indirectly, warming can reduce the time that water is available for uptake by establishing seedlings by increasing evapotranspiration (Lloret *et al*., 2004; Chidumayo, 2008; Hovenden *et al*., 2008; Grossiord *et al*., 2018). As an early response to water deficiency, plants close their stomata to reduce water loss and by doing so, the reciprocal flow of CO_2 is inhibited and photosynthetic rate decreases. Consequently, the limited manufacture of assimilates and thus energy available for plant growth (Lambers *et al*., 2008) results in a decline in growth rate and successful establishment. Additionally, warming may alter the balance between grasses and woody plants in a savanna system (Volder *et al.,* 2013). With warmer conditions, C4 grasses are expected to photosynthesise at a greater rate than C3 plants (woody plants) due to increased photorespiratory losses from C3 leaves at high temperatures (Atwell *et al.,* 1999). Resulting increases in grass biomass is expected to enhance the competitive effect of grasses on establishing seedlings (Cramer *et al.,* 2012) and increase the chances of fire, leading to the death of seedlings and saplings within the fire trap (Bond, 2008). Warming can also intensify the competition between establishing seedlings and C4 grasses by limiting soil water availability through increased evapotranspiration (Hovenden *et al.,* 2008; Grellier *et al*., 2012; Grossiord *et al*., 2018). In addition to this, warming increases the rate at which grass is cured (Archibald *et al.,* 2013), leading to a higher frequency of fires which further restricts escape of tree saplings from the fire trap. Although not investigated in this study, other global change drivers can interact with, and alter the direct and indirect effects of, warming (Blumenthal *et al.,* 2018). Elevated CO_2 is one such driver that has been found to increase soil water availability through reduced rates of community evapotranspiration (Owensby *et al.,* 1997; Polley *et al.,* 1997; Morgan *et al.,* 2001). This has a counteracting effect to warming on soil water availability and the competitive relationship between trees and C4 grasses (Xu *et al.,* 2013).

Woody savanna plants are adapted to warm temperatures and periods of limited water supply (Stevens *et al.,* 2014a), but the severity of warming predicted and the vulnerability of communities in southern African savannas to such changes, makes understanding the system's response to forecasted global change a necessity in light of their preservation. This study therefore aimed to investigate the response of establishing woody plant seedlings to experimental warming. It aimed to investigate the indirect effect of warming on tree seedling establishment by understanding the role of warming on soil water availability and how this influences the relationship between tree seedlings and C4 grasses. The study also set out to understand the direct effect of warming on tree seedling mortality and growth rates and how this too influences the relationship between tree seedlings and C4 grasses. To test the main hypothesis that there is an interactive, adverse effect of warming and grass competition on tree seedling establishment, questions central to the two data chapters as follows:

Chapter 2

- How did warming by passive open-topped chambers (POTCs) change the ambient and soil temperature and humidity in the surroundings of savanna tree seedlings in the presence and absence of C4 grasses?
- How did warming change the water relations of savanna tree seedlings and C4 grasses in a semi-arid savanna system?

Chapter 3

- How did warming affect the mortality of African savanna seedlings grown in the presence and absence of C4 grasses?
- How did warming affect the growth of African savanna seedlings grown in the presence and absence of C4 grasses?

This thesis is composed two major chapters, each with a theoretical introduction that includes ideas discussed in this introduction, resulting in some repetition. Each chapter includes data collection, analysis and write-up.

1.2   Study Species

The Fabaceae family is the largest and most prominent tree family of southern Africa with it being a dominant family in drier savannas (Osborne *et al.,* 2018). They have pods as fruits, nodulated roots with nitrogen-fixing bacteria and most species are deciduous, losing their leaves during the dry winter
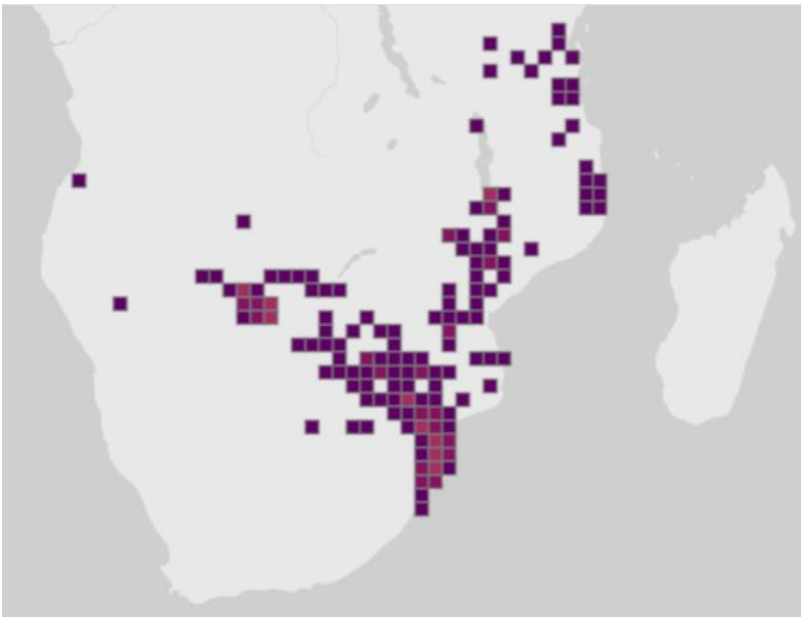
Figure 1.1: Current distribution of Senegalia nigrescens *across southern Africa (GBIF Secretariat 2017).*

Figure 1.2: Young Senegalia nigrescens *trees are browsed by savanna herbivores including African elephants. Photograph taken by the author in the Kruger National Park, South Africa.*

season. *Vachellia* and *Senegalia* were previously known as *Acacia* but have since been split into the two genera and fall within the *Mimosoideae* (thorn tree) subfamily, which is the largest in the region with approximately 40 species (Moll, 2012). They are important components of natural savanna areas and are useful to local people and their domestic animals (Midgley & Bond, 2001). All thorn tree species are spinescent with spines mostly paired and either hooked or straight (with a few exceptions) (Moll, 2012). *Vachellia* and *Senegalia* species exist along a continuum of mean annual rainfall (MAP), some species favouring certain regions and some species showing no preference for particular moisture levels.

Senegalia nigrescens (Oliv.) P. J. H. Hurter was the focal species of this study. This species is common to the area but has a widespread distribution (*Figure 1.1*). It is a semi-arid savanna species that favours the well-drained, rocky areas of the granite lowveld (Coates Palgrave and Coates Palgrave, 2002). It is a deciduous, small to medium-sized tree that has a wide distribution range from KwaZulu-Natal, South Africa northwards to Tanzania. It has thorny, characteristic knobs on its trunk and bears a cylindrical yellowish white flower in spring. The flowers and leaves are browsed by numerous savanna herbivores, especially giraffe, impacting the development and survival of this species (Fornara and Du Toit, 2007).

1.3 Site Description

The experiment took place at Wits Rural Facility, situated on the outskirts of the Kruger National Park, South Africa (~31°23′28E, 24°28′ 29S) (594m ASL). The experiment was conducted within a ~ 100m^2 fenced site located on an old airstrip. Prior to the experiment, the soil was thoroughly loosened and turned on two separate occasions. The first occasion took place approximately one year prior to the experiment, when the entire site was dug up to roughly 20cm using hand implements.

Figure 1.3: Photographs of the site layout at Wits Rural Facility (top left), a warmed plot with grass (top right) a control plot (bottom left) and a Senegalia nigrescens *seedling (bottom right).*

On the second occasion, roughly three months prior to starting the experiment, the site was watered and turned over again using hand implements. The site was then watered on a weekly basis in order to facilitate the establishment of a grass sward.

This site falls within the lowveld bioregion, in the granite lowveld specifically (Mucina and Rutherford, 2006). The area is composed mainly of granite and gneiss that weathers into sandy soils in the uplands and clayey soils in the lowlands. The deep sandy uplands are dominated by tall shrubland (*Combretum hereonse*, *Dichrostachys cinerea*, *Euclea divinorum*) with few trees

(*Senegalia nigrescens, Sclerorya birrea* subsp. *caffra*, *Vachellia nilotica*, *Terminalia sericea* and various *Combretum* species), to moderately dense low woodland. In the bottomlands, dense thickets to open savanna occur. The herbaceous layer is dominated by *Digitaria eriantha*, *Panicum maximum* and *Aristida congesta* in the sandy soils whereas *Sporobolus nitens*, *Urochloa mosambicensis* and *Chloris virgata* dominate the clayey bottomlands. This area experiences characteristically hot, wet summers (October –April) and warm, dry winters (May-September) (mean annual temperature of 20.9°C with a maximum temperature of 38°C in January and a mean minimum temperature of 3.7°C in June/July) with approximately 633mm of rainfall received per annum (Mucina and Rutherford, 2006).

1.4   Warming Method

Passive open-topped chambers (POTCs) were used to create a warming treatment. The ecological effects of POTC - enhanced temperature changes correspond well with natural warming of the climate (Hollister and Webber, 2000). With application on a global scale, especially in the International Tundra Experiment (ITEX), they are the most popular method used to warm low-statured vegetation (Godfree *et al.,* 2011) under realistic field conditions (Yin *et al*., 2008). They are however limited to use in these low-statured plant communities (Godfree *et al.,* 2011). They can raise daytime ambient temperatures by as much as 5°C (Musil *et al.,* 2005). There are potential limitations to the use of POTCs which involve the possibility of the chambers reducing light intensity, relative humidity and/or wind speed within the warmed plots (Kaarlejärvi *et* al., 2012, Godfree *et al*., 2011). These conditions were carefully monitored during the experiment. Chambers were made out of six 1.6mm thick polycarbonate panels, 1.15m long at the base and 0.8m long at the top and approximately 0.6m high (*Figure 1.4-A*) with a total diameter of 6.8m at the base of the chamber and 4.8m at the top of the chamber. At the joining of each panel, cable ties were used to fix a 0.7m long aluminum rod and hold the panels together.
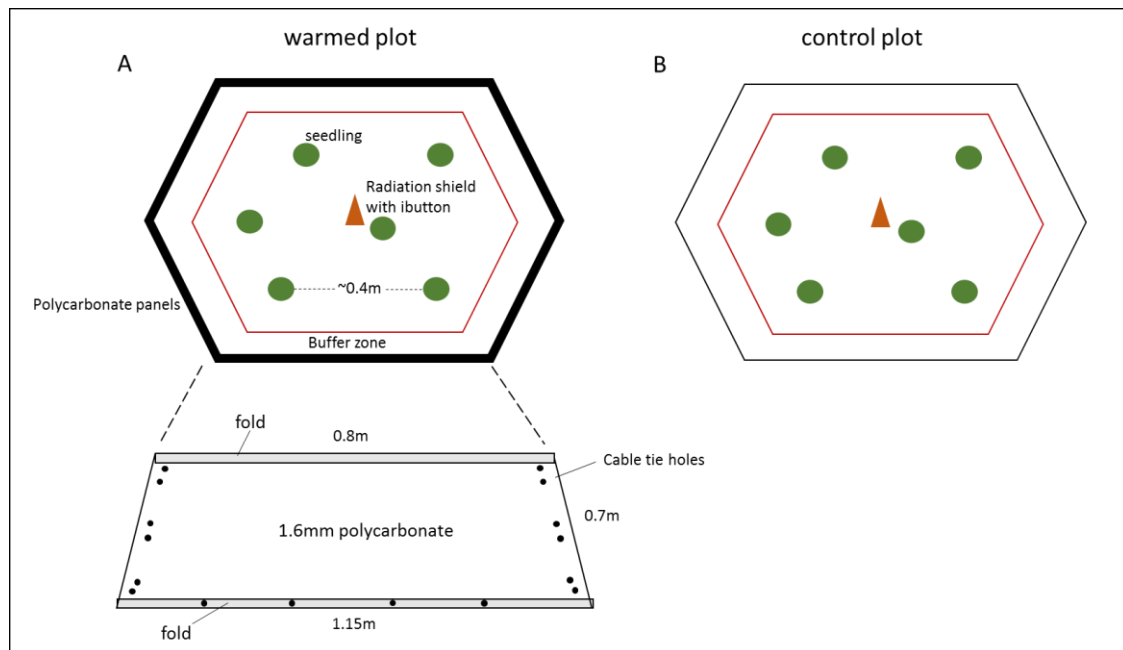
Figure 1.4: A diagrammatic representation of a warmed plot (A) and a control plot (B). Warming was created by passive open-topped chambers (POTCs) made up of six 1.6mm thick polycarbonate panels, each 1.15m long at the base and 0.8m long at the top and approximately 0.6m high. Aluminium rods (0.7m long) were used at the joining of each panel, fixed together by cable ties. Seedlings were planted further than 0.2m away from the edge of the panels. The control plots was marked out with string in the same dimensions of the POTCs.

Seedlings were planted further than 0.2m away from the edge of the panels to create a buffer zone.

1.5   Statistical Analysis

The statistical approach and analyses are described in context in the two data chapters that follow.

2. Chapter 2: The effect of warming on the water relations of grass and establishing tree seedlings in a semi-arid African savanna

2.1 Abstract

Tropical grassy ecosystems in sub-Saharan Africa are warming at twice the global rate. Establishing savanna tree seedlings face competition from C4 grasses for water, nutrients and light. Competition for water is especially strong in arid and semi-arid savanna systems, where plant growth is limited by water availability. Warming effects on establishing seedlings could act directly on their physiology and growth, or indirectly, through the availability of water. Indirect warming effects through increased community evapotranspiration would be expected to increase aridity and reduce seedling growth. To test for such indirect effects of warming on savanna tree seedling establishment, I grew savanna seedlings (*Senegalia nigrescens)* in passive open-topped warming chambers (POTCs) in the presence and absence of grass. I investigated the effect of warming on the water relations of grass and establishing tree seedlings. The POTCs significantly warmed ambient conditions by 1-2°C (data for daily average warming) which is within the range of warming projected to occur this century. Results show that the presence of grass significantly reduced seedling leaf RWC regardless of the warming treatment, but that warming did not change soil water, leaf relative water content (RWC) of tree seedlings or grass growth. These findings therefore do not support a significant role for indirect warming effects acting to suppress tree seedling success via increased community evapotranspiration in this situation. This study confirms that competing grasses reduce the amount of water available to tree seedlings but suggests that the intensity of this competitive relationship will not be affected by atmospheric warming of 1-2°C.

2.2   Introduction

On average, the mean global surface temperature has increased by ~ 0.2°C - 0.3°C over the last 40 years (IPCC, 2014), but there are some hotspots in the subtropics where temperature over land has increased at double this rate (Engelbrecht *et al.,* 2015). One such climate change hotspot is in the semi-arid and arid savannas of southern Africa. In these systems, along with disturbances like fire and herbivory, water availability, rather than temperature, is the main determinant of the structure and function of savannas, shaping the growth of trees and grasses and the interactions between them (Sankaran *et al.,* 2005; Lehmann *et al.,* 2014). Yet given the projected likelihood of further warming, little research exists which investigates how warming will interact with the drivers of savanna structure, functioning, and changing distribution into the future (Lehmann and Parr, 2016).

High temperatures are projected to increase evaporation of surface soil water (Wan *et al*., 2002; Hovenden *et al*., 2008), and several ecological studies already show warming – induced increases in aridity in water-limited systems (Niu *et al.,* 2008; Bowman *et al.,* 2014; Blumenthal *et al*., 2018). Increased soil evaporation reduces the volume of water within the system and it reduces the time it is available to plants (Polley *et al.,* 2013). Warming also increases the rate at which water is lost from plants through transpiration, further reducing water in the system (Quan *et al.,* 2018). Modifications to the amount and seasonal availability of soil water can differentially alter the growth of trees and grasses and the interactions between these two growth forms (Volder *et al.,* 2013).

Much of our knowledge on climate warming effects on plant growth has been centred on temperate systems where alleviation of low temperature limits may directly increase plant growth rates (Nemani *et al.,* 2003; Jolly and Running 2004; Lin *et al.,* 2010). There has not been a strong research focus on the impacts of warming on savanna plant growth, possibly because water availability is seen as the primary structuring climatic component of savannas, thus few studies have investigated the role of warming on plants in warm systems (Lehmann and Parr, 2016). In tropical systems, warming increases evaporation, which mostly affects water in the surface soil layers. Early successful establishment of woody plant seedlings is highly dependent on water availability, (Zavaleta, 2006; Mazzacavallo and Kulmatiski, 2015), especially water in the surface soil layers (Stevens *et al.,* 2014a). Given this, sufficient soil moisture in the top soil layers is a critical bottleneck to savanna tree seed germination and seedling recruitment (Bond, 2008; February *et al.,* 2013).

Changes in soil moisture can also alter the competitive interactions between trees and C4 grasses. There is strong competition for water (Ludwig *et al.,* 2004; Holdo *et al.,* 2015) at the tree seedling stage because grasses are shallow-rooted and have a dense rooting system, which accesses water from the upper soil layers for their growth. The occupation of the same rooting space is an additional barrier

to tree seedling establishment and growth (February and Higgins, 2010). Based on this, I suggest that this competitive interaction is likely to intensify under future warming, in the favour of grasses, which can grow quickly in response to water pulses (Scholes and Walker, 1993), and extract surface water efficiently with their dense root system.

To test for indirect effects of warming via soil water effects on establishing tree seedlings, I used POTCs as a warming method. I planted seedlings of the dominant legume *Senegalia nigrescens* in the absence and presence of grass in warmed and non-warmed plots. I measured how warming altered the ambient humidity, air and soil temperature, and the impacts of warming on the plant-soil water relations of establishing seedlings in the presence and absence of C4 grasses. C4 grasses are highly responsive to warming and have a high temperature optimum for photosynthesis (Bond & Midgley, 2000; Ripley *et al.,* 2010; Lehmann *et al.,* 2014). Consequently, I expected an increase in grass growth in plots that were warmed. I predicted that warming would reduce soil water content as a result of increased evaporation (Wan *et al*., 2002; Hovenden *et al*., 2008) and I expected an increase in ambient air and soil temperatures as both the surrounding air and soil are heated by the chambers. I predicted a reduction in leaf relative water content (RWC) of warmed seedlings because I expected warming to increase evapotranspiration as a result of increased vapour pressure deficit (VPD) (Kirschbaum, 2000). I also predicted a reduction in RWC of seedlings in the presence of grass, because competition with grasses (Ludwig *et al.,* 2004; D'Onofrio *et al.,* 2015) would limit the availability of water for seedlings.

2.3   Methods & Materials

2.3.1 *Study Site*

The experiment was established at Wits Rural Facility in Acornhoek, South Africa (~31°23′28E, 24°28′ 29S) (594m ASL). The site was located on granitic soils of the lowveld bioregion (Mucina and Rutherford, 2006). This area has wet, hot summers (October-April) and cool, dry winters (May-September) with an average 633mm of rainfall received per year. The area is composed mainly of granite and gneiss that weathers into sandy soils in the uplands and clayey soils in the lowlands. The deep sandy uplands are dominated by tall shrubland (*Combretum hereonse*, *Dichrostachys cinerea*, *Euclea divinorum*) with few trees (*Senegalia nigrescens*, *Sclerocarya birrea* subsp. *caffra*, *Vachellia nilotica*, *Terminalia sericea* and various *Combretum* species), to moderately dense low woodland. In the bottomlands, dense thickets to open savanna occur. The herbaceous layer is dominated by *Digitaria eriantha*, *Panicum maximum* and *Aristida congesta* in the sandy soils whereas *Sporobolus nitens*, *Urochloa mosambicensis* and *Chloris virgata* dominate the clayey bottomlands. Plots were positioned on a long-abandoned airstrip on which grasses had established, in a ~100m^2 fenced area. Prior to planting, the soil was loosened and turned manually using picks and hoes. POTCs were used as the warming method.

2.3.2 *Experimental Set-up*

I used a full factorial design with warming and grass presence as the two primary axes of treatment. The treatments were warming with grass (WG), warming without grass (WNG), control/not-warmed with grass (CG) and the designated control was control/not-warmed without grass (CNG). Each treatment was replicated five times. POTCs were made up of six polycarbonate panels based on the design for the International Tundra Experiment (ITEX) (Henry and Molau 1997; Hollister *et al.,* 2015) and have been recently trialled in the South African Nama Karoo (Edwardes, 2018) (Chapter 1, *Figure 1.24*). In each replicate plot, I planted eight *Senegalia nigrescens* seedlings. The seedlings were germinated on-site and were randomly allocated to the plots at the start of the growing season (November 2017). Temperature (ambient air and soil), humidity and soil moisture in the chambers were monitored and leaf RWC of the seedlings was measured once a month. Final grass biomass was recorded at the end of the experiment. Rainfall data for the region was supplied by the local weather station.
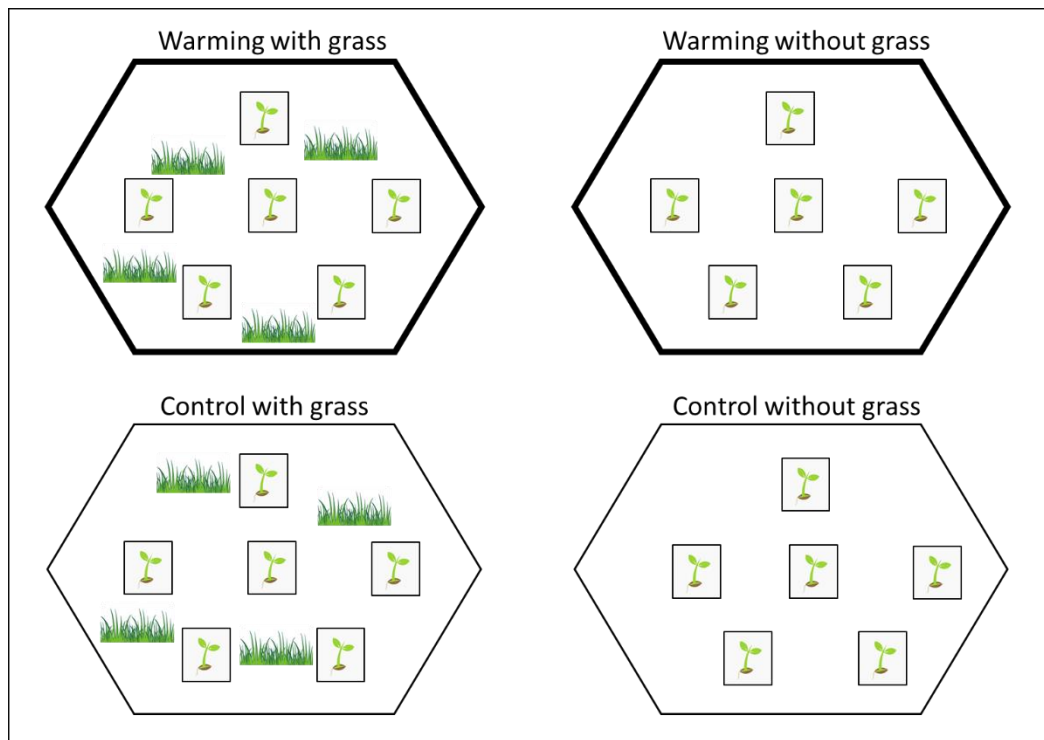
Figure 2.1: Each replicate consisted for 4 treatment plots, warmed plots with one containing grass (a) and one containing no grass (b). Non-warmed plots also contained a treatment with grass (c) and a treatment without grass (d). Each treatment was replicated 5 times.

Ambient air temperature was measured in three randomly selected replicates of each treatment. Within these 12 plots, a plastic radiation shield was placed at the plot's center. DS1923-F5# Hygrochron temperature and humidity loggers (Maxim Integrated, San Jose, California, USA) were placed inside the shield, approximately 20cm above the ground. In the same plots, a waterproofed temperature logger (DS1922) was placed 2cm below the soil surface. All ibuttons logged every 15 minutes to 0.5°C and 0.6% RH resolution for temperature and humidity respectively. Volumetric soil moisture (m^3/m^3) was recorded using four soil moisture probes (EC-5 Soil Moisture Sensor, Decagon Devices Inc, Pullman, WA, USA) placed in the top 5cm of soil. One probe was placed in each treatment. These data were supplemented by multiple handheld soil moisture readings taken at monthly intervals, comprising 15 recordings taken in each plot using a Decagon GS1 volumetric sensor (Decagon Devices Inc, Pullman, WA, USA) connected to a handheld reader (Decagon Procheck). The soil moisture probe extended approximately 5cm into the soil.

Two months after replanting the seedlings in the plots, I started recording leaf relative water content (RWC), for each seedling every month. RWC gives a measurement of the 'water deficit' of the leaf and may indicate a degree of stress expressed under drought and heat stress (Yamasaki and Dillenberg, 1999). Two leaflets were picked from two individuals within each plot (eight readings of each treatment) and their fresh mass was recorded. Leaves were taken back to the lab and rehydrated in de-ionised water for four hours. Their saturated mass was recorded before being placed in a drying

oven for 12 hours at 80°C. Their dry mass was recorded and the following equation was used to calculate relative water content (RWC).

$$RWC(\%) = \frac{[F - D]}{[S - D]} X 100$$

Where F is fresh mass, D is dry mass and S is saturated mass.

At termination of the experiment, grasses from plots were harvested and their biomass was recorded.

2.3.3 *Statistical Analyses*

All graphics and statistical analyses were carried out using R (version 3.5.0) statistical software (R Development Core Team, 2016). To determine how ambient temperature varied between treatments, I calculated the mean daytime temperature for each month (October 2017-May 2018) for every treatment. The treatment with the lowest mean daytime temperature was used as a baseline treatment for each month and variation of the other treatments from each month's figure of this treatment was calculated as the change in temperature. In other words, the lowest figure of all treatments and months was set as 'zero'. The mean daytime soil temperature was calculated for each treatment and the same process of taking a baseline treatment and using its figure for each month to calculate variation in soil temperature between treatments. This procedure was also used to calculate the change in humidity between treatments except averaging was done over a one hour period (1300 – 1400hrs) to account for the large day – night variation of humidity.

To determine the effect of warming (yes/no), the presence of grass (yes/no) and the interaction of these two variables on mean ambient temperature, mean soil temperature and mean humidity, a general linear mixed model (GLMM) was used, with the month being accounted for as a random factor. Non-parametric methods were used to account for the unequal distribution of ambient and soil temperature and humidity. Although often these variables are normally distributed, this is not always the case (Harmel *et al.,* 2002) and these findings prove that. The penalized quasi-likelihood (PQL) method was used for all three models with both ambient and soil temperature fitting a gamma distribution and humidity fitting a log-normal distribution. PQL is a flexible technique that can deal with non-parametric data, unbalanced designs and crossed random effects (McCulloch, 1995).

A GLMM was used to determine the effect of warming, grass presence, sand percentage and the interaction of warming with grass on soil moisture. The soil moisture data fitted a log-normal distribution and the penalized quasi-likelihood (PQL) method was used in the GLMM. Fixed variables included warming (yes/no), grass presence (yes/no), percentage sand within the plot and the

interaction of warming with grass. The plot and date were included in the model as random variables, accounting for variation between day of measurement and plot.

Model selection (MuMIN package) (Barton and Barton, 2018) using GLMMs was used to determine the best-fit models to explain leaf relative water content (RWC) of *S. nigrescens* seedlings. Model averaging of the best-fit models was then used to determine the best predictors of leaf RWC. Akaike's Information Criterion (AIC) was used for model selection. To compare different models, the difference (Δ_i) between the AIC value of the best model and the AIC value for each of the other models was calculated. Model averaging was carried out when Δ_i (delta) < 2. The natural average method was used to compute the model averaged parameters where the parameter estimate for each predictor was averaged only over the models in which that predictor was present and where it was weighted by the summed weights of these models (Grueber *et al.,* 2011). Model averaging consisted of four fixed variables and two random variables. To test for a treatment effect, warming (yes/no), grass presence (yes/no) and their interaction were averaged and held constant. Soil moisture and sand percentage were also included since water availability influences water in plants and the composition of sand was expected to influence soil water (Holdo *et al.,* 2018). Date and plot were included as random factors.

A Wilcoxon – Mann – Whitney test was used to investigate the differences in final grass biomass between plots that were warmed and those that were not.

2.4 Results

Measurements of ambient and soil temperature, and humidity were summarised by month and are presented below (*Table 2.1*).

Table 2.1: Daily maximum and minimum temperatures, mean day and night temperatures, mean soil temperatures and mean humidity (1300-1400hrs) values for each treatment for every month from October 2017 to May 2018. WG are warmed plots with grass, CG are control plots with grass, WNG and CNG are warmed and control plots respectively, without grass.

	Daily Min Temp (°C)	Daily Max Temp (°C)	Mean Daily Temp (°C)	Mean Night Temp (°C)	Mean Soil Temp (°C)	Mean Humidity (%)
October						
WG	10.54	49.52	30.39	19.06	32.88	34.57
CG	10.07	45.56	28.74	18.41	32.78	46.33
WNG	10.61	51.09	30.93	19.21	34.29	33.62
CNG	10.03	46.06	28.83	18.73	32.74	36.67
November						
WG	13.54	50.02	30.25	19.88	31.92	39.22
CG	13.58	47.54	28.98	19.4	32.74	46.64
WNG	14.11	54.07	31.28	20.14	34.06	35.78
CNG	13.03	48.06	29.1	19.69	33.67	40.41
December						
WG	16.09	48.055	30.48	21.36	31.14	48.34
CG	15.58	44.58	29.18	21.11	32.35	55.7
WNG	16.16	52.59	31.63	21.77	33.5	41.61
CNG	16.09	47.56	29.29	21.16	32.57	50.21
January						
WG	15.09	49.05	31.45	21.38	32.48	40.28
CG	15.1	46.54	30.81	20.98	34.07	48.44
WNG	16.12	51.08	33.71	21.85	36.74	30.54
CNG	15.03	47.06	31.85	21.25	36.89	34.02
February						
WG	17.05	50.02	32.08	22.71	32.5	36.55
CG	16.6	45.08	30.93	22.28	33.5	45.81
WNG	18.12	50.1	32.65	22.98	34.92	34.16
CNG	16.53	45.56	31.38	22.55	34.17	38.43
March						
WG	14.45	53.01	32.31	21.61	32.66	35.36
CG	14.08	49.05	30.99	21.05	33.91	38.4
WNG	15.12	55.57	32.9	21.84	34	32.97
CNG	14.03	50.05	31.63	21.28	33.39	36.97
April						
WG	11.58	45.53	28.01	18.63	26.96	42.09
CG	10.07	44.06	27.21	18.07	27.6	47.14
WNG	11.11	48.09	29.04	18.8	28.46	37.89
CNG	9.53	45.56	28.12	18.25	28.43	41.46
May						
WG	9.08	45.53	25.51	15.26	24.8	35.01
CG	8.07	42.09	24.73	14.54	24.39	37.91
WNG	9.61	44.63	26.06	15.44	24.67	33.59
CNG	8.03	42.07	25.17	14.77	24.75	36.74
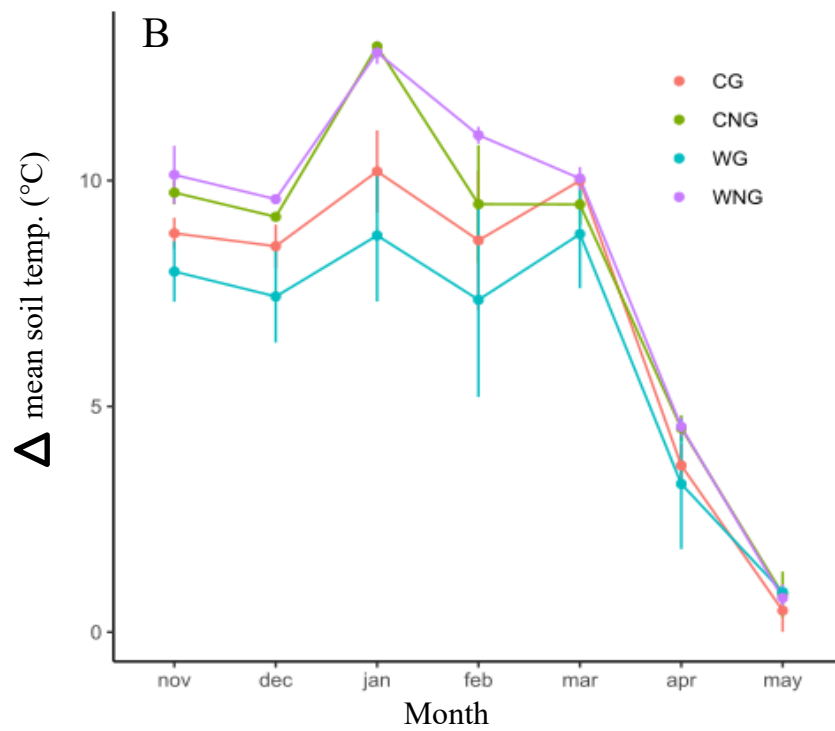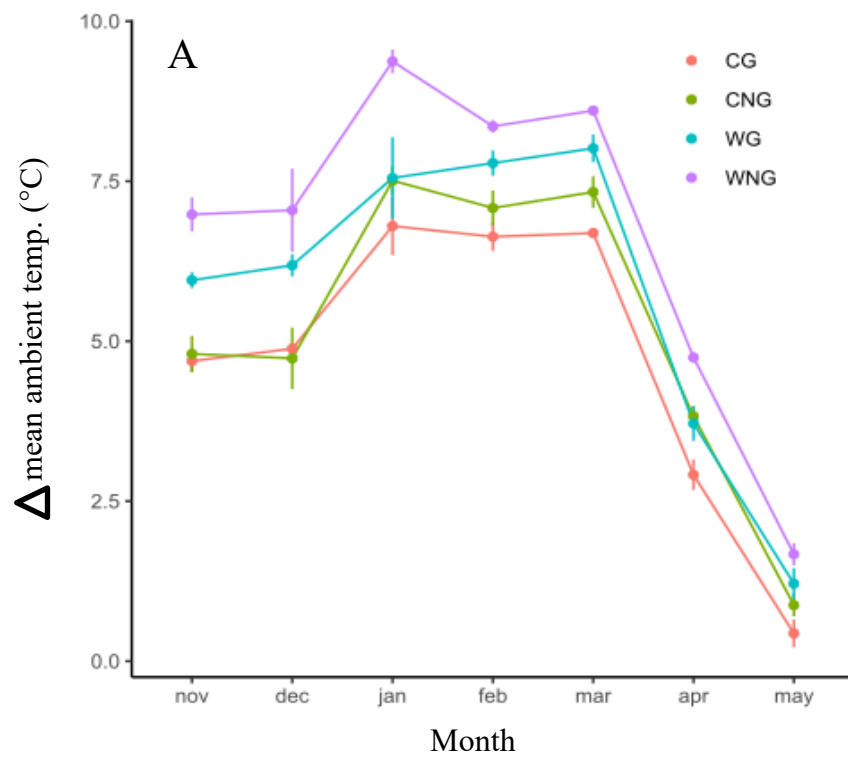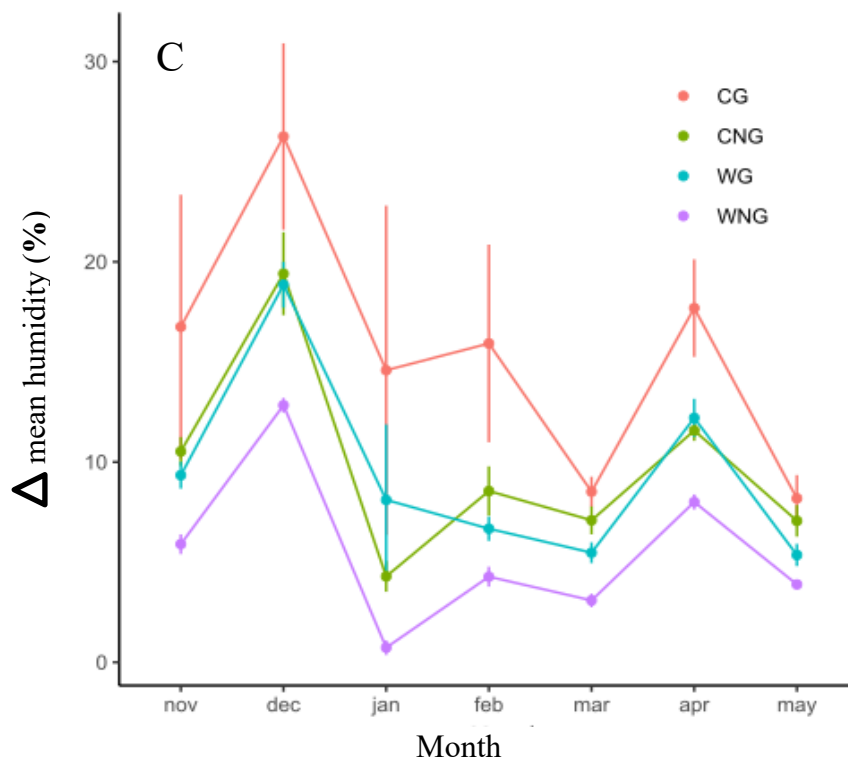
Figure 2.2: **A)** *The difference (Δ) in mean daily ambient temperature between treatments from November 2017 to May 2018. CG is the unwarmed, with grass treatment, CNG is the unwarmed, without grass treatment, WG is the warmed with grass treatment and WNG is the warmed, without grass treatment. A baseline figure was used, that being the lowest value of all treatments and months for each variable. The bars at each point indicate the standard error of the mean. The same is true for the B and C.* **B)** *The difference in mean daily soil temperature between treatments from November 2017 to May 2018.* **C)** *The difference in mean humidity at 1300-1400hrs between treatments for the same period.*

Warming significantly increased daily mean temperature ($T_{83, 0.627}$ = 9.255, p<0.00) (*Table 2.1*), with seedlings experiencing an increase of between 1 - 2.1°C during the experiment. On average, warmed plots were 1.6°C warmer than control plots. The presence of grass significantly reduced ambient temperature ($T_{83, -0.262}$ = -3.824, p<0.00). Warmed plots without grass (WNG) were, on average, 2.08°C warmer than control plots (CNG and CG) but warmed plots with grass (WG) were 1.11°C warmer than control plots (CNG and CG). Plots not warmed and without grass (CNG) were 0.48°C warmer than control plots (CG). On average, warmed plots without grass (WNG) experienced the highest temperature. Grass plots (WG and CG) were, on average, 0.73°C cooler than plots without grass (WNG and CNG) (*Table 2.1* and *Figure 2.2-A).*

The difference in temperatures (ΔT) between treatments was greatest during the warmest part of the day and differences in temperature between treatments were largest in the warmer months relative to the cooler months (*Table 2.1*). Likewise, as the daily temperature increased the warming effect increased. Maximum ambient temperatures differed, on average, by 4.4°C between treatments

without grass and by 3.4°C between treatments with grass (*Table 2.1*) showing that grass reduced the effect of warming. The results also show that as the temperature increases the warming treatments experience greater extreme temperatures (*Figure 2.2-A*).

Neither warming nor the presence of grass significantly affected soil temperature but the interaction of these effects did ($T_{79, -0.354}$= -2.411, p= 0.018). The soil of warmed plots without grass were, on average, 1.91°C warmer than the warmed plots with grass (the coolest treatment). Soil of control plots with grass were 0.78°C warmer than the warmed plots with grass (*Figure 2.2-B*).

Likewise, humidity was affected by the warming treatment ($T_{82,-1.25}$= -2.230, p=0.029) and the presence of grass ($T_{82, 0.75}$=4.355, p<0.00), where warming reduced humidity but the presence of grass increased humidity. Control plots with grass had, on average, midday humidity that was higher by 10.8% RH than warmed plots without grass (*Figure 2.2-C*).

2.4.1 *Soil Moisture*

Although warming changed the ambient temperature, it did not cause a significant difference in soil moisture between treatments (*Figure 2.3*).
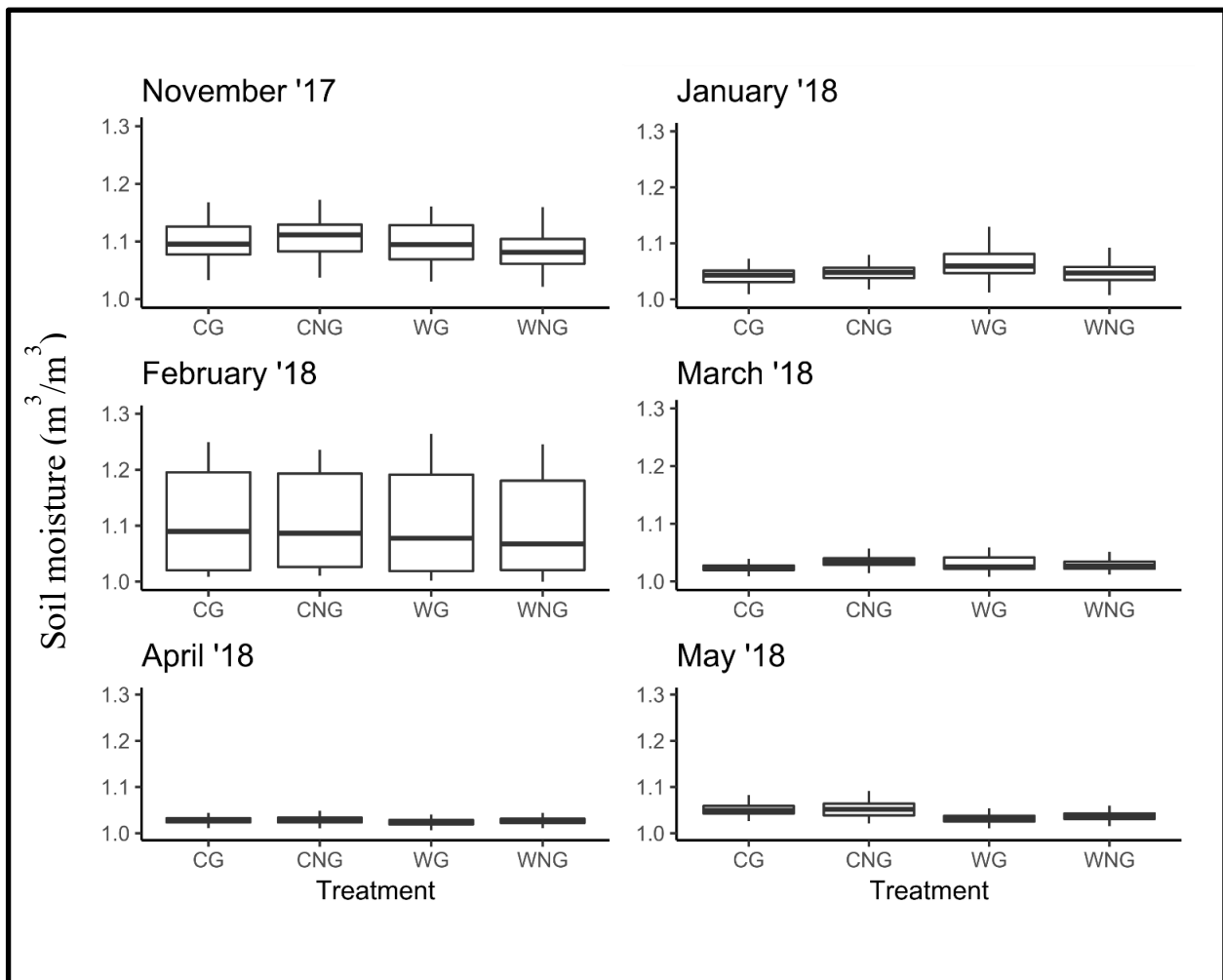
Figure 2.3: Box and whisker plots of soil moisture for all treatments for six months (excluding December 2017). The lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles respectively). The upper whisker extends to the largest value no further than 1.5 times the inter-quartile range (IQR) from the hinge and the lower whisker extends from the hinge to the smallest value at most 1.5 times IQR of the hinge (R v 3.5.0.)

2.4.2   *Leaf Relative Water Content of Tree Seedlings.*
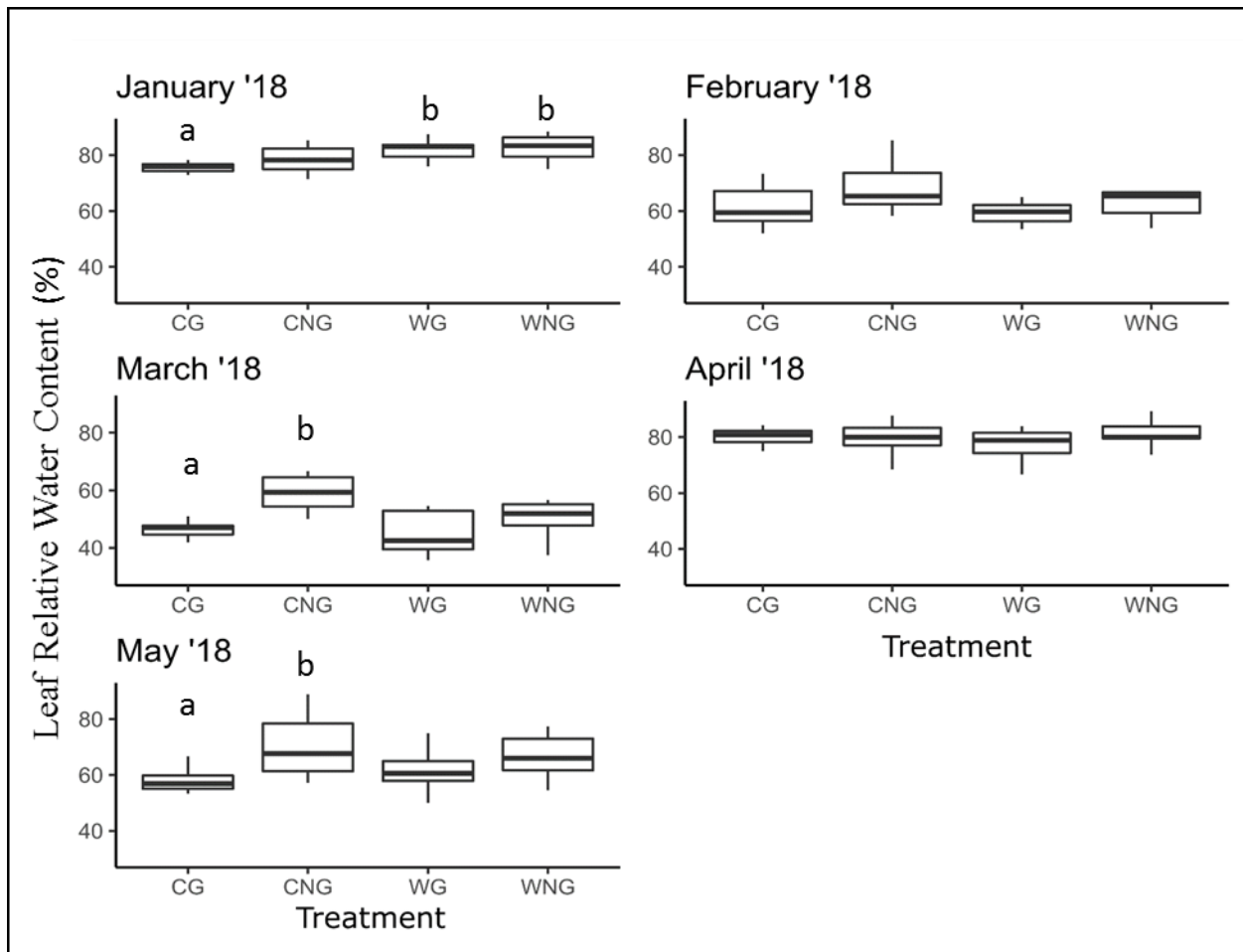
Figure 2.4: Box and whisker plots of leaf relative water content (%) of Senegalia nigrescens *seedlings for all treatments from January to May 2018. The letters above certain treatments indicate significant differences.*

The seedling leaf relative water content (RWC) was unaffected by warming but was significantly reduced by the presence of grass. The differences were most clearly observable in January, March and May 2018 (*Figure 2.4*). The decline in RWC as a result of grass presence was increased by drier conditions, as seen in March and May where little rainfall had been received the month before the measurements (*Figure 2.4*). The best models of leaf RWC included the following variables: warming, grass, soil moisture, sand percentage and the interaction of warming and grass. Model averaging showed the presence of grass to be a significant predictor of leaf RWC ($Z = 4.63$, p<0.00), reducing seedling leaf RWC by 7.69% (*Table 2.2*). Therefore, regardless of warming, the presence of grass negatively influenced seedling water content. The results also showed that warming has no effect on final grass biomass ($W_{(9)} = 8$, $p = 0.421$).

Table 2.2: Results of model averaging of leaf RWC using a GLMM. Parameter estimates are given, standard error (SE) indicates the degree of uncertainty due to sampling error and the z value gives the statistics for the Wald test. Bold type indicates a significant predictor of RWC. The asterix indicates a significant p-value (p<0.05).

Coefficients	Estimate	SE	z value	p
(intercept)	71.6	5.77	12.4	<0.00*
Soil moisture	0.292	1.21	0.241	0.809
Grass (yes)	**-7.69**	**1.66**	**4.63**	**<0.00***
Warming (yes)	-1.83	1.67	1.10	0.274
Grass: Warming	3.94	2.33	1.69	0.091
Sand	-0.0798	0.360	0.222	0.825

2.5   Discussion

This study found that warming did not change soil moisture or leaf RWC of seedlings but it does demonstrate that the presence of grass significantly reduced leaf RWC of establishing African tree seedlings. POTCs in this study simulated natural warming of the climate by increasing ambient temperatures by 1-2°C on average. At maximum temperatures, the chambers warmed the microclimate by approximately 5°C. As predicted, warming reduced humidity and increased ambient and soil temperature.

Whilst warming is expected to reduce soil water availability by increasing evapotranspiration and creating more arid conditions (Bowman *et al.,* 2014; Blumenthal *et al.,* 2018), this study did not find warming to influence soil moisture. This was an unexpected finding considering the many reports of drier conditions as a result of warming. It is possible that the experiment did not run for a length of period suitable to detect this or the frequency of soil moisture recordings and the position of the soil moisture probe and hand-led measurements did not allow for detection of an effect. If warming and grass did not change soil moisture availability, one would not expect an effect of either warming or grass on the relative leaf water content (RWC) of seedlings. However, I did find a significant effect of grass presence on leaf RWC, which suggests a lack of precision in measurements of soil moisture in this experiment.

Warming did not affect leaf RWC of seedlings, and in fact seedlings that were warmed the most, (in the absence of grass) were the least water-stressed. However, the presence of grass in both warmed and non-warmed plots significantly reduced the leaf RWC of tree seedlings. This finding supports the large evidence base for competition between C4 grasses and woody plant seedlings for soil water (Ludwig *et al.,* 2004; D'Onofrio *et al.,* 2015) where grass suppresses savanna tree seedling growth (Riginos, 2009, Manea and Leishman, 2015). Grass within treatments limited excessive warming by keeping plots cooler (Wan *et al.,* 2002) by an average 0.73°C, suggesting a potential facilitative effect. This effect, however, reduced as grasses reduced the water content of seedlings. The belowground relationship between these two growth forms is known to be highly asymmetrical (February *et al.*, 2013; Tedder *et al.,* 2014) with the dense rooting system of grasses out-competing the developing root system of seedlings (Cramer *et al.*, 2012; D'Onofrio *et al.,* 2018). A reduction in the water available for uptake increases the water stress in tree seedlings. Seedlings in this experiment (*Senegalia nigrescens*) tolerated low leaf RWC (as low as 31%) but it is highly likely that their growth rate declined (Otieno *et al.,* 2001) as they suffered from stomatal and non-stomatal limitations of carbon assimilation (Chaves *et al.*, 2002; Bernacchi *et al.*, 2013).

This study's results show that warming does not alter soil moisture availability, neither does it increase the competitive effect of C4 grasses on tree seedlings. The fact that warming does not interact with this competitive relationship is surprising because it is known to be an important process through which climatic drivers mediate ecosystem responses (Volder *et al.*, 2013). Elevated CO_2, warming and drought are drivers that have changed the relationship between C4 grasses and trees, predominantly by altering the availability of soil water (Polley *et al.,*1997; Morgan *et al.*, 2001; Volder *et al.,* 2010; Volder *et al.,* 2013; Blumenthal *et al.,* 2018). Often, these drivers act in opposing ways (Blumenthal *et al.,* 2018) for example, warming and drought may eliminate the improvement of soil water under elevated CO_2 (Xu *et al.,* 2013).

Although the presence of grass reduced the water content of seedlings, it is likely that many African savanna trees are able to operate at relatively high levels of water stress. (Chaves *et al.,* 2002; Zhou *et al.*, 2014). For example, the arid savanna species *Colophospermum mopane*, has adapted to grow well in water-limited conditions, and has even been found to be more resilient to water-stress in warmer conditions (Stevens *et al.*, 2014b). The extent of global warming will determine which species are able to persist. It is suggested that mesic species are more likely to suffer than arid species because they have lower thermal limits and are not adapted to high levels of water-stress. Arid and semi-arid species, on the other hand, which are mechanized to survive water-limited conditions, have the best chance at persisting or evolving further water-stress resilient strategies. Future research needs to be

focused on a diversity of plant functional types, across a range of savanna types to gain a thorough understanding of how warming is likely to affect African savanna systems.

Not only are there multiple drivers of global change but the responses of plants are likely to be interactive (Xu *et al.,* 2013). Although not investigated in this study, it is important to consider the effects of warming interacting with CO_2, to fully understand interactions into the future (Xu *et al.,* 2013). Elevated CO_2 may increase the water efficiency of plants, offsetting the potential negative effects of warming (Swann *et al.,* 2016). Although this study found grass biomass unaffected by warming, an experiment conducted by Manea and Leishman (2015) found increased CO_2 to reduce tree seedling growth because grass growth was more responsive to CO_2 fertilization than tree seedlings. Potentially, there may be more water available but intensive competition with C4 grasses which, as seen in this study and many others, has a significant detrimental effect on tree seedling growth. These contradictory findings highlight the need for further research to clarify the effects of climate change drivers (CO_2 fertilization and warming) on plant growth in savanna systems.

2.6   Conclusion

The findings of this study show a lack of an effect of warming on soil water and tree seedling water content, suggesting that warmer conditions are unlikely to directly increase the water-stress in establishing seedlings, nor are they likely to disrupt the competitive relationship between C4 grasses and tree seedlings by modifying soil water availability. The presence of grass negatively influences the water content of establishing seedlings, regardless of the presence of warming. This shows the major role that this asymmetric relationship plays in determining tree seedling establishment and thus savanna structure. Understanding the effect of warming on soil water availability and uptake by both establishing seedlings and grass is paramount to predicting the response of savanna plant growth to climate warming. More accurate research is required to detect subtle warming effects on various plant growth determinants, but this study gives useful, early insight into the potential warming effect on the water relations of grasses and tree seedlings in an African savanna system.

3. Chapter 3: Warming and C4 grass competition suppress the establishment of an African savanna tree.

3.1 Abstract

Savannas are characterised by the co-dominance of trees and grasses. Tree seedling establishment is a key plant demographic bottleneck, and affects savanna structure and functioning. How the tree-grass balance will shift, if at all, in a warmer world has not yet been well quantified for many savanna systems. This study investigated how warming may alter the establishment of an African savanna tree, *Senegalia nigrescens (Oliv.)* P. J. H. Hurter in a semi-arid savanna setting. I grew seedlings in both the presence and absence of grass in experimental warming chambers and control plots to examine how warming affected seedling growth and subsequent survival. Tree seedlings that were warmed in the absence of grass showed no significant reduction in growth, but in the presence of grass, growth was significantly reduced by warming above a threshold of 30°C. Warming reduced the survivorship of tree seedlings grown in the absence of grass only by approximately 15%, but by approximately 65% for seedlings grown in the presence of grass. These results show that the presence of grass and warming reduce seedling growth and subsequent survivorship in an additive way, possibly due to a combination of reduced water availability and direct warming effects on seedling respiration. The results of this study show that the combined effects of grass competition and warming may therefore reduce successful African savanna tree seedling establishment. Further research involving a greater diversity of species and functional types, from a wider variety of savannas is required to confirm this result more broadly.

3.2   Introduction

Climatic variables are important in determining the biological and chemical processes in plants (Shaver *et al.,* 2000; Beier *et al*., 2004) and the availability of resources (Scheiter and Higgins, 2009). As such, climatic variables have been used to understand the distribution of biomes, and by extension, project future climate change impacts upon them (Prentice *et al.,* 1992; Motew and Kucharik 2013). This notion has led to a strong focus of research on northern hemisphere temperate species, investigating how plants respond to a changing climate, particularly a reduction in low temperature limitations (Milbau *et al*., 2009).

Savanna ecosystems, however, make up the largest biome that does not appear to be in equilibrium with climate. Tree cover of savannas is almost always below a theoretical maximum predicted by water availability (Bond and Midgley, 2012). The distribution and structure of savannas is thought to be shaped by interactions between disturbance, mainly fire and herbivory, and climate (Lehmann *et al*., 2014; Lehmann and Parr, 2016). The current understanding of changing climate effects on savannas invokes disturbance dynamics and how this affects establishing saplings in a flammable grass sward.

The thermal limits of plant species determine the response of ecosystems to warming (Sage and Kubien 2007; Volder *et al*., 2013). Plants in cool environments benefit from warming because they grow at faster rates and for longer periods (Yin *et al*., 2008; Wilson and Nilsson, 2009; Kaarlejarvi *et al*., 2012; Greenwood *et al*., 2015). Studies suggest that this mechanism is at play in driving woody encroachment in the tundra (Myers-Smith *et al.,* 2015) where increases in shrub cover, tree growth and plant productivity is correlated with warmer temperatures (Shaver *et al.,* 2000; Rustad *et al.,* 2001; Walker *et al*., 2006; Bronson *et al*., 2009; Munier *et al.,* 2010; Myers - Smith *et al.,* 2011; Elmendorf *et al.,* 2012; Henry *et al*., 2012; Hollister *et al.,* 2015; Bjorkman *et al.,* 2018). However, the warming response of plants in warm ecosystems, where temperature is not the limiting growth factor, depends on the species ability to tolerate direct and indirect warming effects (Chidumayo, 2007; Volder *et al.,* 2013) and remains largely uninvestigated, particularly in savannas.

In African savannas especially, the effect of temperature on tree: grass interactions is largely unknown (Lehmann and Parr, 2016). Existing work on warming of savanna trees has produced variable results. Some studies demonstrate that climate warming can significantly increase seedling mortality of African savanna woody species (Chidumayo *et al.,* 2007) whilst other studies indicate that warming can increase the establishment success of certain seedlings through enhanced growth rates (Choinski and Tuohy 1991; Stevens *et al.,* 2014a). Warming can increase evapotranspiration (Grossiord *et al.,* 2018, Chapter 1) and reduce soil water (Scheiter and Higgins, 2009; Xu *et al.,* 2010) which is crucial

to successful tree seedling establishment. It can also directly increase seedling mortality by increasing rates of dark respiration, creating temperatures above their critical limit for photosynthesis and directly damaging cellular components (Sage and Kubien, 2007). Warming also increases water stress in seedlings by increasing transpiration rates. When exposed to these temperature extremes, to prevent further water loss, seedlings close their stomata but succumb to carbon starvation (Volder *et al.,* 2013).

However, warming in savannas can have important indirect effects through altering the competitive nature between trees and grasses, an interaction which has also been poorly investigated. With sufficient water, C4 grass growth is likely to increase with warming (Edwards and Smith, 2010) as their photosynthetic pathway enables them to minimize photorespiration under warming, consequently increasing the intensity of competition for soil water with tree seedlings (Hovenden *et al.,* 2008). However, the presence of grass may have a positive effect on tree seedling establishment as they can reduce the direct heat stress on seedlings by protecting seedlings from direct sunlight and creating a microclimate more suitable for successful seedling establishment (Grellier *et al.,* 2012; Volder *et al.,* 2013).

The numerous different effects of warming on establishing seedlings makes it difficult to predict the overall effect of warming on savanna tree seedlings. Different leaf traits and physiological tolerances (Volder *et al.,* 2013) of trees and grasses may cause the two life forms to respond differently. However, the competitive interaction between trees and grasses is greatest at the seedling establishment phase (Riginos, 2009; Scheiter and Higgins, 2009) and it is likely that warming will have the strongest effect on plant growth and mortality at this stage.

Significant focus on tree:grass interactions within savannas has been dedicated to understanding the role of water in this interaction, primarily as water is one of the primary determinants of savanna structure and function (Sankaran *et al.,* 2005). Earth's climate is predicted to be warmed by an average of 2°C (IPCC, 2014) yet the savanna region of southern Africa is projected to be a climate change hotspot, with average temperatures 4°C - 6°C warmer (Engelbretch *et al.,* 2011). Given this projected increase I aimed investigate how warm-adapted tree seedlings are likely to respond to further warming in both the presence and absence of savanna grasses. I investigated how experimental warming alters the mortality and growth of African savanna tree seedlings grown with and without grasses. To understand this, I warmed establishing *Senegalia nigrescens* seedlings using passive open-topped chambers (POTCs) for 8 months, from October 2017 to May 2018. Environmental variables were monitored, including ambient and soil temperature, humidity and soil moisture. The mortality and growth rate of the seedlings were recorded, and growth measurements of plant height,

extended height, basal stem diameter and total plant length where measured. I predicted that seedling mortality would be greatest in warmed plots in the presence of grasses. Although grasses may have a facilitative effect on establishing savanna tree seedlings (Volder *et al.,* 2013), I predicted that the competitive effect of grass would out-weigh its facilitative shading effect (Bond, 2008). Several studies show increased survival and growth of tree seedlings with the removal of grass (February *et al.*, 2013; Bond, 2008), sometimes reducing seedling survival probability by approximately 50% (Stevens *et al.,* 2018). I suggest that warming will exacerbate this impact and further intensify competition between seedlings and grass as it is likely that warming will reduce water availability in the soil (Quan *et al.,* 2018; Polley *et al.,* 2013). I predict that warming will initially increase seedling growth rates as photosynthesis rates increase (Lambers *et al.,* 2008). I also expect that seedling growth may decline after some time of warming, where either reductions in water availability (as a result of warming) induce water stress within the seedlings (Grassi and Magnani, 2005) or damage to photosynthetic machinery from high temperatures (Lambers *et al.,* 2008), limits growth rate.

3.3   Methods and Materials

3.3.1 *Study Site*

The experiment was carried out at Wits Rural Facility, situated adjacent to The Kruger National Park in Limpopo Province, South Africa (~31°23′28E, 24°28′ 29S) (594m ASL). This site occurs in the granite lowveld bioregion, and is characterised by sandy soils in the uplands and clayey soils in the lowlands. This area experiences characteristically hot, wet summers (October –April) and warm, dry winters (May-September) (mean annual temperature of 20.9°C with a maximum temperature of 38°C in January and a mean minimum temperature of 3.7°C in June/July) with approximately 633mm of rainfall received per annum (Mucina and Rutherford 2006).

3.3.2 *Site Preparation*

Twenty hexagonal plots ($3.42m^2$ each) were established within a $100m^2$ site. As the site was a abandoned landing strip, prior to planting, the soil was loosened and turned manually using picks and hoes. All vegetation was removed and plots were prepared for planting by dampening the soil every day for two weeks. This was done until the seasonal rains arrived. I created five replicated treatment plots. Each treatment plot consisted of a warmed plot with and without grass and a non-warmed plot with and without grass. Plots receiving warming treatments were warmed using POTCs. These chambers are made up of six polycarbonate panels, approximately 800mm high. Cable ties were used to fasten the panels together at their edges and an aluminum rod was placed at this join to prevent the panels from folding. POTCs are the most popular method used to warm low-statured vegetation (Godfree *et al.,* 2011). They increase ambient temperatures on average, by approximately 1-2°C and

extreme maximum temperatures by approximately 5°C (Chapter 1), corresponding well with projected natural climate warming.
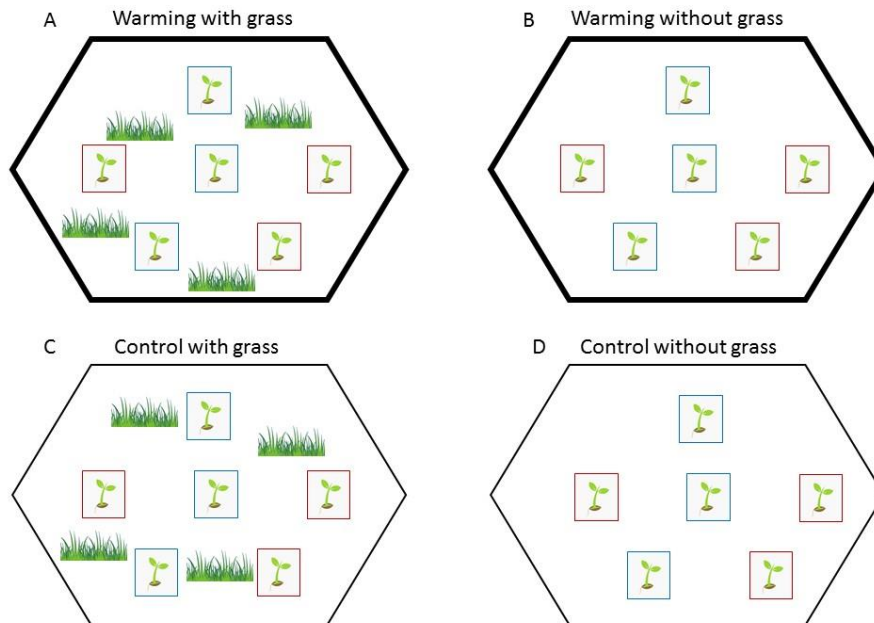
Figure 3.1: Each replicate consisted for four treatment plots, warmed plots with one containing grass (A) and one containing no grass (B). Non-warmed plots also contained a treatment with grass (C) and a treatment without grass (D). Each treatment was replicated 5 times.

3.3.3 *Measurements*

Within each treatment group, I planted eight *Senegalia nigrescens* seedlings. Seeds of this species were collected from trees in the area between October 2016 and April 2017 and were stored in manila envelopes before they were sown into seedling boxes. Seeds received no treatment prior to sowing. They were germinated in an outdoor greenhouse in October (2017), at the start of the growing season and were then transplanted into 1l seedling bags when they were 2 weeks old. All seedlings germinated and grew under the same environmental conditions and received the same water treatments.

In November, seedlings were randomly transplanted into the plots. Eight seedlings were positioned towards the center of the plot, approximately 20cm apart. At monthly intervals, the mortality, plant height, extended height, basal stem diameter and total plant length were recorded. Plant height was the measurement of the top of the plant from the ground. Total length was the measurement of the stem, added to the measurement of combined length of woody branches. A tape measure was used to record seedling height and length and digital calipers were used to measure stem diameter. These measurements were taken for the duration of the growing season (November-April). A tape measure

was used to record lengths and digital calipers were used to record basal (1cm from the ground) stem diameter.

Ambient temperature was measured in three randomly selected replicates of each treatment. Within these 12 plots, a plastic radiation shield was placed at the plot's center. DS1923-F5# Hygrochron (Maxim Integrated, San Jose, California, USA) temperature loggers (ibuttons) were placed inside the shield, approximately 20cm above the ground. The ibuttons logged every 15 minutes to 0.5°C resolution. Volumetric soil moisture (m^3/m^3) was recorded using four soil moisture probes (EC-5 Soil Moisture Sensor, Decagon Devices Inc, Pullman, WA, USA) placed in the top 5cm of soil. One probe was placed in each treatment. These data were supplemented by multiple handheld soil moisture readings taken at monthly intervals comprising 15 recordings taken in each plot using a Decagon GS1 volumetric sensor (Decagon Devices Inc, Pullman, WA, USA) connected to a handheld reader (Decagon Procheck). The soil moisture probe extended approximately 5cm into the soil (Chapter 2).

3.3.4 *Statistical Analyses*

All graphics and statistical analyses were carried out using R (version 3.5.0) statistical software (R Development Core Team, 2016).

To examine the survival of seedlings throughout the experiment, I used the survfit function to create Kaplan-Meier survival curves. I used the mixed-effects Cox proportional hazards model using the coxme (version 2.2-10) (Therneau, 2018) package to quantify the effect of treatment as a predictor of seedling survival and I used the 'Efron' method for calculating ties in the data. To investigate treatment effect on the final survival count of seedlings, a two-way ANOVA was conducted.

Relative growth rate of total seedling length (RGR_L) was calculated using the increment in ln (total length) (Kohi *et al.,* 2010; Vadigi and Ward, 2013). The following equation was used (Beadle, 1993),

$$RGR_L = \frac{\ln(L2) - \ln(L1)}{t2 - t1}$$

where L2 and L1 indicate the final and initial lengths, respectively, for that growing period and t2 and t1 indicate the time (in days) when these measurements were taken. Although seedling growth measurements were taken each month, the time between measurements was not consistent. To account for this variation, time was calculated in days, hence the units for RGR_L are as mm/mm/days. It is important to use RGR_L because fast growth is crucial for successful seedling establishment (Vadigi and Ward, 2012). The seedlings with the highest growth rates are those most likely to establish successfully (Wakeling *et al.,* 2011; Bond *et al.,* 2012), thus, in addition to the mean RGR_L,

the 90th percentile was investigated to look at the relationship between RGR_L and various variables for the top 10% of the fastest growing individuals.

The average soil moisture for each period and treatment was determined and a regression was plotted of seedling RGR_L against soil moisture for both the mean and the 90th percentile. The relationship between RGR_L and mean daily temperature was investigated by plotting regressions for both seedlings grown with grass and seedlings grown without grass. For seedlings grown with grasses, a segmented regression was plotted using the 'segmented' package to identify a break point at which the relationship changed. Regressions of the mean and 90th percentile were plotted using ggplot (version 2.2.1), with the function geom_quantile ('rqss' method). The function 'segmented' (R package, segmented) was used to estimate a break point for both percentiles.

General linear mixed effect models (GLMMs) with non-normal distribution errors were constructed to investigate the role of certain variables on both seedling growth rate and final seedling length. Growth rate exhibited a gamma distribution and final seedling length had a log-normal distribution. Variables to predict growth rate included mean daily temperature, grass presence, and the interaction of mean daily temperature with grass. Plot, sand percentage and date of measurement (month) were included as random factors. These variables were included in the model because temperature affects the growth rate of seedlings and the proximity of the seedlings to their upper thermal limits (Myers-Smith *et al.,* 2011; Elmendorf *et al.,* 2012; Bjorkman *et al.,* 2018), and grass interacts with seedlings by competing for resources or facilitating seedling growth (Grellier *et al.,* 2012; Volder *et al.,* 2013). The interaction of temperature with grass was also included as a predictor because the relationship between temperature and growth rate was expected to change with the presence of grass. Spatial and temporal variation was accounted for by including the plot and the date of the measurement as random factors in the model. Predictors of final seedling length included warming (yes or no), grass presence and the interaction of warming with grass. Plot was included as a random factor. These variables are all factors expected to influence seedling growth and thus, their final length. The penalized quasi-likelihood (PQL) method was used in the GLMMs because both seedling growth rate and final seedling length exhibited a non-normal distribution. Avoiding transformation, the PQL method helps to better capture the variation of seedling RGR_L and final length (McCulloch, 1995).

3.4 Results

3.4.1 *Seedling Growth Patterns*

Seedling relative growth rate (RGR_L) was affected by temperature and soil moisture. A regression of the mean monthly RGR_L was plotted against the mean monthly soil moisture for every plant and each month. The regression showed that growth rate significantly increased as water availability increased ($F_{1, 609} = 44.71$, p <0.00), with RGR_L increasing by 0.064 mm/mm/day for every $1m^3/m^3$ increase   in   moisture.
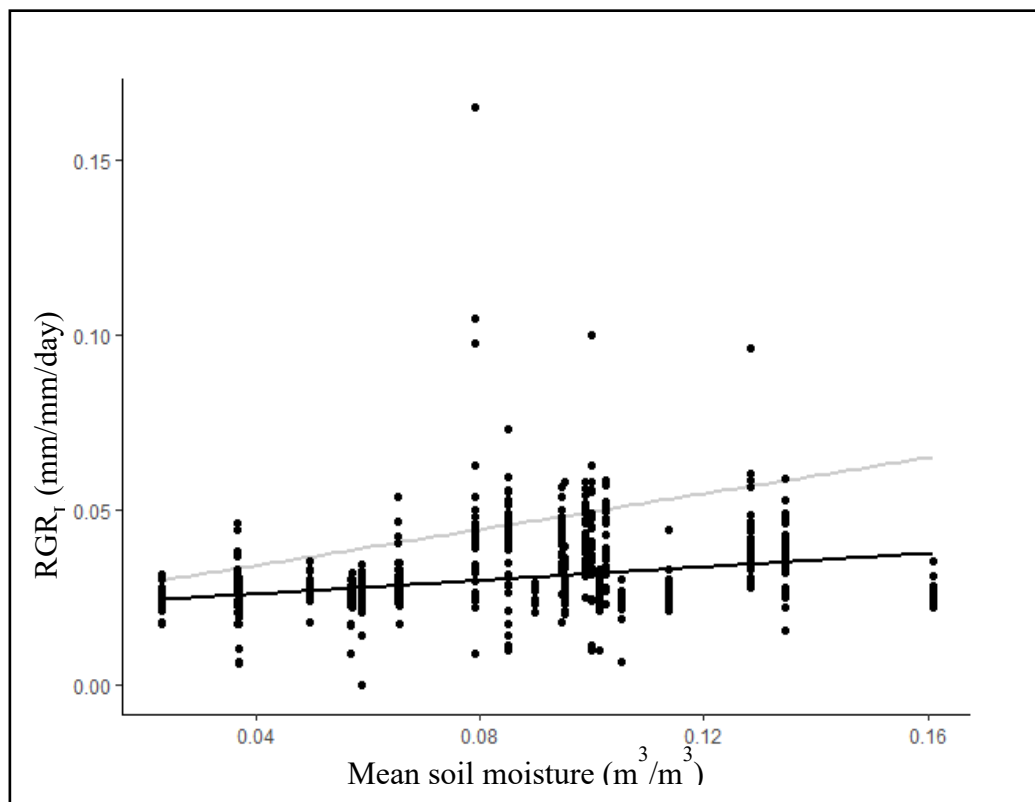
Figure 3.2: A mean (black line) and 90th (grey line) percentile regression between mean soil moisture and relative growth rate for seedlings from all treatments. RGRL significantly increased with mean soil moisture (F1, 609 = 44.71, p <0.00). The 90th percentile regression was not significant.

The relationship of monthly mean daily temperature with RGR_L was investigated separately for seedlings grown with, and seedlings grown without grass (*Figure 3.2*). Seedlings grown without grass exhibited no significant relationship between RGR_L and monthly mean daily temperature. For seedlings grown with grass, growth rate of the 90th percentile was unaffected by increasing temperatures until temperatures exceeded 30.25°C (breakpoint defined using segmented regression, (*Figure 3.3*)) after which the relationship changed from being non-significant to significant. When temperatures exceeded 30.25°C, plant growth rates declined significantly ($F_{1, 2} = 5075$, p<0.00) as temperature increased. For every unit increment of temperature, seedling growth rate declined by 0.034 mm/mm/day (*Figure 3.3*, *with grass*). The 50th percentile showed RGR_L to increase with

temperature ($F_{1, 142}$ = 22.18, p <0.00) until a change point of 30.13°C (R = 0.22). Beyond this temperature, RGR_L significantly decreased ($F_{1, 149}$ = 68.49, p <0.00) (*Figure 3.3*, *with grass*). For every unit increment of temperature (°C), seedling growth rate decreased by 0.007 mm/mm/day. These results clearly show that the combined effect of warming above 30.25° and the presence of grass significantly reduces seedling RGR_L.
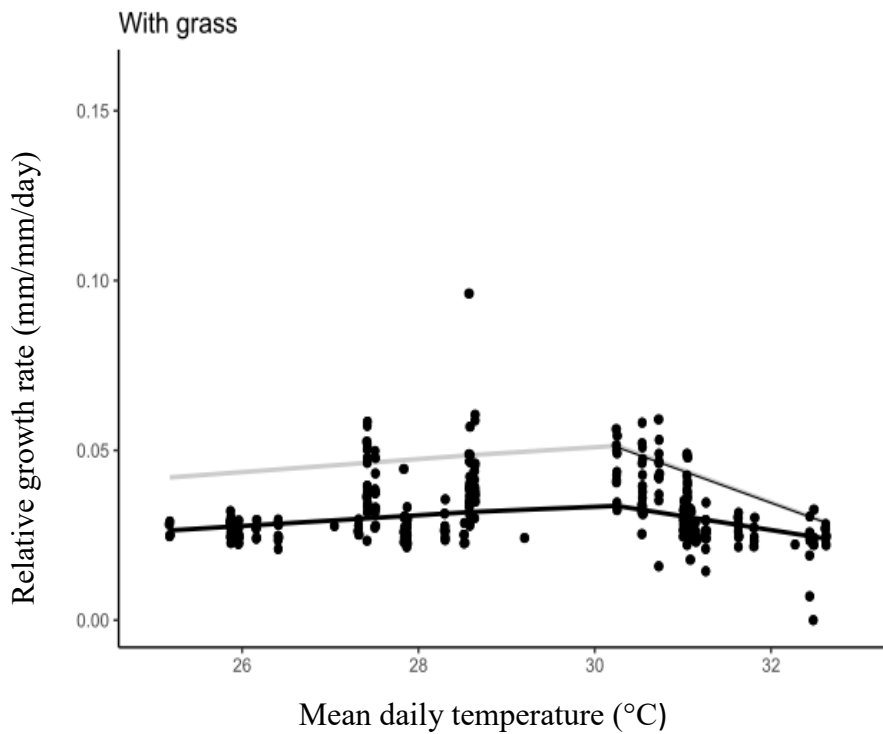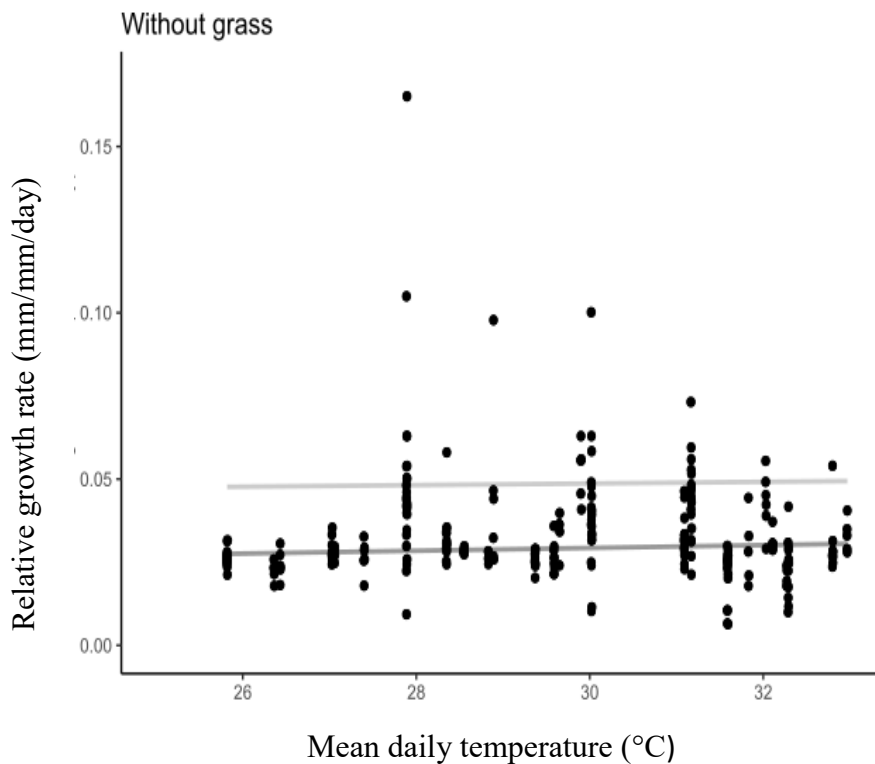
Figure 3.3: The relationship between mean daily (0600hrs -1800hrs) temperature and relative growth rate (RGR$_L$) for seedlings grown in the presence of grass (left) and in the absence of grass (right). There was no relationship between RGR$_L$ and mean daily temperature for seedlings grown without grass (right). The RGR$_L$ of seedlings grown with grass increased significantly (F$_{1, 142}$ =22.18, p <0.00) until 30.13°C, after which it decreased with rising temperatures (F$_{1, 149}$ =68.49, p<0.00). Maximum growth rates (90th percentile) had no significant relationship (grey line) until 30.25°C, after which it decreased significantly (thin black line) (F$_{1, 2}$ =5075, p <0.00) with increases in temperature.

3.4.2 *Seedling Mortality*

Kaplan-Meier survival curves show that both the presence of grass and warming changed the seedling survival probability during the experiment (*Figure 3.4*). Warming reduced the survival of seedlings with the strongest reductions in survival occurring when grass was absent. Tree seedling survival was highest in non-warmed plots with grass (CG) (±95% survival by the end of the experiment). However,
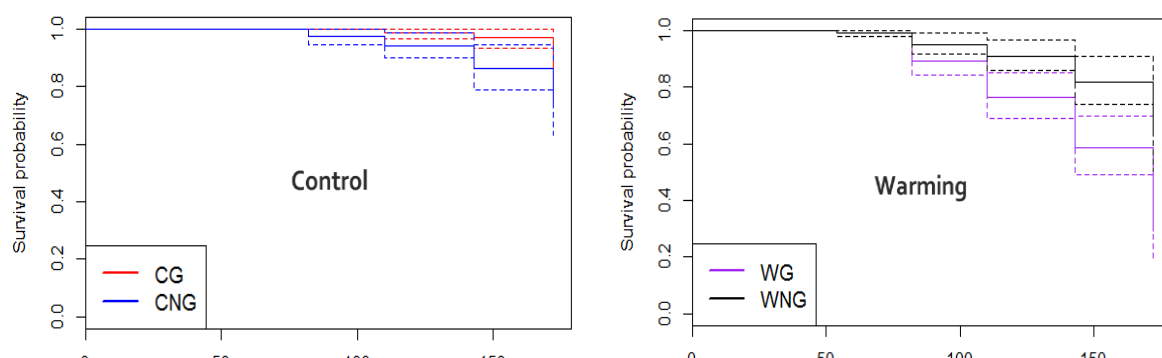
Figure 3.4: Kaplan Meier estimates of survival rates for seedlings grown without warming (left) and with warming (right), grouped into those grown in the presence of grass and those grown without grass.

when grass plots were warmed, seedling survival declined significantly ($Z_{4, 840} = 3.92$, $p < 0.0001$) to ± 30% by the end of the experiment. Seedling survival was also significantly lower in warmed plots without grass ($Z_{4, 840} = 2.47$, $p < 0.013$) than control plots with grass. These results of seedling mortality align with the findings of Section 3.4.1 (Seedling Growth Patterns), where the combination of both the effect of warming and the presence of grass significantly reduces seedling survival probability.

The final model of treatment effects on total seedling length at the end of the experiment included warming, the presence of grass and their interaction as predictors. None of these variables were found to significantly influence final seedling length.

3.5 Discussion

The growth of seedlings grown with grass was reduced by warming above a threshold of 30°C. The growth of seedlings grown without grass was unaffected by warming. Survivorship of African savanna tree seedlings grown in the absence of grass was only approximately 15%, but survivorship of seedlings grown in the presence of grass was approximately 65%. These results show that the additive presence of grass and warming reduce seedling growth and subsequent survivorship, possibly due to a combination of reduced water availability due to grass competition, and direct warming effects on seedling respiration.

Seedling growth over time displayed a negative interaction with warming, but only above the temperature threshold of 30°C and in the presence of grass. This contrasts with studies in temperate systems where warming has been shown to increase successful tree seedling establishment (Franklin *et al.,* 2016) through increased growth rates and longer growing seasons (Henry *et al.,* 2012; Kaarlejarvi *et al.,* 2012; Volder *et al.,* 2013). This result is most likely explained by the additive effect of grass competition and warming as two independent stressors, where warming affects the carbon balance of the seedlings and grass limits the availability of soil water (Cramer *et al.,* 2012; Ketter and Holdo, 2018). Subsequent to declines in growth rate, this study found warming reduced the survival of savanna tree seedlings, especially in the presence of grass. The decline in growth rate and increase in mortality of seedlings is likely driven by direct temperature effects where high temperatures increase dark respiration, may adversely affect the carbon balance (Midgley *et al.,* 2004) and directly damage the thermo sensitive cellular components of seedlings (Volder *et al*., 2013; Zhou *et al.,* 2013; Yamori *et al.,* 2014).

A direct effect of warming on soil water availability has been excluded in this experiment (See Chapter 2), implicating the additive effect of warming and grass presence on tree seedling mortality. Unlike cooler temperate systems, plant growth in tropical savannas is highly sensitive to water availability (Bond and Midgley, 2003; Fensham *et al.,* 2005; Sankaran *et al.,* 2005). Consequently, the effect of soil water competition with C4 grasses plays an important role in tree seedling survival and growth (Grellier *et al.,* 2012; Volder *et al.,* 2013), which, in this study, adds to the direct warming effects on seedlings.

These findings support the large body of literature reporting the competitive effect of grass on tree seedlings (Higgins *et al.,* 2000; Bond, 2008; Bond *et al.,* 2012; Cramer *et al.,* 2012; Wakeling *et al.,* 2012). Instead of an interactive effect, where warming reduces the competitive effect of C4 grasses on tree seedlings (He *et al.,* 2013), this study shows warming has an additive effect on this interaction by enhancing the competitive effect of grass. Warming above 30°C reduced the growth rate of seedlings grown in the presence of grass and warming reduced seedling survivorship of those grown with grasses. I propose that the cumulative stresses of carbon imbalance and grass competition for soil water drives this decline in tree seedling growth, resulting in higher seedling mortality and fewer successful establishment events.

Competition is the predominant relationship between tree seedlings and grasses, making the seedling establishment phase one of the most important bottlenecks determining savanna structure and function (Midgley and Bond 2001; Bond *et al.,* 2012). However, there is evidence that the presence of grass may benefit the establishment of tree seedlings (Volder *et al.,* 2013; Greenwood *et al.,* 2015). In this study, seedlings grown in control plots had a higher chance of surviving if they were in the presence of grass. This beneficial role may be a result of intermittent shading by grasses, protecting seedlings from direct sunlight and high temperatures (Volder *et al.,* 2013), but it appears to fall away when temperatures increase. Grass cover may also reduces the rate at which soil moisture is lost to the atmosphere by evaporation (van Langevelde *et al.,* 2011), but its presence also means less water is available for uptake by establishing seedlings. Although grass may be beneficial at times, the competition for soil water by grasses (Cramer *et al.,* 2012) is much greater than the facilitative effect of shading because water is the major limiting factor to plant growth (Sankaran *et al.,* 2005).

This study shows that direct warming effects add to the negative effects of C4 grass competition on establishing seedlings. As the severity and frequency of drought increase with global change (Allen *et al.,* 2015), the addition of warming to these scenarios will likely exacerbate water stress and increase seedling mortality (Will *et al.,* 2013; Franklin *et al.,* 2016). A study in the forest - grassland ecotone found warming of 3°C (from 30°C to 33°C) decreased the period of survival of tree seedlings

by 13% (Will *et al.*, 2013). Elevated CO_2 is another global change driver that has been shown to increase the water-use efficiency of plants (Donohue *et al.,* 2013) and improve soil water availability (Owensby *et al.,* 1997; Polley *et al.,* 1997; Morgan *et al.,* 2001; Blumenthal *et al.,* 2018). Although it has been proposed to counteract the effects of warming (Xu *et al.,* 2013) a complete offset of the negative effects of both high temperatures and water stress on establishing seedlings, is unlikely (Leuzinger *et al.,* 2011; Allen *et al.,* 2015). Additionally, the responsiveness of C4 grasses to elevated CO_2 (Scheiter and Higgins, 2009; Manea and Leishman, 2015), high temperatures and water-limited conditions (Sage, 2004; van der Waal *et al.,* 2009; Edwards and Smith, 2010; February *et al.,* 2013) allows them to intensify their role as formidable competitors for soil water. For example, a recent study found that under elevated CO_2, *Shea* seedlings were unable to offset the effects of grass competition and drought (Tom-Dery *et al.,* 2018).

The additive effect of warming and C4 grass competition on seedling mortality suggests that in a warmer world there will be a decline in the establishment of African savanna trees. However, it is important to understand the relative significance of functional variations both between and within savanna communities (Osborne *et al.,* 2018), thus it would be useful to extend this work to a more diverse range of species and plant functional types. The effects observed in this study may be more pronounced in drier savannas where competition for water is greater, but this requires further investigation to understand the warming effects on tree – grass relationships in both wetter and drier savannas. These findings suggest a change in the balance of trees and grasses of savannas to more grass - dominated ecosystems. However, in some savanna regions a decline in tree establishment may be desirable to slow down and prevent further encroachment of woody plants, a phenomenon that is threatening savanna systems across southern Africa (Myers – Smith *et al.,* 2011; O'Connor *et al.,* 2014; Stevens *et al.,* 2017). Either way, savanna structure and function is likely to change as global warming progresses. Credible predictions of how they are likely to change are crucial to successful management and conservation of these systems.

3.6   Conclusion

This study appears to be the first field-based experiment in southern African savannas to investigate how warming will impact tree seedling growth and survival in a grassy system. The results of this study show that warming is likely to limit successful African savanna tree seedling establishment by acting as an additional stressor to C4 grass competition. Whilst this study shows a reduction in tree seedling establishment, it needs to be extended for different functional plant types and across a spectrum of savanna types. It provides an early suggestion that warming could counteract the negative impacts of woody encroachment but this requires further research as warming has also been found to

correlate with increasing encroachment (Shekede *et al.,* 2018; Venter *et al.,* 2018). This study, however, makes an early and important contribution to help start understanding the complexities of predicting the future of our savannas under a changing climate.

4. Synthesis: Warming and C4 grass competition as independent stressors of savanna tree seedling establishment

4.1 Background

A warming climate is a prominent feature of global change, with increased temperatures documented worldwide (IPCC, 2018). In temperate ecosystems where plant growth potential is primarily determined by climatic factors, experimentally applied and observed anthropogenic warming has resulted in increased plant establishment and growth (Yin *et al*., 2008; Wilson and Nilsson, 2009; Myers – Smith *et al.,* 2011; Elmendorf *et al.,* 2012; Kaarlejarvi *et al*., 2012; Greenwood *et al*., 2015; Hollister *et al.,* 2015; Bjorkman *et al.,* 2018). In warm desert ecosystems, warming treatments have been shown to increase mortality of endemic plant species, through exacerbating water stress (Musil *et al*., 2009). The potential role of warming is understudied in sub-tropical savanna ecosystems (Lehmann *et al*., 2014), where ecosystem structure and function may be controlled by the interacting impacts of fire, herbivory and climate, all acting on tree seedlings and saplings establishing within a grass sward (Scholes and Archer 1997; Sankaran *et al.,* 2005; Staver *et al.,* 2011).
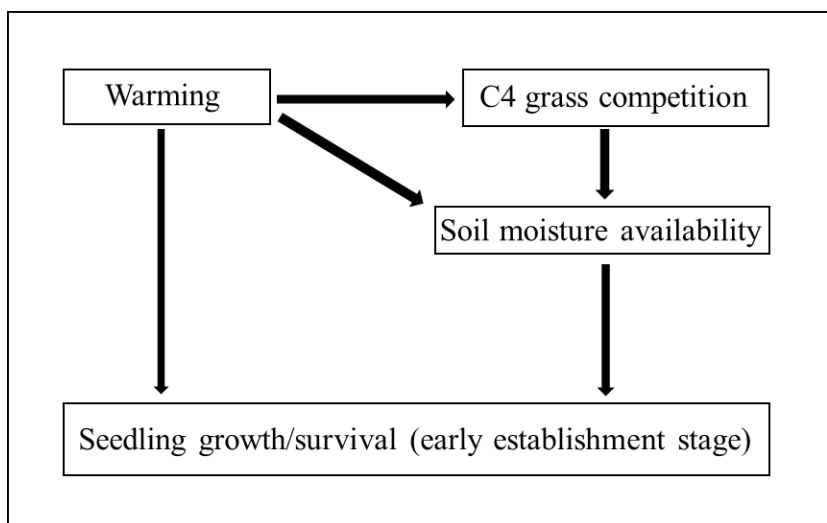
Figure 4.1: My conceptual model of the effects of warming on the growth and survival of establishing tree seedlings. Direct and/or indirect effects via C4 grass competition and soil moisture availability were expected to influence seedling growth and survival. Warming was predicted to increase seedling growth directly but enhance competition with C4 grasses by increasing grass growth and reducing soil moisture availability.

Disentangling these interactive impacts (*Figure 4.1*) will help predict ecosystem level impacts of warming. I developed a conceptual model with the understanding that seedling establishment is highly dependent on adequate water supplies (Walck *et al.,* 2011; Stevens *et al.,* 2014a) and studies show that warming increases evapotranspiration (Dai, 2013; Blumenthal *et al*., 2018), consequently reducing soil water availability (Wan *et al*., 2002; Hovenden *et al*., 2008; Polley *et al.,* 2013). Also, warming is expected to increase competition for water by stimulating the growth of C4 grasses (Edwards and Smith, 2010). Contrary to these expectations, direct effects of warming can increase plant growth (Volder *et al.,* 2010; Volder *et al.,* 2013) until a critical temperature. Not investigated in this study but important to consider is the rising CO_2 level associated with climate change, which may increase

the water – use efficiency of plants (Donohue *et al.,* 2013), and improve soil water availability (Blumenthal *et al.,* 2018), offsetting the potential negative effects of warming (Xu *et al.,* 2013). To begin unravelling the complexity of factors interacting to influence savanna structure and function, the effect of warming on tree seedling – grass relationship was investigated in this study.

4.2 Summary

4.2.1 *The effect of warming on the water relations of C4 grasses and establishing tree seedlings in an African savanna system.*

I investigated the effects of experimental warming on the water relations of tree seedlings as modified by the presence of grasses in a semi-arid savanna system. Warming of 1-2°C by passive open-topped chambers (POTCs) reduced humidity and increased soil and ambient temperature (see Chapter 2, *Figure 2.1*). Warming did not affect the soil water content, neither did it affect the water content of establishing tree seedlings. However, seedlings that were grown in the presence of grass suffered a reduction in leaf relative water content (RWC) (see Chapter 2, *Figure 2.2*). I conclude that, in an African savanna, warming does not change the soil water content, neither does it change the water content of tree seedlings. Reductions of water content in tree seedlings in the presence of C4 grass suggests competition for water between the two plant forms (Bond, 2008; Cramer *et al.,* 2012), regardless of warming.

4.2.2 *Direct and indirect effects of warming on the early establishment (growth and mortality) of an African savanna tree.*

In the second data chapter, I investigated the effect of warming on the growth and mortality of tree seedlings, grown with and without C4 grasses. Tree seedlings grown in the absence of grass were unaffected by warming, yet those that were grown in the presence of grass suffered a reduction in growth rates above a threshold of 30°C (see Chapter 3, *Figure 3.4*). Warming reduced seedling survivorship with highest mortality occurring when seedlings were grown with grasses (see Chapter 3, *Figure 3.4*). These findings show that warming and grass presence act as two independent stressors and have an important additive effect in suppressing seedling growth and increasing seedling mortality in African savanna trees.

4.3 Implications

4.3.1 *Implications of warming in a savanna system on growth and mortality of African savanna tree seedlings.*

I modelled both direct and indirect warming effects on establishing seedlings. Warming was expected to increase the growth of seedlings (Volder *et al.,* 2013) but also increase the rate at which water is

lost to the atmosphere (Dai, 2013; Bowman *et al.,* 2014), reducing the availability of soil water (Harte *et al.,* 1995; Wan *et al.,* 2002) crucial for successful seedling establishment. The model proposed that warming would increase grass growth as well, increasing the competitive effect of C4 grasses on establishing seedlings. The results obtained supported the model only to some extent, but added new understanding from a number of unexpected findings.
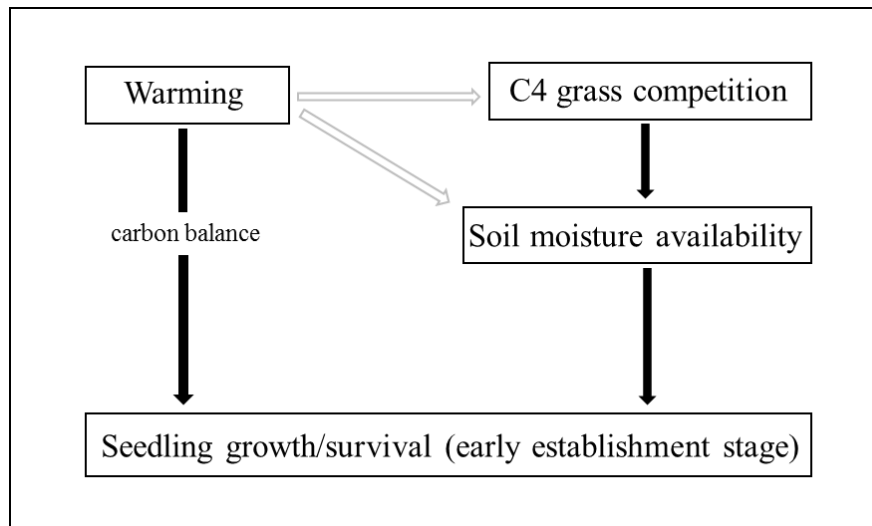
Figure 4.2: My conceptual model showing the results of warming on seedling growth and survival. The bold arrows indicate an effect seen and the white arrows indicate that no effect was seen in this study. C4 grasses had a strong negative effect on seedling survival and growth. It reduced the water content of seedlings, suggesting competition for soil water. Warming directly reduced seedling growth and survival which I suggest was caused by an increase in dark respiration as a result of temperatures above 35°C.

It was unexpected that grass competition would play such a large role in reducing seedling survival (*Figure 4.2*). The strong negative effect of grass on the water content of the tree seedlings lends support to the idea that this occurs via competition for soil water (Ludwig *et al.,* 2004; D'Onofrio *et al.,* 2015). Surprisingly, soil water content and seedling water content were unaffected by warming, leading me to infer a

direct effect of warming on seedling survival and growth by disruptions of their carbon balance where carbon output exceeds carbon uptake (Midgley *et al*., 2004). A drop in photosynthetic rate and an increase in dark respiration rate, as conditions warmed above 30°C, most likely accounts for the reduction in seedling growth. Higher temperatures were most likely responsible for seedling mortality, caused by carbon starvation or damage to their photosynthetic machinery (Zhao *et al.,* 2013). Warming also had no effect on grass biomass. This study makes an important and currently, a rare contribution to understanding the impact of warming on African savanna species, highlighting that warming can directly reduce the survival and growth of seedlings.

4.3.2 *Implications for savanna biodiversity in a warmer world.*

These findings suggest a future decline in the establishment of some African savanna tree species with warmer conditions. In savanna systems, establishing seedlings already face competition by C4 grasses and the addition of direct impacts of warming, amplifies the difficultly in establishing successfully. With an increase in drought duration predicted for southern Africa (IPCC, 2012) competition for water with C4 grasses is expected to increase. With the extra stress of high

temperatures, it is unlikely that seedlings can avoid desiccation by warming because a rapid adaptation rate is required to keep up with the increasing temperatures (Walck *et al.,* 2011). Adaptation however, is more likely to occur in herbaceous species such as grasses, due to their rapid life cycles, than woody species (Dalgleish *et al.,* 2010). This study's results suggest a decline in tree establishment, yet the question is raised of how widespread this phenomenon is in savanna woody species. Are my observations restricted to the study species, to other *Senegalia* species or is it a widespread occurrence that will cause a fundamental change in savanna systems and a shift to more grass-dominated systems? The effects of warming may be more severe in dry savannas where plants experience high levels of water stress and live near their thermal limits, however counter to that, these arid adapted species may be more resilient to increases in heat-caused drought (Stevens *et al.,* 2014a). Therefore, I emphasise that this study has shown important potential impacts of warming on savanna species but it also highlights the need for a greater focus on understanding the impacts of warming on a range of different savanna functional groups across the rainfall gradient in order to improve these estimates of how warming will impact woody tree growth in savannas.

If warming does cause a widespread increase in seedling mortality, will the failure of trees to establish come as a benefit and assist in the restoration of ecosystem function by acting against encroachment, a global phenomenon, threatening many open systems across southern Africa (O'Connor *et al.,* 2014)? This needs to be further investigated however, as studies such as these contradict other regional and global scale analyses which suggest that warming is likely correlated with an increase in encroachment (Shekede *et al.,* 2018; Venter *et al.,* 2018). These regional analyses suggest that warming may impact encroachment through direct positive effects of warming on C3 plant growth (Lehmann *et al.,* 2014) These differences though, highlight just how much more work needs to be done on this topic.

4.4   Future Research

This study showed that the presence of grass increased water stress within tree seedlings and that warming had a direct negative effect on seedling survival and mortality, potentially by disrupting their carbon balance. I provide evidence for an additive effect of grass competition and warming, severely reducing successful tree seedling establishment. Although identified in other studies (Stevens *et al*., 2014a; Engelbrecht *et al*., 2015; Xu *et al*., 2015,), this study on the other hand, found no significant reduction in soil moisture as a result of warming. Future research is required to clarify the role of warming on soil moisture availability in different environments. Importantly, physiological studies will be useful to better understand the effect of warming on seedling growth and survival and

will aid in determining the warming specifics responsible for declines in seedling growth and their demise. Investigation of a variety of plant functional types and species, from all types of savanna systems will provide a more thorough understanding of the warming effects on African savanna systems as a whole.

Whilst many studies suggest that warming is likely to result in a drier world (Dai, 2013; Bowman *et al.,* 2014; Blumenthal *et al*., 2018), this needs to be investigated with the interaction of increasing atmospheric CO_2. In African savanna systems, elevated CO_2 is expected to increase the water-use efficiency of plants (Donohue *et al.,* 2013) and so, as may be happening in this study, tree seedlings remain unaffected by changes in water availability. Although it is a possibility, there is little evidence to show for it (Tom-Dery *et al.,* 2018) and in fact seedling growth has been found to be negatively influenced by increases in CO_2 as a result of grass growth being more responsive to elevated CO_2 (Scheiter and Higgins 2009; Manea and Leishman 2015). These contradictory findings require further clarification on the combined effect of warming and CO_2 fertilization on plant growth.

Savannas are found along a gradient of rainfall (Sankaran *et al.,* 2005), with temperature playing a different role in vegetation growth across this spectrum. Consequently, the effect of warming is likely to vary across different savanna types. I predict that the additional effect of competition for water with C4 grasses will be greater in arid savannas than mesic savannas, where water is more readily available. Thus, fewer tree seedlings will be able to establish in arid savannas because the direct effects of warming will be experienced in conjunction with severe competition for soil water with C4 grasses. This concept however, contradicts the idea that drying will favour tree growth by limiting grass growth and that seedlings will struggle to establish in more mesic savannas where there is high grass biomass (February *et al.,* 2013; Morrison *et al.,* 2019). It is important to remember that as an establishing seedling, competition for water occurs in the upper soil layers with grasses (February and Higgins, 2010), so if grasses are unable to establish, tree seedlings, as the weaker competitor (Scholes and Archer, 1997), most definitely will not have access to water and will be unable to establish. Across this spectrum of savanna types, different species structure the coexistence of grasses and woody plants. Species are likely to vary in their ability to cope with warmer temperatures and their susceptibility to grass competition (Morrison *et al.,* 2019). Research in both drier and wetter savanna types, focusing on a variety of functional plant types and species is important for determining the persistence of savanna systems under a warming climate.

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