



UNIVERSITY OF THE
WITWATERSRAND,
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**The interactive effect of elevated CO₂ and rising temperatures on the growth of
Leucosidea sericea, *Festuca costata* and *Themeda triandra*.**

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A dissertation submitted to the Faculty of Science,
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in partial fulfilment of the requirements for the degree of Master of Science.

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DECLARATION

I declare that this Dissertation is my own, unaided work. It is being submitted to the Degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

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ABSTRACT

The atmospheric concentration of greenhouse gases such as CO₂ have been rising for the past two centuries, leading to a rise in global air temperature. Both atmospheric CO₂ and temperature are projected to rise further in the twenty-first century and changes in either are known to affect plant performance (specifically net primary production) which differs between different plant functional types, for instance trees and grasses, or plants with a C3 or C4 photosynthetic systems. They are also predicted to drive the expansion of plants to higher altitude and extinction rates, as a result, they drive the depletion of the earth's global biodiversity which is a major challenge of the twenty-first century. It has been noted that the tree *Leucosidea sericea* is encroaching into the formerly treeless high-altitude C4 grassland historically dominated by C4 grasses like *Themeda triandra* in the Drakensberg, South Africa. The expansion of patches of C3 grasses *Festuca costata* has also been observed and it is thought that the distribution of both these grasses and trees is changing due to elevated CO₂, high temperature, or an interaction between the two as a result of climate change. Therefore, the aims of this study were to: (1) test the individual and interactive effect of elevated CO₂ and temperature on the growth rate of a shrubby C3 tree *L. sericea*, a C3 grass *F. costata* and a C4 grass *T. triandra*; (2) to determine how variations in the competitive ability of *L. sericea*, *F. costata* and *T. triandra* are influenced by elevated CO₂ and temperature, projected to be reached later in this century. A full-factorial experiment, conducted in growth chambers, with two levels of CO₂ (400 ppm (ambient) and 650 ppm (elevated)) and two levels of temperature (ambient and +2°C above ambient) monitored the growth performance of the three species each representative of a plant functional type over a six-month period. The leaf traits (total chlorophyll content and leaf area) for each species were also measured at the end of the experiment. The plants were grown in pairs in separate pots interacting with individuals of their own species and the other species. The competitive interaction of the three species examined in pairwise combination were measured by calculating the relative yield per-plant (RYP) for each target and neighbour species. The results showed that there was no significant effect due to the independent and interactive effect of CO₂ and warm temperature on the growth rate and competitive interaction between any of the three species studied. This study suggests that there is no evidence that the overall effect of CO₂ at 650 and warm temperature will result in growth enhancement in *L. sericea*, *F. costata* and *T. triandra* nor will they influence the competitive interaction between the three species. As a result, they will not

help explain the observed upward movement of *L. sericea* into higher altitudes nor the spread of *F. costata* in the north-facing slopes previously dominated by C4 grasses like *T. triandra* in the Drakensberg. This study acknowledges that, apart from the limitations of pot size, success in plant establishment and sample size, there may have been other climatic and non-climatic factors such as precipitation, light availability, the functional traits of species, human disturbances, herbivory/grazing pressure and fire frequencies which were not considered in this study but might drive changes in community compositions in the Drakensberg. Therefore, it is suggested that future studies look into these factors which are likely to explain the upward movement of *L. sericea* and the spread of *F. costata* in high altitude of the Drakensberg Mountain.

Keywords: climate change; C3 and C4 plants; Drakensberg; growth response

DEDICATION

*“You are worthy, O Lord, To receive glory and honor and power; For you created all things,
And by Your will they exist and were created” – Revelations 4:11 (NKJV)*

This dissertation is dedicated to my sweet and loving mother Mirriam Maphuthi Lebese, whose unyielding support, encouragement, spiritual and emotional support inspired me to complete this study. Thank you for teaching me all that you have and for your endless prayers and sacrifices.

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LIST OF ABBREVIATIONS

AIC – Akaike’s Information Criterion

ANOVA – Analysis of variance

CO₂ –Carbon dioxide

FACE - Free air CO₂ enrichments experiments

GLMER – Generalized linear mixed-effect model

LME – Linear mixed effect model

LM – Linear regression model

OAA – Oxaloacetic acid

PAR – Photosynthetically active radiation

RYP – Relative yield per plant

SLA – Specific leaf area

WUE – Water use efficiency

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1. CHAPTER 1: General introduction

1.1. Introduction

1.1.1. *Effect of global change on biodiversity*

Global climate change continues to be a major threat to natural ecosystems and biodiversity (Díaz *et al.* 2019). Climate change is predicted to have serious implications for global biodiversity, including how species interact with one another and their habitats which results in changes in plant range and their community structure (Thuiller 2007; Bellard *et al.* 2012). These impacts have been and continue to be observed at the individual and population level, through changes in behaviour and morphology in species, distribution range shifts, and changes in primary production at the ecosystem level (Weiskopf *et al.* 2020). Assessing the magnitude and direction of these impact will help us predict and adapt to unwanted changes in the future (Weiskopf *et al.* 2020).

An increase in the concentration of atmospheric CO₂ as a result of climate change leads to a rise in global air temperature and recent projections predict a temperature increase of more than 2°C or more and increasing annual precipitation at higher altitude later in this century (IPCC 2014). In the southern African region, temperature is projected to rise by 0.8°C to 3.6°C (Tadross *et al.* 2011). The impacts arising from changes in the level of CO₂ and temperature are likely to be more evident and immediate in mountains (Halpin 1994) because plants that grow well in mountains are restricted by or have already adapted to a narrow variety of environmental conditions (Beniston 2003). Additionally, these changes are predicted to drive the expansion of plants to higher altitude and extinction rates, as a result, they drive the depletion of the earth's global biodiversity which is a major challenge of the twenty-first century (Parmesan and Yohe 2003; Parmesan 2006; Thuiller 2007; Butchart *et al.* 2010; Bellard *et al.* 2012).

1.1.2. *The impacts of climate change on the expansion of species into higher altitudes in mountain regions.*

The impact of climate change pose a significant threat in montane biodiversity (Bhusal *et al.* 2016; Taylor *et al.* 2016; Mukwada and Manatsa 2018). Global warming is generally considered a major driver of these impacts because vegetation in mountain regions is limited by low temperatures (Klanderud and Birks 2003; Walker *et al.* 2006; Elmendorf *et al.* 2015). As a result, the progression of grass species and expansion of shrubs into higher altitude is

generally attributed to an increase in the average air temperature which has the potential to change the structure of mountain ecosystems and the interaction between species and their habitats (Myers-Smith *et al.* 2011; Mayor *et al.* 2017).

Although several studies conducted in mountain regions found that species expand upward along the elevation gradient due to warming effect of climate change (Walther, Beißner and Burga 2005; Gottfried *et al.* 2012; Zu *et al.* 2021), there are other non-climatic factors such as grazing and land management which are considered as important in influencing the expansion of species into higher altitudes (Vanneste *et al.* 2017). Pajunen *et al.* (2013) who found that grazing pressure can potentially buffer the effect of climate warming-driven changes on shrub expansion as it can slow down an increase in species richness and abundance at higher elevations. Evidence for the effect of grazing on species richness along the elevation gradient was provided by Speed *et al.* (2013) in a field experimental study who found that when grazing was excluded at low elevation, species richness declined by 3.7 species and at high elevation it increased by 3.5 species, but where grazing was increased or maintained, the changes were less extreme suggesting that grazing can reduce changes in species richness and plant communities. However, some studies suggest that grazing and other non-climatic factors might intensify the impact of climate change on these expansions (Christensen *et al.* 2004).

While species richness may decline at high altitudes as a result of climate change, Jump *et al.* (2012) showed that species endemism doubled from 23 to 55% with increasing altitude and this correlation has also been reported in other mountain regions around the world. However, at even higher elevations, the species endemism may plateau or decline gradually because families include more species at lower elevation (Vetaas and Grytnes 2002; Grau *et al.* 2007; Wang *et al.* 2007). As species migrate to higher altitude, whether they are influenced by climatic factors (e.g., rising temperature) or non-climatic factors (such as grazing or increased number of competitors), their population numbers and size may decline due to unsuitable habitats which raises concerns of some species experiencing stochastic extinctions although there has not been evidence of this extinction occurring (Pauli *et al.* 2007; Sekercioglu *et al.* 2008; Chen *et al.* 2009).

The Drakensberg mountain region in Kwa-Zulu Natal, South Africa, is known for its species richness of rare species and high levels of endemism (Carbutt and Edwards 2003) and like other mountain regions, the Drakensberg is also susceptible to the impact of climate

change due to rise in average temperatures (Morris 2017): the rate of temperature rise in the Drakensberg in the 1960 – 2016 period is estimated to be between 0.03°C/annum and 0.01°C/annum (Mohamed and Mukwada 2019). In addition to climate change, other non-climatic factors including intense grazing is expected to intensify the effect of rising temperatures on species distributions in the Drakensberg (Quinlan and Morris 1994; Christensen *et al.* 2004).

The Drakensberg is dominated by vegetation with different functional groups such as C3/C4 grasses and C3 shrubs which co-occur in this region (Mucina and Rutherford 2006). Shifts in the distributions of these plants is attributed to changes in the level of CO₂ and temperature which vary between different functional groups (Morris 2017). For instance, C3 plants benefits from CO₂ enriched environment and C4 plants are highly favoured by warm temperatures relative to C3 plants which are disadvantaged by warm temperatures (Pearcy and Ehleringer 1984; Ehleringer *et al.* 1997). As a result, the type of vegetation in the Drakensberg mountain regions can be used to assess changes in the level of CO₂ and temperature (Mukwada and Manatsa 2018).

Morris (2017) proposed how changes in the level of CO₂ and temperatures may alter the distribution of C3/C4 plants by suggesting that longer growing seasons and warm temperatures in mountainous areas should enable C4 grasses to expand to higher altitudes. This correlates with Ehleringer *et al.* (1997) quantum yield of C3 and C4 plants which predicts that the quantum yield of C3 and C4 plants at any temperature increases with concentrations of CO₂ although C4 plants generally have a higher optimum temperature than C3 plants because their distribution is limited by low temperatures (Angelo and Daehler 2015). However, their expansion is influenced by competition from C3 grasses which benefit more from the effect of elevated CO₂ (Pearcy and Ehleringer 1984; Ehleringer *et al.* 1997) because of the relative benefit they get from reduced photorespiration at high CO₂. Hence, the growth response of C4 plants is expected to be lower than C3 plants at high CO₂ because C4 plants already have a CO₂ concentrating mechanism which enable them to concentrate high levels of CO₂ of up to 2000 ppm in their bundle sheath cells (Poorter and Navas 2003; Ehleringer 2005; Mishra *et al.* 2019). The effect of CO₂ will also interact with other extreme environmental conditions (too dry, wet or hot) that are projected to correspond with climate change in the future (IPCC 2014; Obermeier *et al.* 2017). Intense overgrazing pressure by grazers (e.g., small ungulates wildlife grazers) on palatable grasses such as *T. triandra* (Joubert

et al. 2017) may attenuate any changes driven by climatic variables on the distribution of C4/C3 grasses. However, warming and selective grazing may favour grazing resistant C4 grasses more relative to palatable C4 grasses like *T. triandra* (Morris 2017).

1.1.3. Observed distribution of woody plants, C3 and C4 grasses in the Drakensberg, and trends over time

C3 and C4 grasses have been observed to co-occur in the montane grassland of the Drakensberg, South Africa (Mucina and Rutherford 2006). As expected, C4 grasses are more dominant on north-facing slopes and at low altitude (both of which are associated with warmer climates); conversely, C3 grasses are more dominant on south-facing slopes and higher altitudes (Morris 2017). However, it has also been observed that the native C3 species *F. costata* is now more abundant in montane areas of the Drakensberg, including on the north-facing slopes historically dominated by C4 grassland including grass species like *T. triandra* (Bentley and O'Connor 2018). This change has been attributed to the effect of elevated CO₂, on the argument that if it was a response to rising temperatures only, the abundance of C3 grasses should decrease on the cooler, south-facing slopes (Bentley and O'Connor 2018).

The abundance of *F. costata* in the Drakensberg could have a negative impact on species diversity overall as they move into formerly C4 dominated grassland. This is because it forms large tufts that may outcompete the more diverse C4 grass communities by out shading them (Hilliard and Burt 1987). Furthermore, some C3 grass species like *F. costata* are evergreen (Werger and Van Bruggen 2012) and continue to transpire even over the dry winter season. Therefore, they are expected to use a greater quantity of water than C4 plants. The presence of green grass in the dry season could limit the occurrence and spread of fires, which helps maintain C4 dominated grasslands (Bentley and O'Connor 2018; McGranahan *et al.* 2018).

The change in the upward movement of C3 grasses into the high altitude of South Africa could be due to either elevated CO₂ or warmer temperatures, or an interaction between the two (Sieben *et al.* 2010). Additionally, the encroachment of shrubs into higher altitude has also been documented to be due to these changes (Morgan *et al.* 2007; Angelo and Daehler 2015) and one such species that has also been observed to be creeping upward on the eastern escarpment margins of the highveld in the Drakensberg is a C3 woody shrub *Leucosidea sericea* (Adcock 1990; De Villiers 2013). Morgan *et al.* (2001) found that woody

species benefit relatively more from elevated levels of CO₂ than herbaceous plants because of their larger roots, increased stem diameter, more branches, multi-stems and below-ground storage which increases their carbon sinks (Kgope *et al.* 2010), but Bond and Midgley (2000) suggests that these benefits are only noticeable during the initial phase of CO₂ enrichment only. Burning experiment in the Drakensberg also show that the abundance of *L. sericea* shrubs is affected by fire (De Villiers and O'Connor 2011), so again, there are potential interactions with non-climatic drivers that remain to be explored.

1.2. Rationale

The expansion of mountain species in response to the impact of climate change have significant impact on the mountain ecosystem and biodiversity (Gottfried *et al.* 2012; Zu *et al.* 2021). Zu *et al.* (2021) shows that the expansion of species in higher elevations is influenced by changes in climate variables such as warmer temperatures (Angelo and Daehler 2015; Feeley *et al.* 2017; Morris 2017), as well as changes in atmospheric CO₂. However, these global change drivers can sometimes have opposite effects and are expected to have different impacts on different plant functional types. Therefore, range expansions due to the effect of global change remain equivocal (Mukwada and Manatsa 2018). Additionally, Rowe *et al.* (2015) suggest that climate change may not only influence the expansion of species into higher altitude but may also limit their ability to expand which will likely lead to local extinction of endemic species (La Sorte and Jetz 2010; Freeman *et al.* 2018). These studies show that there are still controversies associated with the shifts and drivers of the distribution of these species as they expand into higher altitudes (Zu *et al.* 2021). Therefore, identifying the main climatic variable that influence the distributions of mountainous species along the elevation gradient is important in assessing how climate change will influence the individual response of these species and their interaction with other species (Grytnes *et al.* 2014; Serra-Diaz and Franklin 2019).

1.3. Aims and objectives

The aim of this study was to investigate the effect of elevated CO₂ and warm temperatures (individually and in combination) on the growth of *Leucosidea sericea*, *Festuca costata* and *Themeda triandra*.

The objectives of this study were to:

1. Test the individual and interactive effects of warm temperatures and elevated CO₂ on the survival, productivity and leaf traits of *Leucosidea sericea*, *Themeda triandra* and *Festuca costata*, grown alone, over a six-month growth season.
2. To determine the competitive interactions between a tree, *Leucosidea sericea*, a C3 grass *Festuca costata* and a C4 grass *Themeda triandra*, in response to the individual and interactive effect of elevated CO₂ and warm temperature by testing differences between their biomass accumulation over a six-month growth period.

1.4. Study approach and thesis structure

Chapter 1: General introduction – The first chapter of this dissertation consists of a general introduction to global change impacts in mountains, and a summary of what is known about the range shifts of the tree *Leucosidea sericea* and C3 grass *Festuca costata* into higher altitude grassland previously dominated by C4 grasses like *T. triandra*.

Chapter 2: Research Methodology – The second chapter describes the methods that were used to conduct this dissertation which will serve both Chapter 2 and Chapter 3.

Chapter 3: Growth response – The third chapter investigates the response of *Leucosidea sericea*, C3 grass *Festuca costata* and C4 grass *Themeda triandra* to elevated CO₂, warm temperature and the interaction between two.

Chapter 4: Competitive relationships – The fourth chapter investigates the effects of competition between a tree, a C3 grass and a C4 grass under the individual and interactive effect of elevated CO₂ and warm temperature.

Chapter 5: Synthesis and conclusions – Lastly, this chapter includes general discussion of the research findings and further research suggestions and recommendations.

2. CHAPTER 2: Research methodology

2.1. INTRODUCTION

The purpose of this chapter is to describe the research process followed in this study. It describes the methods that were used to conduct the research and serves to support both Chapter 2 and Chapter 3. It covers the various stages of the research and includes descriptions of the study species and where they were sourced, the experimental design and the type of data that was collected.

2.2. METHODS

2.2.1. Origin of the plant material

The grass plant material was obtained from wild populations growing at Mike's Pass (under a permit issued by eZimvelo Wildlife), at an elevation of 1700m, close to the current treeline, near Cathedral Peak in the Natal Drakensberg (28.961125°S, 29.231068°E). The grass specimens were sampled by cutting out sods of earth in which tufts of the grasses were established. The two species were growing adjacent to one another, and *Leucosidea sericea* was growing one hundred meters away, at the same altitude. The *Leucosidea sericea* seedlings used in the experiment were purchased from the nursery at the Pietermaritzburg Botanical Gardens, where they were established in 5 litre plastic bags, from seeds of Drakensberg origin.

2.2.2. Study species

Leucosidea sericea is a shrubby C3 tree known as *ouhout* in Afrikaans and *umTshitshi* in Zulu (Foden and Potter 2005), is the only species in the genus *Leucosidea* (Pooley 2003), in the family Rosaceae. The Rosaceae are typically the family which provides the highest altitude trees in Afromontane ecosystems (White 1980). *Leucosidea sericea* usually grows as a short semi-evergreen shrub or tree with a height ranging from 2 - 9 m (Mafole *et al.* 2017). The leaves are simple, dentate, slightly hairy and approximately 20 x 10 mm in size. *Leucosidea sericea* grows in a broad range of habitats including damp sandy or clay soil, riverbanks, grasslands, rocky soils and rocky ridges (Mafole *et al.* 2017). It flowers between August and December. The small yellow flowers are arranged in terminal sprays (Mafole *et al.* 2017). It has a wide distribution in high-altitude areas of southern Africa: Mpumalanga, Limpopo, Gauteng, Kwa-Zulu Natal, Free state, North West and Eastern Cape (Raimondo *et al.* 2009).

Leucosidea sericea has been suggested to be invading into montane grasslands, particularly at its upper distributional limit.

Festuca costata is a C3 grass species that occurs in montane grassland in South Africa (McGranahan *et al.* 2015). The fibrous tillers of *F. costata* species are considered by livestock farmers to be poor forage and unpalatable to livestock. However, it has economic value to the local populations in southern Africa as it is used to make brooms and traditional baskets (Chippindall and Crook 1976). Generally, very little is known about *F. costata* (McGranahan *et al.* 2015). *Festuca costata* has been anecdotally suggested to be invading higher altitude grasslands.

Themeda triandra is a C4 perennial tussock grass species endemic to Asia, Australia and Africa (Snyman *et al.* 2013). In South Africa it is commonly known as *rooigras* or Red Oat Grass and is widely distributed, though perhaps in ecotypic variants (Raimondo *et al.* 2009). *Themeda triandra* occurs in a wide range of habitats including areas with a wide range of rainfall (300 – 6250 mm), altitudes, and soil types including, amongst others, silty-clay loam, heavy clays, sandy and alluvial soils (Snyman *et al.* 2013). It is well adapted to fire, sensitive to uninterrupted selective grazing regimes and has a substantial economic and ecological value as it is considered a keystone species for grazing systems, due to its high palatability and productivity (Snyman *et al.* 2013). In this study, it is a ‘representative C4 grass’ of the high-altitude montane grasslands.

2.2.3. Experimental design and protocol

The study was conducted at the University of the Witwatersrand, Johannesburg, South Africa (26°11'27"S 28°1'49"E) in four walk-in CONVIRON growth chambers. The chambers were programmed to provide a complete factorial interactive design of two levels of CO₂ by two levels of temperature (Table 2.1). The two CO₂ levels imposed were 400 ppm (close to the current ambient) and 650 ppm, which is a level that could be reached later in this century (IPCC 2014). A diurnal cycle of temperature was used for the duration of this experiment, based on the mean summer diurnal temperature range at the altitude of the current treeline in the Drakensberg where grass species were collected. This location is one where upward movement of *Leucosidea sericea* and *Festuca costata* has been anecdotally observed. The elevated temperature treatment was obtained by adding 2°C to this range, which is approximately the increase anticipated between the current temperature and the

temperature corresponding with a concentration of CO₂ of 650 ppm (IPCC 2014). Light was provided using a combination of LED and incandescent bulbs at PAR (Photosynthetically Active Radiation) of 140 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ to 1063 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$, during the daylight hours starting at 06:00 am and ending at 18:00 pm.

Table 2.1. Complete 2 x 2 factorial experimental design with three species, *L. sericea* (L), *F. cosata* (F) and *T. triandra* (T) and six pairwise combination (LL, TT, FF, TF, LF and LT) replicated three times under each sub-treatment comparing current (400 ppm) and elevated CO₂ (650 ppm) at current ‘ambient’ and warmer temperature (2°C).

		Temperature	
		Ambient	Warm (2°C)
CO ₂	400 ppm	3 spp, 6 pairwise-combination, 3 reps	3 spp, 6 pairwise-combination, 3 reps
	650 ppm	3 spp, 6 pairwise-combination, 3 reps	3 spp, 6 pairwise-combination, 3 reps

The plants and treatments were swapped around between the chambers at the end of the third month of the experiment to avoid a chamber effect. Therefore, although we only had one treatment in each chamber, we minimised issues associated with pseudoreplication. The water supplied to the pots was based on the daily average summertime rainfall (from 2015 to 2019) of 2.724 mm at Cathedral Peak which equates to 532.90 ml/day of water for each rectangular pots size of dimensions: 320mm L x 150mm W x 106mm H that the plants were grown in. The soil in the pots was a moderately fertile sandy clay loam, similar to the soils at the Cathedral Peak site where the grass plants were sourced.

Two plants were placed in each pot, either of the same species, or two of the three species in combination, resulting in six possible combinations: LL, TT, FF, TF, LF and LT where L = *Leucosidea sericea*, F = *Festuca costata*, T = *Themeda triandra* (Figure 2.1). Each plant combination was replicated three times in each chamber (6 x 3 = 18 pots per chamber). The physical layout in each chamber consisted of two tables 1000 mm high tables (thus about 1.5 m below the lights) of dimensions 760 x 1210 mm, on which the treatments and replicates were randomly placed (Figure 2.1).

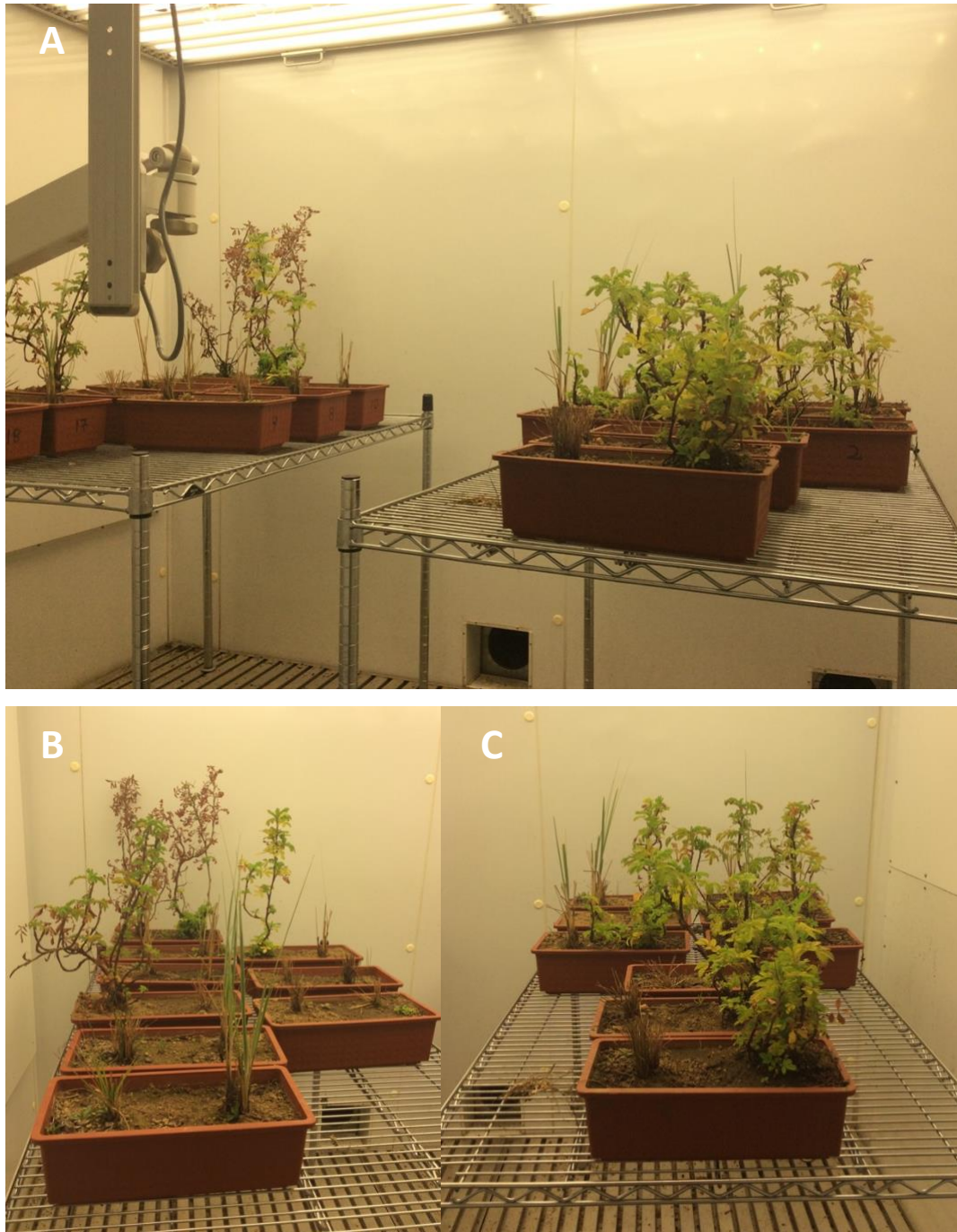


Figure 2.1. The experimental set up in each chamber (A) consisting of two tables (B and C) with six possible combinations (LL, TT, FF, TF, LF and LT where L = *Leucosidea sericea*, F = *Festuca costata*, T = *Themeda triandra*) that were replicated three times and randomly placed on each table (Photos: EM Lebesse).

The *Leucosidea sericea* seedlings, initially of about 15 cm in height, and tussocks consisting of three height-trimmed culms each of *T. triandra* and *F. costata* grasses, were

allowed to grow for six months (March - August 2019), which is approximately the length of a growing season at an altitude of 2000 m asl on the eastern escarpment.

2.2.4. Data collected

2.2.4.1. At the time of experiment establishment

The ground-level stem diameter and stem lengths of each tree seedling was measured with a digital calliper (0-150 mm, 0.01 mm resolution) and a clear ruler respectively (1 mm resolution). If the seedling branched, the main stem, then the diameter and length of all the subsidiary branches was measured. Additionally, the number of leaves on each seedling of *Leucosidea sericea* were also counted. For each grass species, the aboveground biomass was monitored by using a proxy based on counting the number of individual tillers, measuring the height of the green material and the basal circumference of the tuft, measured using a tape with resolution of 1 mm.

2.2.4.2. Once a month during the 6-month experiment

The apparent death of individuals was noted, and the plants were not replaced. The aboveground growth of *Leucosidea sericea* trees was monitored by measuring the basal diameter of each stem, height of the longest and subsidiary stems, where present, when gently stretched out. For each grass individual, the number of tillers, height of the tallest green point when stretched gently and the basal circumference of the tuft were measured.

2.2.4.3. At termination of the experiment

Three fully expanded leaves were cut from each species and weighed (wet) on a balance to 0.01 mg. The leaf was then transferred to test tube containing 10 mL of 95% ethanol in order to extract the chlorophyll (Chl). The test tubes were sealed with a parafilm and left at room temperature for 72 hours. After 72 hours, 3 mL of the ethanol extractant was transferred to quartz cuvette and characterized using a dual-beam spectrophotometer at wavelengths A_{470} , $A_{648.8}$, $A_{663.2}$ (Lichtenthaler 1987). To correct for turbidity and contaminating coloured compounds, the absorbances at A_{750} were also recorded (Pompelli *et al.* 2013), since, Chl 'a', Chl 'b' and carotenoids do not absorb at this wavelength. If turbidity was found, the test tubes with samples were centrifuged for 10 minutes at 1 120 g force before they were reanalysed as described by Lichtenthaler and Buschmann (2001). The zero absorbance was reset on the spectrophotometer using a blank consisting of a quartz cuvettes filled with pure 95% ethanol after each absorbance reading. The concentration of the extracts was then converted

(equations 1 to 4) into leaf chlorophyll content (g.kg^{-1} LW) as described by Lichtenthaler (1987).

Equation (1): $\text{Chl "a"} = (13.36 A_{664.2}) - (5.19 A_{648.6})$

Equation (2): $\text{Chl "b"} = (27.43 A_{648.6}) - (8.12 A_{664.2})$

Equation (3): $\text{Car} = ((1000 A_{470}) - (2.13 \text{ Chl "a"})) - (97.64 \text{ Chl "b"}))/209$

Equation (4): $\text{Total Chl} = \text{Chl (a+b)}$

The plants were harvested, and the soil was gently washed off the roots. The plants were then separated into roots, stem and leaves (Figure 2.2), keeping the individuals from each species apart, and recording dead and live plants separately. Thereafter, the subsamples were dried in paper bags for 24 hours at 60 °C in a forced convection oven and weighed on a balance to 0.1 mg for each part, which were then summed to get their total dry biomass. Colour photographs of three leaves from each species were taken with a digital camera against a plain white background. After image analysis, the Leaf Area of each leaf was solved for using the ImageJ version 1.5.3 computer image programming software (Abràmoff *et al.* 2004), which along with the dry mass of the leaves, allowed a specific leaf area (SLA) to be calculated.



Figure 2.2. *Leucosidea sericea* (A) and *Themeda triandra* (B) separated into leaves (l), stems (s) and roots (r) (Photos: EM Lebesse).

Details of the statistical analyses applied are to be found in the individual data chapters.

3. CHAPTER 3: The response of *Leucosidea sericea*, C3 grass *Festuca costata* and C4 grass *Themeda triandra* to elevated CO₂ and temperature.

3.1. ABSTRACT

The atmospheric concentration of greenhouse gases such as CO₂ have been rising for the past two centuries, leading to a rise in global air temperature. Both CO₂ concentration and near surface air temperature are projected to continue in the coming decades, at rates dependent on the degree to which the countries of the world implement climate change mitigation measures. Both of these environmental drivers are known to affect plant performance (specifically net primary production), and the response differs between different plant functional types, for instance trees and grasses, or plants with a C3 or C4 photosynthetic systems. This study sets out to test the effect of elevated CO₂, rising temperatures and the interaction between the two on the growth rate, total biomass, leaf area and chlorophyll content of *Themeda triandra* (C4 grass), *Festuca costata* (C3 grass) and *Leucosidea sericea* (shrubby C3 tree). A full-factorial experiment, conducted in a growth chamber, with two levels of CO₂ (400 ppm (ambient) and 650 ppm (elevated)) and two levels of temperature (ambient and +2°C above ambient) monitored the growth performance of the plants over a six-month period. The results showed that the independent and interactive effects of elevated CO₂ and warm temperature did not influence the growth rate and biomass accumulation in any of the three species. However, the growth increment of both *L. sericea* and *T. triandra* was higher and that of *F. costata* was slightly lower under ambient conditions. This study suggests that the overall effect of CO₂ at 650 ppm (150 ppm higher than at present), and temperature 2°C warmer than at present are unlikely to result in growth enhancement in *L. sericea* and *T. triandra*. However, more follow up work is needed under more realistic conditions and with more replicates to confirm this.

Keywords: biomass accumulation, chlorophyll, growth rate, leaf area, survival.

3.2. INTRODUCTION

3.2.1. *Plant responses to elevated atmospheric CO₂ concentrations*

CO₂ is an important resource for plants, that affects photosynthesis and growth rates (Long *et al.* 2004). The concentration of CO₂ in the atmosphere has been increasing since the start of the Industrial Revolution, nominally about 1750, and is predicted to increase even further in this century (IPCC 2014). Current levels of CO₂ are over 200 ppm above the pre-industrial levels of 280 ppm (WMO 2017). In the absence of strenuous, worldwide efforts to reduce CO₂ emissions, models project an increase to 650 ppm by the middle and 900 ppm by the end of the century (IPCC 2014).

Many studies have shown that the concentrations of CO₂ in the present time are already impacting productivity in a range of different ecosystems (Ainsworth and Long 2005; van der Kooi *et al.* 2016; Dong *et al.* 2020). CO₂ is the source of carbon for the plant, from which it synthesises the carbohydrates that ultimately become both the structure of the tissues, and the energy source for their construction (Taub 2010; Thompson *et al.* 2017). Plant biomass is approximately half carbon (Frank *et al.* 2004). Plants take up CO₂ from the atmosphere through their stomata, and convert into sugars through the process of photosynthesis (Taub 2010; Thompson *et al.* 2017). These sugars then form the feedstock for all other carbon-based compounds.

The atmospheric concentration of CO₂ can affect plant water use by controlling the opening of the stomata (Taub and Wang 2013). Water diffuses out of the leaves principally via the stomata, thus CO₂ assimilation inevitably results in water loss (Taub and Wang 2013), and the ratio of the two is known as the 'water use efficiency'. One widely reported plant response to elevated CO₂ concentrations is to reduce the stomatal conductance (which then also reduces water loss) while maintaining the same CO₂ assimilation rates (Morison and Gifford 1983; Morison 1985).

A second effect is that at low CO₂ concentrations, much of the CO₂ that is initially fixed from the atmosphere is subsequently lost through photorespiration, which is the result of competition between CO₂ and O₂ at the key binding site on the enzyme Rubisco (Taub and Wang 2013). Therefore, an increase in the concentration of atmospheric CO₂ increases net photosynthesis, by reducing the strength of this competition, and thus the magnitude of photorespiration (Kramer 1981). When combined with the reduced water loss owing to reduced stomatal conductance (Bernacchi *et al.* 2007), the water use efficiency increases,

along with the productivity of plants, as revealed by their total dry mass accumulation over a given period of time (Marschner 2011; Ziska 2008). Since C4 plants have much reduced photorespiration, that effect is much weaker for them, but the stomal conductance reduction still partly occurs (Sage and Kubien 2007), and can result in increased productivity overall under high CO₂ conditions. C4 grasses already have a much higher WUE than C3, so in theory gain less from rising CO₂ (Morgan *et al.* 2011).

Jongen and Jones (1998) investigated the effects of interspecific relationships on the responses of plants to elevated CO₂ and found that the development and growth responses are altered in all species, but to differing degrees. This leads to changes in their relative competitive ability. In grasses, the positive response is usually indicated by an increase in the number of tillers (Nicolas *et al.* 1993). In other plant species, including trees, a typical response to elevated CO₂ is a reduction in the specific leaf area (i.e. leaf surface area per unit mass) that is caused by denser cell packaging (Ryle and Powell 1992). Due to the differential responses, the species composition of natural plant communities is likely to change at elevated CO₂. The studies also show that plant species ability to respond to elevated CO₂ is influenced by the environmental conditions, such as the light intensity, temperature and nitrogen supply, which further results in different responses to elevated CO₂ among different plant species (Jongen and Jones 1998; Wand *et al.* 1999), both under experimental and natural conditions.

This difference has also been noted in plants possessing the C3 and C4 photosynthetic pathway. A lot of studies have been published on the response of C3 plants to elevated CO₂ and rising temperatures, most of which found that C3 plants will be favoured under elevated CO₂ (Mishra *et al.* 2019). This is because C3 plants are more affected by photorespiration than C4 plants (C4 plants already have a CO₂ concentrating mechanism), so C3 plants get a greater relative benefit from reduced photorespiration at high CO₂ (Sage and Kubien 2007). Elevated CO₂ increases the growth rate of C3 plants. In contrast to C3 plants, the response of C4 plants to elevated CO₂ is expected to be lower than C3 plants because C4 plants already pre-concentrate CO₂ internally to 2000 ppm in their mesophyll tissues (Poorter and Navas 2003; Mishra *et al.* 2019). As a result, the growth rate of C4 plants under elevated CO₂ is not expected to rise at levels of CO₂ below 2000 ppm (Mishra *et al.* 2019), although they can still theoretically benefit through increased water use efficiency and this has been demonstrated experimentally (Morgan *et al.* 2011).

3.2.2. *Plant responses to warmer temperatures.*

An increase in the level of atmospheric concentration of greenhouse gases including CO₂ leads to a rise in the global air temperature (IPCC 2014). The present global mean air temperature near the Earth's surface is currently about 1°C above the inferred 1750 level, and in southern African interior sites such as Cathedral peak, nearly 2°C higher (Engelbrecht and Landman 2010). The global mean air temperature is projected to rise to 2°C – 5°C above the pre-industrial mean in this century (IPCC 2014), despite Paris Agreement ambitions of keeping the rise to less than 1.5°C (UNFCCC 2015). Plant carbon assimilation generally shows a unimodal response to temperature (Figure 3.1). Photosynthesis increases with increasing air temperature up to an optimum, after which it rapidly tails off as enzymatic activity is negatively affected and photorespiration reduces carbon assimilation rates (Hatfield and Prueger 2015).

Autotrophic respiration (including respiratory pathways other than photorespiration), on the other hand, continues to rise at exponential rates; thus, respiration rapidly exceeds assimilation at temperatures in the range 30-40°C, putting the plant into net carbon deficit (Piao *et al.* 2010). However, the effect of warm temperatures differs among plants, as each plant species has a specific range of temperature for its minimum, maximum and optimal development (Hatfield *et al.* 2008; 2011). This optimum temperature is affected by the photosynthetic pathway of the plant (C3 plants usually have lower optima than C4 plants, due to photorespiration being more of a problem at high temperatures (Figure 3.1)).

The optimum is generally shown to correlate with the mean annual growing season temperature the plants are exposed to (Ehleringer *et al.* 1997). At a plants' optimum temperature, the vegetative development, including the plants' leaf and the node appearance rate, also increases. When temperature levels increase to level considered to be extreme and harmful to plants, photosynthesis, transpiration, grain formation and pollen viability are all negatively affected (Hatfield *et al.* 2011). This leads to reductions in the plant's biomass and productivity (Hatfield *et al.* 2011). Plant biomass and productivity is also reduced in climates where there is high seasonal variation, with temperatures within the year alternating between too hot or too cold for the growth of plants (Hunt *et al.* 1996).

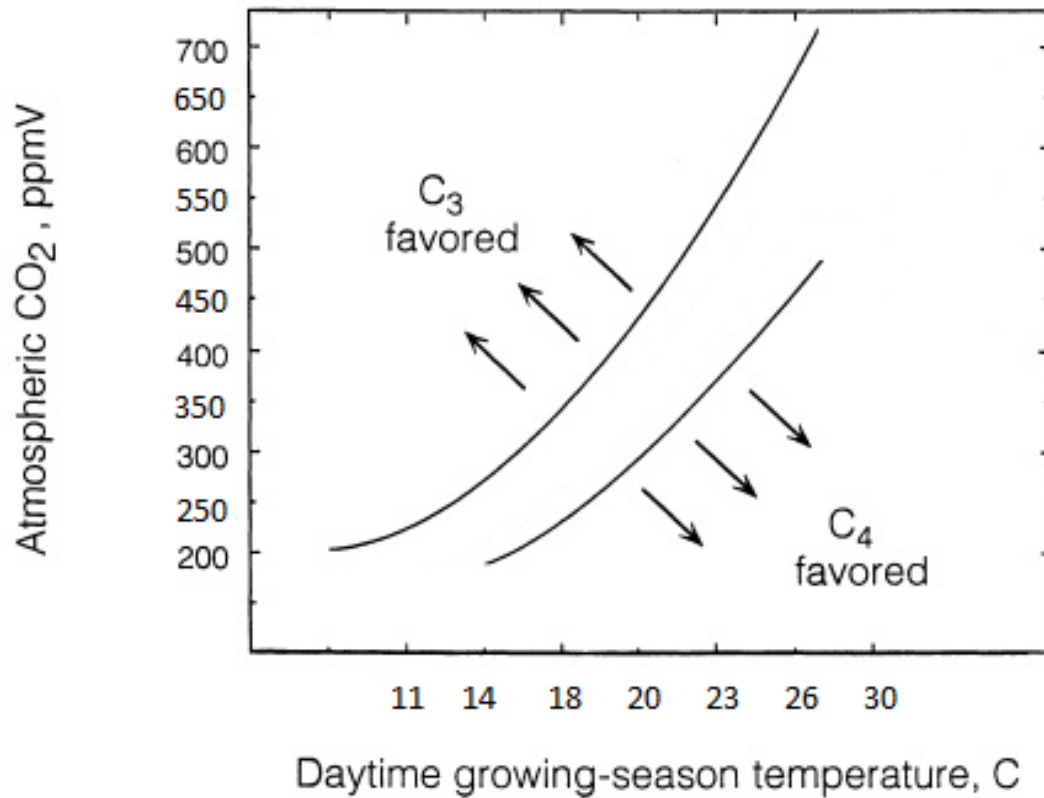


Figure 3.1. The modelled expected response of C₃ and C₄ plants as a function of growing seasonal temperatures for a particular atmospheric concentration of CO₂ (Ehleringer *et al.* 1997).

3.2.3. Whole Plant response to Interactive effect of elevated CO₂ and rising temperatures

Lee (2011) found that the biomass production of C₃ grasses was reduced by 47.3% under warm temperature and increased by 33.9% under the combined effect of elevated CO₂ and warm temperature compared to ambient conditions. Conversely, the biomass production of C₄ grasses grown under the combined effect of elevated CO₂ and temperature was not significantly different from that under ambient conditions, although it was slightly increased under the individual effect of warm temperatures (Lee 2011). These results confirm our physiological understanding on the expected response of C₃ and C₄ functional types which predicts that the quantum yield of C₃ and C₄ plants at any temperature increases with concentrations of CO₂ although C₃ plants generally have a lower optimum temperature than C₄ plants (Figure 3.1) (Ehleringer *et al.* 1997). When warm temperature is combined with

elevated CO₂, the negative effect of temperature on C3 plant could be reduced by the effect of elevated CO₂ (Long 1991).

The independent effects of rising temperatures and elevated CO₂ on plants possessing different photosynthetic pathways have been extensively studied (Pearcy *et al.* 1981; Hunt *et al.* 1996; Ehleringer *et al.* 1997; Ainsworth and Long 2005; Ghannoum *et al.* 2010; Duan *et al.* 2014; Gordijn *et al.* 2018; Mishra *et al.* 2019). The majority of these studies have been conducted using enclosure methods (walk-in growth chambers). A few have used large-scale free air CO₂ enrichments (FACE) experiments (Kallarackal and Roby 2012). Although there are many disadvantages associated with the use of enclosure methods including loosely controlled plant density, height and limited root growth when grown in pots, enclosure methods are better able to control environmental conditions (e.g. temperature, humidity, CO₂) and irrigation than FACE methods (Jablonski *et al.* 2002; Kallarackal and Roby 2012) and are much cheaper to execute; hence, they have formed the basis of our knowledge of plant physiological responses to rising temperatures and elevated CO₂ (Ainsworth and Long 2005).

The interactive effect on plants of multiple drivers is still poorly understood (Morgan *et al.* 2011), as is the net outcome on competition dynamics in mixed communities, which is the topic of the next chapter. The Drakensberg is an interesting place to study these drivers because it has both C3 and C4 grasses and trees and because it is thought that the distribution of both these grasses and trees is controlled by high temperature as a result of climate change (Bentley and O'Connor 2018). Therefore, looking at the specific plant response to CO₂ and temperature will not only help us to understand the interactive effect of CO₂ and temperature, but will also help us predict how this rich biodiversity and important ecosystem will respond to global change. Thus, this chapter focusses on understanding the combined and interactive effects of higher temperature and elevated CO₂, within the range projected to occur with climate change this century, on the growth of plants with different photosynthetic pathways and growth forms, when grown without competition from other species to help explain the upward movement of *L. sericea* and *F. costata* in the Drakensberg.

3.2.4. Aims and objectives

The aim of this chapter is to test the individual and interactive effects of rising temperatures and elevated CO₂ on the survival, productivity and leaf traits of *Leucosidea sericea*, *Themeda triandra* and *Festuca costata*, grown alone, over a six-month growth season.

3.3. METHODS

3.3.1. Origin of the plant material

The origin of the plant material was described in Chapter 2.

3.3.2. Study species

The species selected for study were described in Chapter 2.

3.3.3. Experimental design and protocol

The experimental design of this study was described in Chapter 2.

3.3.4. Data analysis

In this chapter, the analysis considers only those pots where each species is co-planted with itself. The treatments where it interacts with other species are analysed in Chapter 4. R Studio (R Core Team 2020, version 1.3.1073) was used to perform statistical analysis, and the package 'ggplot2' to graph the data. Shapiro-Wilk tests were conducted to explore whether the distribution of the data significantly differed from a normal distribution. Where it did, appropriate transformations were performed using the package 'rcompanion' (either a log, or a Tukey transformation). Some individuals recorded zero, or even negative growth. We therefore added a constant to the raw data (+20 and +9 for the growth rate and biomass accumulation data respectively) to produce positive values that could then be log-transformed to fit a normal distribution.

Survival of the three species in the four different treatments was assessed using Generalized Linear Mixed Effects Models (glmer) using the lme4 package, with survival (1/0) as a binomial response variable, and treatment (AMB.400, AMB.650, WARM.400, WARM.650) as a fixed effect.

We fitted a relationship between cross sectional area (basal circumference for grasses) and above-ground biomass for the final harvested plants in order to be able to convert our non-destructive measures of growth to a measure of plant biomass. This calibration was used to quantify the biomass of the initial planted individuals and calculate biomass change. The predictive power of this relationship was poor: stem diameter predicted total biomass with an R^2 of 0.17 for *L. sericea*. Basal area predicted total biomass with an R^2 of 0.12 for *F. costata* and R^2 of 0.31 for *T. triandra*. Therefore, although we do report our results in terms of biomass, we also report changes in stem length and basal area. To account for differences in the initial size of the plants the change in biomass ($\text{Biomass}_{\text{final}} - \text{Biomass}_{\text{initial}}$) was used for all

analyses. The total stem length of the tree *L. sericea* and height of the grasses was used to determine the growth rate ($GR = (\text{Length}_{\text{final}} - \text{Length}_{\text{initial}}) / \text{experiment duration (months)}$). Linear regression models were used (using the function `lm()`) to assess how treatment affected the logged biomass accumulation independently for each species (i.e., a separate model was fitted for each species - mixed effects models with species as a random effect produced the same results but were more complex to interpret). Linear regression models were also used to analyse how treatment affected the leaf traits data (leaf area and the total chlorophyll) for each species. For each model the assumptions required for linear modelling (homoscedasticity, normal response variable, and linear relationships) were checked by testing the constancy of variance and normality of the residuals (Appendix C).

3.4. RESULTS

3.4.1. Survival

Leucosidea sericea had lower mortality rates than either of the grass species, across all treatments, followed by *Festuca costata* (Figure 3.2). *Themeda triandra* had the highest mortality rate (Figure 3.2). In the warmest treatments, no individuals of *T. triandra* survived after three months (Table 3.1). However, the survival rate reported in each group of species is apparently not significantly influenced by the conditions in each treatment (Table 3.2).

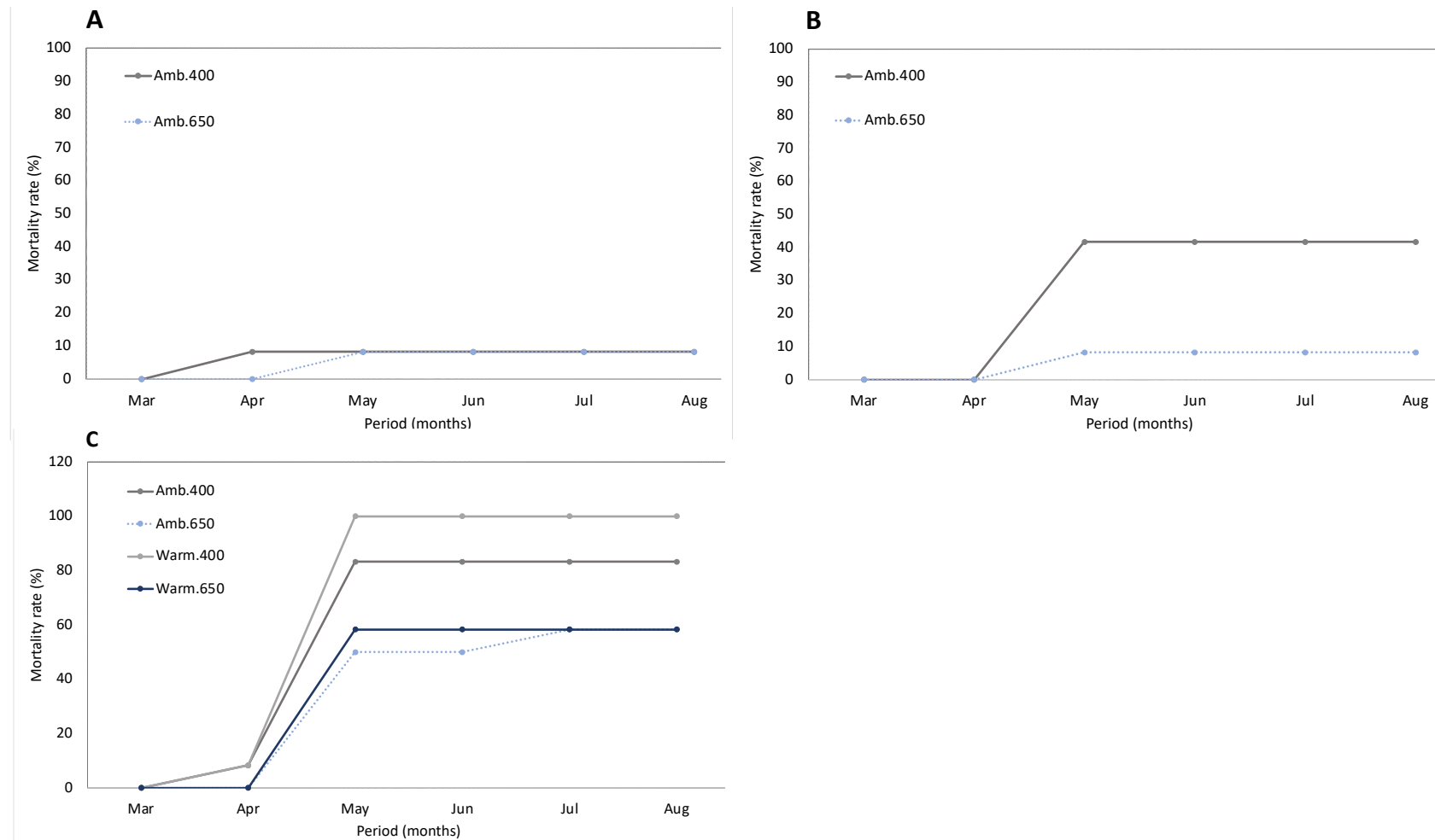


Figure 3.2. The mortality rate (%) of *Leucosidea sericea* (A), *Festuca costata* (B) and *Themeda triandra* (C) over a 6-month growth period in response to two levels of CO₂ (400 ppm and 650 ppm) and two levels of temperature (ambient (AMB) and +2°C above ambient (WARM)). Where treatments are not shown in the graphs there was no mortality.

Table 3.1. Total number of individuals per species at the start of the experiment and the percentage of individuals that survived over a six-month period under individual and interactive effect of elevated CO₂ and warm temperature. Statistical test used Generalized Linear Mixed-Effect Model (glmer) summarized in Table 3.2.

Species	Treatment	Total	Survived	% Survived
<i>L. sericea</i>	Amb.400	12	10	83
	Amb.650	12	11	92
	Warm.400	12	11	92
	Warm.650	12	12	100
<i>F. costata</i>	Amb.400	12	7	58
	Amb.650	12	9	75
	Warm.400	12	12	100
	Warm.650	12	8	67
<i>T. triandra</i>	Amb.400	12	4	33
	Amb.650	12	5	42
	Warm.400	12	0	0
	Warm.650	12	5	42

Table 3.2. Generalized Linear Mixed-Effect Model (glmer) of the effects of treatment on survival, controlling for species. (NS means ‘not significant’).

Response	Treatment	Estimates	Std. Error	z-value	p-value
Survival	Intercept	0.6223	1.0315	0.603	NS
	Amb.650	0.6574	0.6024	1.091	NS
	Warm.400	0.4543	0.5982	0.759	NS
	Warm.650	0.6574	0.6023	1.091	NS
	Random effect	Intercept			
	<i>L. sericea</i>	1.74787632			
	<i>F. costata</i>	0.04943663			
	<i>T. triandra</i>	-1.9357601			

3.4.2. Growth

The initial response of *L. sericea* to transplant from its nursery bag into the growth chamber pots was a reduction in stem length, but all treatments had fully recovered by month three. In contrast, *F. costata* initially gained a lot of height, with the high CO₂ treatments seeming to grow more quickly – but it subsequently died back, and the final stem height was only slightly higher than the initial one in all treatments (Figure 3.2). So many of the *T. triandra* died that it was not possible to assess its growth trajectory reliably.

The growth rate over a six-month period of *L. sericea*, *F. costata* and *T. triandra* varied greatly across treatments, with some individuals even displaying negative growth (Figure 3.2). However, the independent and interactive effects of elevated CO₂ and warm temperature did not influence the growth rate of *L. sericea* (Table 3.3). The only treatment where the growth rate was influenced by the conditions of the treatment was the ‘ambient conditions’ treatment, where the total stem length increment of *L. sericea* was higher, and that of *F. costata* was slightly lower (Figure 3.2). The influence on *T. triandra* is statistically insignificant as a result of low sample size, due to the low survival rate recorded for this species. Similarly, there were no significant effects of treatment on biomass accumulation (derived from an allometric model based on the basal cross-sectional area of each plant, separately calibrated per species) for any of the species studied (Table 3.3).

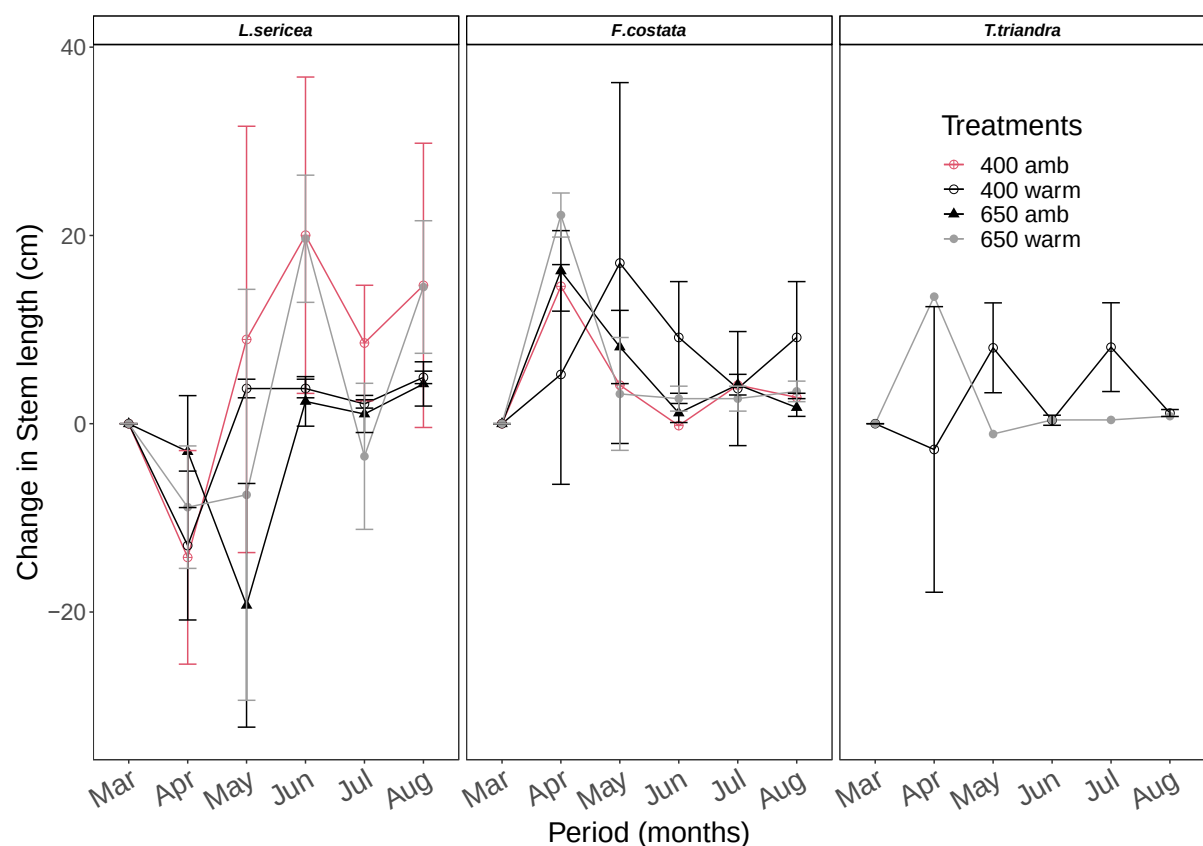


Figure 3.3. Growth – represented as the difference from initial stem length (*Leucosidea sericea*) and grass height (*Festuca costata* and *Themeda triandra*) - in response to two levels of CO₂ (400 ppm and 650 ppm) and two levels of temperature (ambient (AMB) and +2°C above ambient (WARM)) over a 6-months growing season. ‘Ambient’ CO₂ and temperatures in the

growth chamber refers to a fixed CO₂ concentration of 400 ppm (close to the actual atmospheric CO₂ concentration at Cathedral peak at the time of the experiment) and a diurnal temperature profile (obtained by adding 2°C to this range) based on the average summer values for the habitat in which all three species coexist, as recorded at a weather station a few hundred metres from the site, and a similar altitude and aspect.

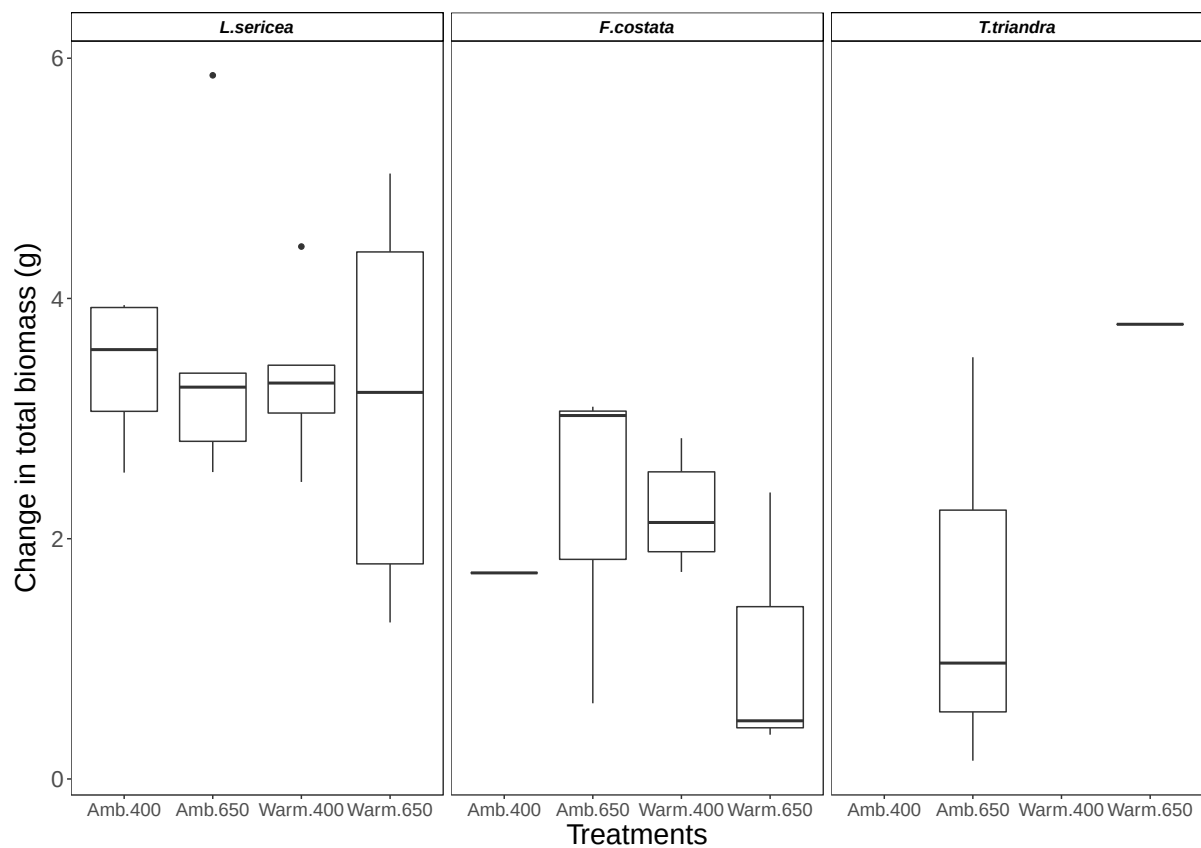


Figure 3.4. The interactive effect of two levels of CO₂ (400 ppm and 650 ppm) and two levels of temperature (ambient (AMB) and +2°C above ambient (WARM)) on total biomass accumulation (g) in *Leucosidea sericea*, *Festuca costata* and *Themeda triandra*. None of these responses were significant. The results of a statistical test on these data using linear regression model are summarized in Table 3.3.

Table 3.3. Stem length growth and biomass accumulation by each species under the four treatments, over a six-month period, tested using a linear regression model (lm) (NS=not significant; ***: $p < 0.001$; **: $p < 0.01$).

Response	Species:	Treatment	Estimates	Std. Error	t-value	p-value
Stem Length Growth rate	<i>L. sericea</i>	Intercept	3.0383	0.60929	4.987	***
		Amb.650	-0.04306	0.81745	-0.053	NS
		Warm.400	-0.24004	0.81745	-0.294	NS
		Warm.650	-0.59187	0.7866	-0.752	NS
	<i>F. costata</i>	Intercept	3.18773	0.07463	42.713	***
		Amb.650	0.11961	0.08618	1.388	NS
		Warm.400	0.03805	0.08061	0.472	NS
		Warm.650	0.05727	0.08618	0.665	NS
	<i>T. triandra</i>	Intercept	3.07754	0.19597	15.704	**
		Amb.650				-
		Warm.400				-
		Warm.650	0.02854	0.39195	0.073	NS
Δ Biomass	<i>L. sericea</i>	Intercept	3.41048	0.59857	5.698	***
		Amb.650	0.16206	0.80306	0.202	NS
		Warm.400	-0.07247	0.80306	-0.09	NS
		Warm.650	-0.26563	0.77274	-0.344	NS
	<i>F. costata</i>	Intercept	1.7152	0.9133	1.878	NS
		Amb.650	0.5361	1.0546	0.508	NS
		Warm.400	0.5077	0.9864	0.515	NS
		Warm.650	-0.6361	1.0546	-0.603	NS
	<i>T. triandra</i>	Intercept	1.542	1.012	1.524	NS
		Amb.650	-	-	-	-
		Warm.400	-	-	-	-
		Warm.650	2.243	2.023	1.109	NS

3.4.3. Leaf traits

The leaf area of *L. sericea* did not respond to any of the treatments (Table 3.4). In contrast, the leaf area of *F. costata* was significantly increased relative to the ambient conditions by warm temperature ($8.77 \pm 1.24 \text{ cm}^2$) and the interactive effect of elevated CO_2 and temperature ($3.75 \pm 0.00 \text{ cm}^2$). The leaf area of *T. triandra* was also higher than ambient in the high temperature and elevated CO_2 treatment ($3.75 \pm 0.00 \text{ cm}^2$). Interestingly, although the leaf area of *L. sericea* did not respond to treatment, the total chlorophyll under the effect of elevated CO_2 alone ($5.77 \pm 1.03 \text{ mg.kg}^{-1}$) and warm temperature ($5.08 \pm 0.82 \text{ mg.kg}^{-1}$) treatment was significantly higher than the total chlorophyll under the ambient treatment ($2.59 \pm 0.32 \text{ mg.kg}^{-1}$), but this was not found for the interactive effect of elevated CO_2 and warm temperatures ($3.52 \pm 0.52 \text{ mg.kg}^{-1}$) (Table 3.4). The total chlorophyll in the leaves of *L. sericea*, *T. triandra* and *F. costata* ranged from $1.09 - 14.06 \text{ mg.g}^{-1}$, $1.05 - 18.66 \text{ mg.g}^{-1}$ and $0.22 - 22.73 \text{ mg.g}^{-1}$ across all treatments respectively (Table 3.5).

Table 3.4. The effects of treatments on selected leaf traits, analysed by linear regression model (lm) (NS: not significant; ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$). Data are logged – for unlogged co-efficient see the text.

Response	Species	Treatment	Estimates	Std. Error	t-value	p-value
Leaf area	<i>L. sericea</i>	Intercept	0.4557	0.4258	1.07	NS
		Amb.650	0.4963	0.6022	0.824	NS
		Warm.400	1.8263	0.9521	1.918	NS
		Warm.650	0.5588	0.6022	0.928	NS
	<i>F. costata</i>	Intercept	1.3097	0.322	4.067	**
		Amb.650	0.6277	0.3719	1.688	NS
		Warm.400	0.8027	0.3478	2.308	*
		Warm.650	1.1952	0.3944	3.03	*
	<i>T. triandra</i>	Intercept	1.54262	0.01999	77.161	***
		Amb.650	-	-	-	-
		Warm.400	-	-	-	-
		Warm.650	0.22219	0.03998	-5.557	*
Total Chlorophyll content	<i>L. sericea</i>	Intercept	0.879	0.1484	5.924	***
		Amb.650	0.7331	0.205	3.576	**
		Warm.400	0.6949	0.2423	2.868	**
		Warm.650	0.305	0.2098	1.453	NS
	<i>F. costata</i>	Intercept	0.9428	0.3053	3.088	**
		Amb.650	0.6124	0.3906	1.568	NS
		Warm.400	0.7357	0.3842	1.915	NS
		Warm.650	-0.2465	0.4318	-0.571	NS
	<i>T. triandra</i>	Intercept	1.2805	0.3273	3.912	**
		Amb.650	0.9647	0.4392	2.196	NS
		Warm.400	-	-	-	-
		Warm.650	-0.4916	0.4392	-1.119	NS

Table 3.5. Mean, standard deviation, standard error, minimum and maximum Chlorophyll values for three study species in each treatment.

Response	Species	Treatment	Mean	Std. Deviation	Std. Error	Min	Max
Total Chlorophyll content	<i>L. sericea</i>	Amb.400	2.6415	1.0895	0.3445	1.0890	4.1373
		Amb.650	5.7748	3.4310	1.0345	2.1792	14.0620
		Warm.400	5.1850	2.3483	0.9587	3.4383	9.5586
		Warm.650	3.5242	1.6358	0.5173	2.0261	7.6278
	<i>F. costata</i>	Amb.400	2.9921	1.7689	0.6686	1.1374	5.5922
		Amb.650	5.7780	3.6599	1.1035	1.9489	11.7692
		Warm.400	7.1958	6.5219	1.8827	1.8089	22.7336
		Warm.650	2.9692	1.9372	0.7322	0.2225	5.4213
	<i>T. triandra</i>	Amb.400	4.5109	3.1413	1.5706	1.7905	7.2314
		Amb.650	10.8887	6.3681	2.8479	5.1752	18.6592
		Warm.400	-	-	-	-	-
		Warm.650	2.5065	1.4182	0.6342	1.0550	4.7109

3.5. DISCUSSION

In this study it was difficult to differentiate between transplant effects and the effect of the environmental treatments. The survival analysis suggests survival of individuals in each species is not influenced by the conditions of each treatment, although there were large differences in survival between species. *Themeda triandra* individuals had the lowest survival rate, with no individuals surviving under the warm temperature treatments. As there was no significant treatment effect, it is likely that *T. triandra* tillers, which were initially much smaller than *F. costata* tillers, did not respond well to the effects of transplanting, rather than that there was differential survival due to environmental conditions. The *L. sericea* specimens were young seedlings, already well established in potting bags, and so could be transplanted with minimal mortality. There was also no evidence that the warm temperature and elevated CO₂ treatments affected growth rate and biomass accumulation over a six-month period for any of the species studied. (Figure 3.2, Table 3.3). These results suggest that it is unlikely that *L. sericea* will expand into higher elevation locations due to rising temperatures later this century, nor dominate over grasses as a result of rising CO₂.

These results differ substantially from those found by other studies, which generally show that the growth rate of plants varies under elevated levels of CO₂ which differs among C3 and C4 plants (Jongen and Jones 1998; Wand *et al.* 1999; Ainsworth and Long 2005). For instance, C3 plants show a 10-20% increase and C4 plants show a 0-10% increase in their yield (Ainsworth and Long 2005). Trees always show an even greater increment in dry matter production (dry biomass) of 28% under elevated CO₂, although this high response is observed mostly in saplings of trees which are very young and are growing rapidly (Ainsworth and Long 2005). It could be that these plants, instead of increasing above-ground production, put more resources into below-ground reserves, as has been suggested by Kgope *et al.* (2010) and (Bond and Midgley 2012). However, there was also no treatment effect on the total biomass at the end of the experiment, which indicates that below-ground resources were also not significantly different.

Most published studies on the response of C3 grasses like *F. costata* to elevated CO₂ and warm temperatures, have found that C3 plants are favoured under the effect of elevated CO₂ (Mishra *et al.* 2019) due to reduced photorespiration caused by stimulated CO₂ which increases the efficiency of photosynthesis (Sage and Kubien 2007). In this study, *Festuca*

costata plants grown at high CO₂ levels did initially appear to increase growth (Figure 3.2) but this response did not continue throughout the 6 months of the experiment.

The biomass production of C3 grasses like *F. costata* is disadvantaged by warm temperatures caused by warm temperature shock (Wilson *et al.* 2000; Wall 2001) that strongly depress their productivity (Pearcy and Ehleringer 1984; Hatfield *et al.* 2011). Additionally, Hunt *et al.* (1996) suggest that the productivity of these grasses in response to warm temperatures may be reduced where there are seasonal variations with temperatures alternating between too hot or too cold for the growth of plants. However, we did not explore seasonal variation in temperature as a treatment in this study, instead, the average temperatures mimicked summertime temperature at Cathedral peak where samples of *F. costata* species were collected. Similarly, the results showed that the combined effect of elevated CO₂ and warm temperature did not influence their growth rate and biomass accumulation, and this is because when in combination, the reduced biomass production is attenuated by elevated CO₂ which stimulates C3 plants (Lee 2011). As a result, this most likely lead to their insignificant effect when compared to ambient conditions.

Similarly, the growth rate and biomass accumulation response of C4 grass *T. triandra* was not influenced by elevated CO₂. The conventional interpretation would be that C4 plants already pre-concentrate CO₂ internally to 2000 ppm in their mesophyll tissues (Poorter and Navas 2003), as a results, their growth rate is not expected to rise at levels of CO₂ below 2000 ppm (Mishra *et al.* 2019). This does not take into account the water use efficiency (WUE) benefit they gain, which in FACE experiments suggest a 10% to 12% growth enhancement between 360 and 700 ppm (Walker and Brian 1996). However, as our pots were well watered the improved WUE of C4 grasses under high CO₂ levels is unlikely to have been an important factor here, so the responses to CO₂ fit with expectations.

Likewise, *T. triandra* grasses did not show any significant response to the warm temperatures which is consistent with Lee (2011)'s finding. Again, this could be because water was not limiting in this study, and the reduction in the growth of C4 plants under warm temperatures is suggested to be due to changes in water relation caused by droughts stress or low water regimes (or less water) experienced by the plants (Leakey *et al.* 2004; Ghannoum 2009). Our interpretations of the data for *T. triandra* are limited due to low sample size as a result of low survival rate observed in individuals of this species (Table 3.1). We would have expected C4 grasses to perform better under the high temperature treatment, as they appear

to be growing at the cold edge of their environmental niche in the Drakensberg. It is possible, however, that it is not the temperature, but the degree of competition with *F. costata* that drives their upper distribution limit in the Drakensberg, and this will be tested in the next chapter.

In conditions with elevated CO₂, plants are expected to have larger leaves and those grown under warm temperatures are expected to have smaller leaves because small leaves are easier to keep cool (Cowling and Sage 1998). However, the canopy productivity depends not only on leaf size, but also photosynthetic efficiency. Although the leaf area of *L. sericea* trees was not influenced by either of the conditions in each treatment, they had higher photosynthetic efficiency under elevated CO₂ and under warm temperatures due to their higher total chlorophyll content. In contrast, the leaf area of *F. costata* increased both under the warming treatment and the combined warm temperature and elevated CO₂ treatment but there was no response of chlorophyll content. The leaf area of *T. triandra* grasses was also higher in the CO₂ high temperature treatment. The increased leaf area of the grass species in the warmed treatments was not consistent with results reported by Cowling and Sage (1998). Again, because water was not limiting as the water supplied to the pots was based on the average daily summertime rainfall of 2.724 mm at Cathedral Peak where samples were collected. Perhaps the plants were able to use evaporative cooling in which case large leaves would be advantageous, rather than risky.

3.6. CONCLUSIONS

In this experiment, the growth rate and biomass accumulation response of the three species were not influenced by the individual or combined effect of elevated CO₂ and warm temperature. Therefore, this study suggests that it is unlikely that rising temperatures or elevated CO₂ are causing *L. sericea* or *F. costata* to increase in dominance. However, fully exploring this hypothesis requires that we understand competitive relations between these growth forms more clearly.

4. CHAPTER 4: Competition between a tree, a C3 grass and a C4 grass under elevated CO₂ and temperature

4.1. ABSTRACT

Elevated CO₂ and higher temperatures go together when they result from anthropogenic climate change. They are each known to affect net primary productivity and range size, individually and interactively, in plant species grown alone. If this is to result in changes in community composition, the effects must be evident in the presence of other species. This study sets out to test how variation in the competitive interaction of *Festuca costata*, *Leucosidea sericea* and *Themeda triandra* in the Drakensberg, South Africa is influenced by changes in the level of CO₂ and temperature projected to be reached later in this century. The competitive interaction of the three species, each representative of a plant functional type, was examined in pairwise combinations in pots, grown in four walk-in growth chambers with two levels of CO₂ (400 ppm (ambient) and 650 ppm (elevated)) and two levels of temperature (ambient and +2°C above ambient) over a six-month growing period. The competitive ability of each species was measured as the relative yield per-plant (RYP) for each target and its neighbour species. It was found that *F. costata* has competitive advantage under all conditions over individuals of *T. triandra* and *L. sericea*. Further, *L. sericea* out-competed individuals of *T. triandra* that survived. This suggest that changes in the level of CO₂ and temperature projected to be reached later in the century will not influence the competitive interaction of any of the three species, instead, their competitive ability is driven by the root morphology of plants. These results imply that other abiotic and biotic factors such as light availability and disturbance, that were not considered in this study, are important in maintaining current community composition in the Drakensberg. There is no evidence from this experiment that higher temperatures or atmospheric CO₂ will alter the competitive dynamics in this system.

Keywords: competitive ability; neighbour species; shrub encroachment; target species

4.2. INTRODUCTION

4.2.1. *Competition between grasses and woody plants under global change*

Global climate change during the twenty first century is predicted to have serious implications for global biodiversity, including altering plant ranges and community structure (Thuiller 2007; Bellard *et al.* 2012). The observed expansion of woody plants into grasslands (Skowno *et al.* 2017; Stevens *et al.* 2017; Venter *et al.* 2018) has been suggested to be due to the effect of climate change linked to changes in average air temperature (Angelo and Daehler 2015) and atmospheric CO₂ which had been increasing since the start of the industrial revolution about 1750 (Kgope *et al.* 2010; Bond and Midgley 2012; Buitenwerf *et al.* 2012). In an environment where trees are competing with grasses, previous studies have shown that elevated CO₂ leads to reduced water use in grasses and deeper rooted trees, as a result, it enhances the growth of trees (Polley 1997). These studies were carried out on seedlings or on the leaves of larger trees and suggest that the effect of CO₂ on woody plants are only noticeable during the initial phase of CO₂ enrichment and as CO₂ rises to higher levels their effects are less noticeable in woody plants (Bond and Midgley 2000). The competitive interaction of shrubs and grasses could also influence shrubland – grassland progression by strengthening the grazing and drought-induced stress on grasses (Pierce *et al.* 2018).

The competitive interaction of woody shrubs and grasses is also mediated by changes in available resources (Pierce *et al.* 2019). However, Goldberg and Werner (1983) suggest that these interactions should be similar for all functional types, because the competing plants require similar resources. The competitive ability of species may be size specific, and adult woody plants should successfully compete against grasses due to their larger size. However, (February *et al.* 2013a) showed that below-ground competition from grass is an important factor slowing tree sapling growth rates in a semi-arid savanna, and (Morrison *et al.* 2019) demonstrated that grass competition could reduce tree seedling survival by over 90%. This is supported by data showing that grass-to-shrub competition does not slow the rate of expansion when shrubs have recruited into larger size classes (Köchy and Wilson 2000; Ward and Esler 2011; Ward *et al.* 2013). This is because in early stages of grass-shrub transition, the shrubs interact mostly with grasses and once they reach a critical size they start to interact with other shrubs as the cover of grasses declines (Pierce *et al.* 2019).

4.2.2. *The responses of C3 and C4 plants to elevated CO₂ and warm temperature*

The photosynthetic pathway adopted by a plant functional type is one of the most significant determinants of species responses to elevated CO₂. Most plant species on earth use the C3 photosynthetic pathway, in which Rubisco fixes carbon dioxide into a three-carbon compound called 3-phosphoglyceric acid (PGA) as its first stable compound (Pearcy and Ehleringer 1984; Ehleringer 2005). Tropical grasses, however, evolved the C4 photosynthetic pathway where PEP carboxylase fixes the carbon dioxide, producing the four-carbon compound called the oxaloacetic acid (OAA) (Pearcy and Ehleringer 1984). Fixation by Rubisco is inefficient at low atmospheric CO₂ concentrations due to the affinity of Rubisco for O₂ as well as CO₂, leading to photorespiration (Barnaby and Ziska 2012). PEP carboxylase is less susceptible to photorespiration and therefore the C4 pathway confers an advantage at low CO₂ levels (180 ppm) which is much less apparent at high CO₂ levels (600 ppm); in fact, the additional steps in the C4 pathway impose a burden (Barnaby and Ziska 2012).

The response of C3 plants to elevated levels of CO₂ is usually noticeable through an increase in their growth and photosynthetic light use efficiency (Sage and Kubien 2007; Rogers *et al.* 2009). In contrast, elevated levels of CO₂ have little- to- no effect on the photosynthetic rate of C4 plants, since they pre-concentrate CO₂ within their bundle sheath cells to the equivalent of about 2000 ppm (Mishra *et al.* 2019). Wand *et al.* (1999) found that C3 and C4 plants show increased productivity in their yield in response to elevated levels of CO₂. However, the response of C3 to elevated CO₂ is normally greater than the response of C4 plants in the absence of other environmental factors (Wand *et al.* 1999).

Optimal temperature (the range of temperature for the plants development, with a minimum, maximum and optimum) is also affected by the photosynthetic pathway of the plant (Hatfield *et al.* 2008; 2011). C3 plants generally have lower optima than C4 plants – again due to increased photorespiration at high temperature. The optimum temperature is also shown to generally correlate with the mean annual growing season temperature the plants are exposed to (Ehleringer *et al.* 1997). As a result, temperature influences the distribution of C4 and C3 grasses, with C4 grasses dominating in areas with warm growing season temperatures (Pearcy and Ehleringer 1984) and C3 grass favouring cool climates: the abundance of C3 grasses is observed to decrease in response to higher temperatures (Hunt *et al.* 1996; Ehleringer *et al.* 1997). Eamus (1991) suggests that the productivity of C3 plants might be expected to increase at warmer temperatures if this increase co-occurs with an

increase in atmospheric CO₂. This is because photoinhibition at high temperatures is not such a problem if CO₂ levels are high (Eamus 1991; Long 1991).

Most organism's range are controlled not only by their capacity to survive certain environmental conditions, but also by interactions with other organisms and these interactions could change as environmental conditions also change (February *et al.* 2013b). Competitive interactions can reduce the dominance of particular species, or even exclude them from environments entirely (Hardin 1960). The effect of CO₂ and temperature on the direct physiological response of plants with different functional types has been extensively studied (Ehleringer *et al.* 1997; Kgope *et al.* 2010), however, their effect on the competitive interaction between trees and grasses is less well studied (February *et al.* 2013b).

4.2.3. *The spread of trees and C3 grasses to higher altitudes in South Africa*

Certain tree species, especially *L. sericea* have been observed to be creeping upward on the eastern escarpment margins of the highveld (Adcock 1990; De Villiers 2013). At the same time, it has also been observed that the native C3 species *F. costata* is now more abundant, especially in north-facing slopes historically dominated by C4 grasslands including grasses like *T. triandra* (Bentley and O'Connor 2018). These changes have been suggested to be due to elevated CO₂ on the argument that if it was a response to rising temperatures only, the abundance of C3 grasses should decrease on the cooler, south-facing slopes (Jump *et al.* 2012; Bentley and O'Connor 2018; Zu *et al.* 2021).

The results of the previous chapter showed minimal responses of *L. sericea*, *F. costata* and *T. triandra* species to the individual and interactive effect of elevated CO₂ and warm temperature. However, ultimately changes in grass communities occur through changes in the competitive interactions between different species, not to their productivity alone. Therefore, it is also important to assess how the competitive relationships between the three main functional types in the Drakensberg – woody plants, C3 grass and C4 grass – are affected by changing environments. If competitive interactions change to the point that new species become dominant, it could have serious implications for the structure of the ecosystem and its biodiversity (Kallarackal and Roby 2012). Therefore, this chapter investigates the competitive dynamics between *F. costata*, *L. sericea* and *T. triandra* and tests how they change under conditions of high CO₂ and warm temperatures.

4.2.4. Aims and Objectives

The aim of this study was to determine the competitive interactions between a tree, *Leucosidea sericea*, a C3 grass *Festuca costata* and a C4 grass *Themeda triandra*, in response to the individual and interactive effect of elevated CO₂ and warm temperature. The competitive response of the species under each treatment was monitored by testing differences between their biomass accumulation over a six-month growth period. The following expectations were based on the reviewed literature: (1) *L. sericea*, in the seedling stage studied here, was expected to be an inferior competitor to both grass species (February *et al.* 2013a), but this competitive disadvantage should be reduced with increased CO₂ (Pierce *et al.* 2019); (2) The competitiveness of the C3 grass *F. costata* is expected to increase at high CO₂ because C3 grasses are favoured by high levels of CO₂ (Pearcy and Ehleringer 1984; Ehleringer *et al.* 1997; Wand *et al.* 1999; Mishra *et al.* 2019); (3) *T. triandra* grasses could outcompete *F. costata* under warm temperatures due to their adaptive ability to respond well to a high light and warm environment (Pearcy and Ehleringer 1984; Hunt *et al.* 1996; Epstein *et al.* 1997). The outcomes of this study will help us understand how the variations in the response of the three species influence their competitive ability and the extent to which their interaction will affect the grass – to – shrub transitions in the Drakensberg in response to higher levels of CO₂ and warmer temperatures projected for later this century.

4.3. METHODS

4.3.1. Origin of plant material

The origin of the plant material was described in Chapter 2.

4.3.2. Study species

The species selected for this study were described in Chapter 2.

4.3.3. Experimental design and protocol

The overall experimental design of this study was described in Chapter 2. Germane to this chapter is that individuals were grown two to a pot, in all combinations: LL, FF, TT, TL, TF and LF (where L= *Leucosidea sericea*, F= *Festuca costata*, T= *Themeda triandra*). In the previous chapter the monospecific combinations were analysed. In this chapter the mixed-species and monospecific combinations are analysed.

4.3.4. Data analysis

The analysis of this chapter considers pots where each species is co-planted with individuals of their own and with the other species. R Studio (R Core Team 2020, version 1.3.1073) was used to perform statistical analysis and to graphically represent the data using the package 'ggplot2'. Shapiro-Wilk tests were conducted to explore whether the distribution of the data significantly differed from a normal distribution; where it did, appropriate transformations were performed (either a log, or a Tukey transformation 'rcompanion').

Survival of the three species in the four treatments (AMB.400, AMB.650, WARM.400 and WARM.650) was assessed using model selection with Generalized Linear Mixed Effect Model (glmer) from 'lme4' package with survival (1/0) as a binomial response variable and both treatment and the type of competition (LL, FF, TT, TL, TF and LF, where L= *Leucosidea sericea*, F= *Festuca costata*, T= *Themeda triandra*) as fixed effects. For each species, four models were compared in terms of their explanatory power: a null model with both the fixed effect deleted, the second with treatment as a fixed effect, the third with competition as a fixed effect and the fourth with both treatment and competition as fixed effects. The model with the lowest possible Akaike's Information Criterion (AIC) value was selected as the best model for predicting survival.

The competitive ability of species was calculated as: the production by species *i* (target species) when grown in combination with species *j* (neighbour species), relative to its production when grown with another individual of its own species. This is called the relative yield per plant (RYP) (Wilson and Keddy 1986):

$$RYP_{ij} = \frac{Y_{ij}}{Y_{ii}}$$

Where: Y_{ij} is the total biomass (see Chapter 3 for calculation of change in biomass overtime (Appendix D)) of individuals of target species *i* grown in combination with individual of neighbour species *j* and Y_{ii} is the total biomass of individual of species *i* grown in the presence of individuals of their own species. Where $RYP_{ij} > 1$ it indicates that species *i* has greater competitive ability over species *j* than when grown with individuals of its own species and where $RYP_{ij} < 1$ it indicates that species *i* has poorer competitive ability over species *j* than when grown with individuals of its own species (Wilson and Keddy 1986). The competitive response of individual species against each other species was analysed using one-way analysis of variance (ANOVA) with each treatment as a fixed factor. Where there was

significant difference in the competitive response of species in each treatment, Tukey's honest Significant difference test was used to test for difference among species under each treatment.

4.4. RESULTS

4.4.1. Survival

The model with the best explanatory powers for *L. sericea* was the null model which had an AIC of 18.454 which is as good as the next best model (reported in this study) that included competition as a fixed factor with an AIC of 19.404 (Appendix A). The best model for both *F. costata* and *T. triandra* included both environmental treatment and competition as fixed effect. These models had an AIC of 41.155 and 49.116 which were smaller than the next best models with an AIC of 45.988 and 51.415 for *F. costata* and *T. triandra* respectfully (Appendix A).

The competitive interaction between the two grass species, and how it influenced their survival, is greater than the competitive interactions between either grass species or the tree species. There was no evidence that competition with *L. sericea* reduced the survival of *F. costata* (Table 4.1), but there was a slight but significant indication that *T. triandra* survival is worse when competing with individuals of *F. costata* grasses under ambient conditions of CO₂ and temperature (Table 4.2). The survival of *F. costata* in competitive combinations was apparently not influenced by changes in the level of CO₂ and temperature.

No individuals of *T. triandra* grown in combination with individuals of *L. sericea* under ambient conditions survived. Only 67% that were grown in combination with *F. costata* survived. There was no evidence that competitive ability of grasses and the conditions in the treatment affect the survival of *L. sericea* individuals (Table 4.2). A high survival rate of *L. sericea* was observed across all treatment, when grown with both potential grassy competitors or individuals of their own species.

Table 4.1. Total number of individuals species competing with individuals of their own and with individuals of the other species at the start of the experiment and the percentage of individuals that survived over a six-month period under ambient conditions, individual and interactive effect of elevated CO₂ and warm temperature. Statistical test used Generalized Linear Mixed-Effect Model (glmer) summarized in Table 4.2.

Target	Neighbour	Total target	AMB.400	AMB.650	WARM.400	WARM.650
			% Survived	% Survived	% Survived	% Survived
<i>L. sericea</i>	<i>L. sericea</i>	6	83	83	100	100
	<i>F. costata</i>	3	100	100	100	100
	<i>T. triandra</i>	3	100	100	100	100
<i>F. costata</i>	<i>F. costata</i>	6	17	50	100	50
	<i>L. sericea</i>	3	100	100	100	100
	<i>T. triandra</i>	3	100	100	100	67
<i>T. triandra</i>	<i>T. triandra</i>	6	0	50	0	17
	<i>F. costata</i>	3	67	67	0	67
	<i>L. sericea</i>	3	0	100	0	67

Table 4.2. Generalized Linear Mixed-Effect Model (glmer) of the effects of treatment and competition on survival ran for each species. NS: not significant; ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$)

measure	Species	Treatment	Estimates	Std. Error	t-value	p-value
Survival	<i>L. sericea</i>	Intercept	2.3026	0.7416	3.105	**
		LS x FC	18.2635	5118.3170	0.004	NS
		LS x TT	18.2635	5118.3170	0.004	NS
	<i>F. costata</i>	Intercept	-1.0829	0.8654	-1.251	0.2108
		Amb.650	1.2011	1.1339	1.059	NS
		Warm.400	20.6524	4322.1706	0.005	NS
		Warm.650	0.6111	1.1175	0.547	NS
		FC x LS	20.6596	4597.0598	0.004	NS
		FC x TT	2.6503	1.2033	2.202	0.0276 *
	<i>T. triandra</i>	Intercept	-2.46e+00	1.04e+00	-2.365	0.0180 *
		Amb.650	1.571.579e+00	1.11e+00	1.425	NS
		Warm.400	-1.80e+01	2.83e+03	-0.006	NS
		Warm.650	1.58e+00	1.11e+00	1.425	NS
		TT x FC	-2.173e+00	9.95e-01	2.185	0.0289 *
		TT x LS	-3.37e-16	1.01e+00	0.0000	NS

4.2.2. Competitive ability

Festuca costata competes well against *L. sericea* and *T. triandra* under most environmental conditions: the RYP values were above 1 for all except the elevated CO₂ and warm temperature treatment, where biomass when grown with *L. sericea* was reduced relative to that of plants grown with themselves (Figure 4.1). Similarly, *F. costata* biomass was greater when grown with *T. triandra* grasses than when grown with its own species under ambient conditions and elevated CO₂, but it competed badly at high CO₂ and high temperatures treatment. Individuals of *F. costata* that grew under warm temperature only were growing by themselves and had no competition, since the individuals of *T. triandra* that were planted into the same pots had 0% survival rate under warm temperature (Table 4.1).

Due to low survival of *T. triandra*, the competitive ability of *L. sericea* in the presence of *T. triandra* over their growing period was not possible to assess except in one treatment (the combined effect of elevated CO₂ and warm temperature) where *L. sericea* had a greater competitive advantage over *T. triandra* (Figure 4.1).

The competitive ability of all three species varies across treatments (Figure 4.1). However, the only situation where the competitive ability of the species was significantly influenced by the environmental conditions was for competition between *F. costata* grasses and *L. sericea* trees: *F. costata* was a significantly better competitor under the high CO₂ levels and high temperature treatments, than under the ambient or high temperature and high CO₂ treatment. ($F_{(1,9)} = 36.66, P < 0.001$) (Figure 4.1).

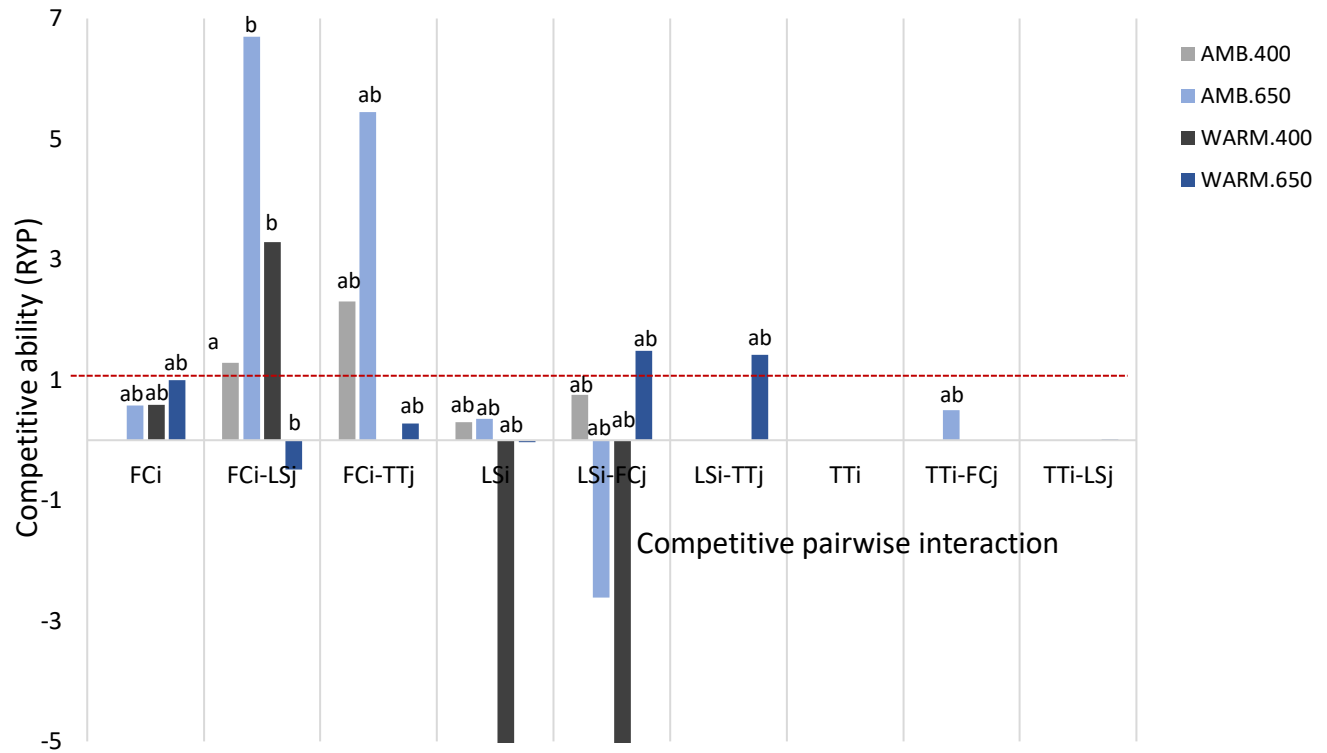


Figure 4.1. The mean response of the target species (*i*) to the competitive ability (measured as the Relative Yield per Plant) of the neighbour species (*j*) grown under interactive effect of two levels of CO₂ (400 ppm and 650 ppm) and two levels of temperature (ambient (AMB) and +2°C above ambient (WARM)) over a six-month growing season. Within each treatment, bars with the same letter were not significantly different in Tukey's honest Significant difference test. The competitive ability of individual species was analysed using one-way analysis of variance (ANOVA) with each treatment as a fixed factor.

4.5. DISCUSSION

Competitive interactions between species play an important role in controlling their distributions (Archer 1995; Scholes and Archer 1997; Sheth *et al.* 2020). Determining how climatic variables particularly temperature and CO₂ modify species interactions is a complicated but important field of research, and some would argue that it is not possible to understand or predict community-level changes without it (Peltzer and Köchy 2001). It is also important for informing conservation and management practices. The aim of this chapter was to determine how changes in the levels of CO₂ and temperature projected to be reached later in this century will influence variations in the competitive ability between a tree species *L. sericea*, a C3 grass *F. costata* and C4 grass *T. triandra* in order to explain the observed upward

movement of *L. sericea* into higher altitudes and an increase in *F. costata* on north-facing slopes.

Pierce *et al.* (2019) argues that the ability of grasses to possess greater competitive advantage over trees are only discernible during early stages of shrub development when their shallow and lateral root system are still less extensive and not yet well equipped to exploit more resources at depth that roots of grasses cannot reach. Therefore, in the present study, *L. sericea* in the seedling stage studied here was expected to be an inferior competitor to both grass species (February *et al.* 2013a) but this competitive disadvantage should be reduced by increased CO₂ (Pierce *et al.* 2019). As expected, the results found in this study confirm previous results that dense tussock grasses are strong competitors for woody seedlings and can reduce their growth rate (February *et al.* 2013a). *Festuca costata* grasses seem to possess greater competitive advantage against individuals of *L. sericea* (Figure 4.1). The extensive mat of fibrous roots of *F. costata* grasses enabled them to take up more available soil resources like nitrogen and water (Caldwell and Richards 1986) and compete successfully against individuals of *L. sericea* trees (Köchy and Wilson 2000; Aerts *et al.* 1991). However, this advantage disappeared in the elevated CO₂ and temperature treatment, indicating that the woody plant is benefitting more from the increased resources than the grassy one.

Growth of *L. sericea* appeared to be unaffected by environmental conditions as there were no significant differences in the RYP of *L. sericea*. It was not possible to assess the competitive ability of *T. triandra* because, so few individuals survived. Interestingly, however, it was found that competition with *F. costata* significantly reduced the probability of survival in *T. triandra* grasses (Table 4.2).

Temperature is a major factor that drives the distribution of C3 and C4 plants along the tropical elevation gradient (Angelo and Daehler 2015). The biomass of C3 grasses is expected to be reduced under warmer climate and will increase in response to CO₂ enrichment (Ainsworth and Long 2005). Furthermore, the biomass of C4 grasses is expected to respond positively to warm temperatures and elevated CO₂ (Morgan *et al.* 2011). Statistical modelling studies also indicate that the biomass of C4 grasses will increase with warmer temperatures at the expense of C3 grasses (Epstein *et al.* 2002). In contrast to these findings, this study found that the individual and interactive effect of elevated CO₂ and warm temperature did not influence the competitive ability of *T. triandra* and *F. costata* when

grown in combination with one another. These results are completely different from those that were expected and found in other studies.

This study acknowledges that, apart from the limitations of pot size, success in plant establishment and sample size, there may have been other factors not controlled for in the experiment that might have influenced these unexpected results. In particular, the frequency and intensity of fire in the Drakensberg, can have a strong effect on species composition (Gordijn and Ward 2014; Gordijn *et al.* 2018). Moreover, although we provided ample water, nitrogen or other key nutrients might have been limiting, which would have affected the ability of the C3 plants to respond to increased CO₂ as expected but should have exacerbated the competitive effect that we were looking for.

During winter season in the Drakensberg both species of *F. costata* and *T. triandra* lose their vigor and *T. triandra* grasses dry out as a result of harsh unfavourable conditions and become less active with less vegetative cover later on in around August (Shoko *et al.* 2018). During summer months, they have a higher above ground biomass where they are very active and productive due to favourable condition. On the other hand, *F. costata* is an evergreen grass (Werger and Van Bruggen 2012) that has high productivity throughout the year but is less active during winter months. In the present study, it was found that *F. costata* grasses outcompete *T. triandra* grasses regardless of changes in the levels of CO₂ and temperatures. Both the results of this study and Shoko *et al.* (2018)'s findings indicate that *F. costata* has the potential to outcompete *T. triandra* grasses due to its available above ground biomass throughout the year. As a result, the survival and competitive ability of *T. triandra* will be compromised as *F. costata* grass encroach into grassland previously dominated by C4 grass *T. triandra* in the Drakensberg.

Additionally, *T. triandra* is considered to be more palatable to livestock than *F. costata* which makes them prone to grazing pressure by grazers. They are also more prone to human disturbance because their fibrous tillers are a good source of thatching, fibre for paper, basketry and fodder, as a result, they have even more reduced biomass (Shoko *et al.* 2018). Although *T. triandra* grasses collected for the purpose of this study were in a conservation area, they also provide forage to small ungulates wildlife grazers (Joubert *et al.* 2017) which also increase grazing pressure on *T. triandra* grasses. There has also been observational evidence that the overgrazing in grassland promotes encroachment of unpalatable shrubs and grasses like *F. costata* into higher altitude (Morris 2011). Therefore, there are many non-

climatic reasons why *T. triandra* dominance might be decreasing in the Drakensberg, which should also be considered before we conclude that global climate change is the main driver.

4.6. CONCLUSIONS

Festuca costata was the most competitive of the three species studied but showed slightly reduced competitive ability under conditions of both high CO₂ and high temperature. At this stage of its life history, *L. sericea* is clearly not a strong competitor against grass, which is to be expected. However, there is no evidence that this competitive ability is increased at high CO₂ or temperatures, which contradicts predictions from Kgope *et al.* (2010) and Bond and Midgley (2012). It was not possible to properly assess how *T. triandra* competition was affected by changing CO₂ and temperatures because of low transplant success. These results do not help to explain why *F. costata* is spreading into areas previously dominated by C4 grasslands, nor why *L. sericea* is expanding its range, which implies that other drivers need to be considered to understand changes in community composition in the Drakensberg.

5. CHAPTER 5: Synthesis and conclusions

5.1. General overview

This study showed how the response of trees and C3/C4 grass species to the individual and interactive effect of elevated CO₂ and temperature influence their distribution shifts along the elevation gradient in the Drakensberg Mountain region and their competitive interaction with one another. This will help us predict how mountain ecosystem and biodiversity will respond to global climate change. The aim of this synthesis is to draw conclusions from the previous chapters which studied how the individual and interactive effects of warm temperature and elevated CO₂ affect the survival, productivity and leaf traits of *Leucosidea sericea*, *Themeda triandra* and *Festuca costata*, over a six-month growth season and how the competitive dynamics between these three species change under conditions of high CO₂ and warm temperatures.

5.2. Discussion

Leucosidea sericea was found to have the highest survival rate followed by *F. costata* and *T. triandra* which had the lowest survival rate (Chapter 3) but was also affected by competition. The rate of survival in each group of species was not clearly influenced by high CO₂ and high temperature treatment conditions (Chapter 3). *Themeda triandra* survives worse in combination with individuals of *F. costata* when grown under 'current ambient conditions' of CO₂ and temperature. However, there was no evidence that *F. costata* survives worse when in combination with individuals of *L. sericea* (Chapter 4).

In the warmest treatments, no individuals of *T. triandra* survived after three months and there was no significant treatment effect. It is likely that *T. triandra* tillers which were initially smaller than *F. costata* did not respond well to the effect of transplanting. As a result, this did not make it possible to assess their response to warm temperature and their competitive ability against *L. sericea* and *F. costata* as so few individuals survived. In the high CO₂ treatment, the individuals of *T. triandra* that survived were also not influenced by elevated CO₂ because C4 plants already pre-concentrate CO₂ internally to 2000 ppm in their mesophyll tissues, therefore, their growth rate is not expected to rise at levels of CO₂ below 2000 ppm (Poorter and Navas 2003; Mishra *et al.* 2019).

In this study, the growth response of the three species contrast sharply with those reported by previous studies which found that the response of C3 and C4 grasses varies under

the individual effect of elevated CO₂ which highly favours C3 grass more (Pearcy and Ehleringer 1984; Ehleringer *et al.* 1997; Mishra *et al.* 2019) and warm temperatures effect (also individually) which benefits C4 grasses due to their adaptive ability to respond well to a high light and warm environment (Pearcy and Ehleringer 1984; Hunt *et al.* 1996; Epstein *et al.* 1997). It is possible that the lack of response to our treatments was a result of insufficient sample size, and errors in the measurements of growth. It was not possible in this study to destructively harvest throughout the experiment, so architectural measurements (stem length, grass height, and grass basal diameter) had to be used as indicators of productivity and biomass gain. This means that increases in below-ground biomass might not have been quantified during the experiment. However, I did use several different indices of growth, and all showed the same pattern (Appendix B). These results suggests that even a 2°C increase in temperatures in the Drakensberg (which have been increasing annually in the 1960 – 2016 (Figure 5.1)) will not reach levels where the growth of the dominant grass and tree species are strongly reduced. However, follow up work into how other factors such as droughts and floods or low versus high rainfall period might influence their distribution is needed to confirm this.

Although treatment effects were hard to discern for mono-specific sample pots there was evidence that the competitive abilities of *F. costata* in particular, were increased at high temperatures or high CO₂ levels (but not both). There may have been other factors not controlled for in this experiment that may have influenced these results including, nitrogen and other key nutrients that might have been a limiting factor and would have influenced the individual and competitive response of C3 grasses to CO₂ as initially expected (Wigley *et al.* 2010; Morrison *et al.* 2019; Russell and Ward 2014). The great competitive response of *F. costata* over both individuals of *L. sericea* and *T. triandra* (Chapter 4) is attributed to differences in the root morphology between grass species and trees (Peltzer and Köchy 2001; Pierce *et al.* 2019). This is because *F. costata* grasses have extensive fibrous roots which enable them to compete successfully against species with less extensive roots like *L. sericea* and *T. triandra* by taking up more available soil resources such as nitrogen and water (Caldwell and Richards 1986). Kulmatiski and Beard (2013) also showed that grasses are competitively superior to trees for water uptake because they extract available water from the shallow soil but when the soil dry out, trees switch to extracting water from deeper soils while grasses continue to extract water from upper soil layers.

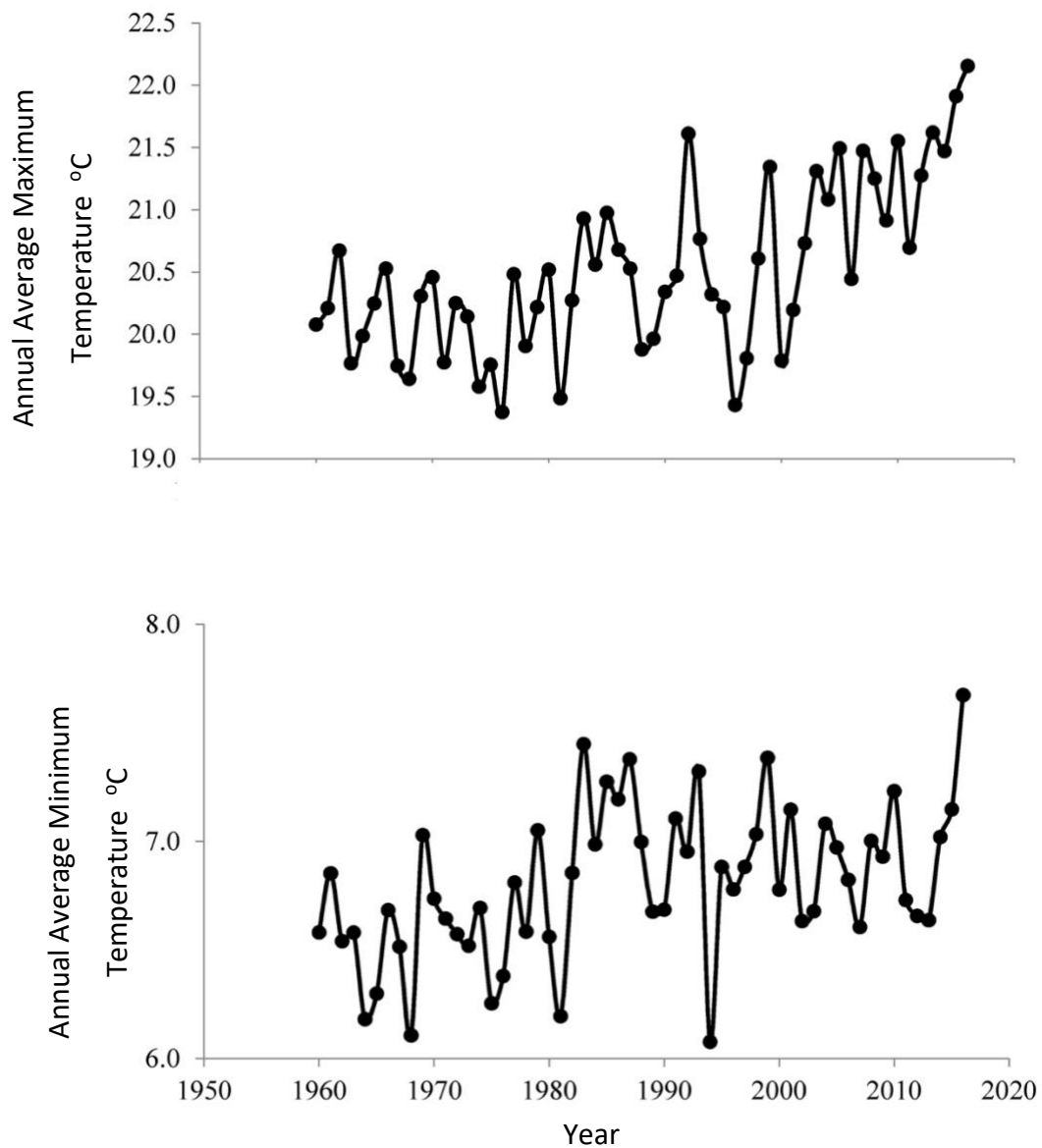


Figure 5.1. Annual average maximum and minimum temperature trend in the 1960 – 2016 period in the Drakensberg mountain region (Mohamed and Mukwada 2019).

Future research and/ recommendations

The results of this study do not help explain why *F. costata* is spreading into grasslands previously dominated by C4 grass *T. triandra*, nor the observed increase in dominance of *L. sericea*. Therefore, this dissertation does not support the growing literatures which suggest

that temperatures drives the expansion of species along the elevation gradient in mountainous regions (Jump *et al.* 2012). Although this study indicates that elevated CO₂ and warm temperatures do not drive changes in the community composition in the Drakensberg, we acknowledge that there might be other climatic and non-climatic drivers not controlled for in this study which are likely to explain the upward movement of *L. sericea* and *F. costata* in the Drakensberg.

Therefore, it is suggested that future studies look into other climatic factors which can be included in growth chamber of the experiment as another factors such as drought since climate change is also predicted to result in severe droughts (O'Connor *et al.* 2014), precipitation and light availability. Non-climatic factors could include increased grazing pressure which leads to reduced competitive effect of grasses on woody plants (O'Connor *et al.* 2014). Grazing pressure also supresses the frequency/occurrence of fires (Archer 1995; van Auken and Bush 2012), as a result, fire can also be included as a treatment because it can have a strong effect on species composition (Gordijn and Ward 2014; Gordijn *et al.* 2018). Due to the constraints of my MSc time available, the experiment took place during the dormant season for growth, but conditions in the chambers replicated growing season conditions. Therefore, future studies could also run the experiments for a longer period of time.

5.3. Conclusion

The work presented in this study shows that changes in the level of CO₂ and temperatures (alone or in combination), projected to be reached later in this century will not affect the growth response of, and competitive interaction between *L. sericea*, *F. costata* and *T. triandra*. As a result, they will not help explain the observed upward movement of *L. sericea* into higher altitudes nor the spread of *F. costata* in the north-facing slopes previously dominated by C4 grasses in the Drakensberg. However, it is likely that the presence of other climatic and non-climatic factors that were not considered in this study drives the current changes in community compositions in the Drakensberg.

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7. APPENDIX

Appendix A. The Akaike's Information Criterion (AIC) values for all four models predicting survival (surv) with both fixed effects deleted (~1), treatment as a fixed effect (~treatment), competition as a fixed effect (~competition) and both treatment and competition as fixed effects (~treatment + competition) of each species and the difference between the best model (lowest AIC) and the next best models.

	Model	AIC	diffAIC
<i>L. sericea</i>	surv~1	18.454	0
	surv~treatment	21.586	3.132
	surv~competition	19.404	0.95
	surv~treatment + competition	22.411	3.957
<i>F. costata</i>	surv~1	55.984	14.829
	surv~treatment	53.073	11.918
	surv~competition	45.988	4.833
	surv~treatment + competition	41.155	0
<i>T. triandra</i>	surv~1	55.984	6.868
	surv~treatment	51.415	2.299
	surv~competition	55.076	5.96
	surv~treatment + competition	49.116	0

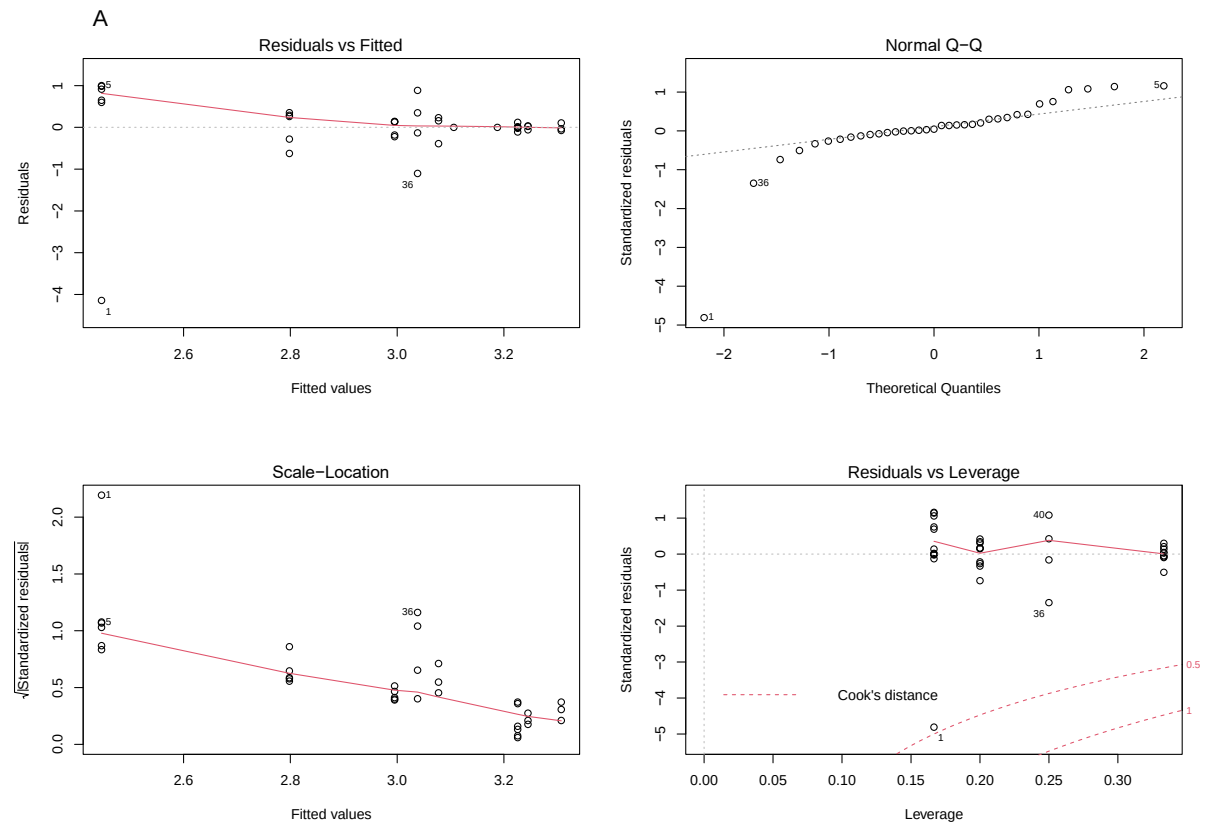
Appendix B. The Basal area (BA) of all species that survived each under each treatment over a six- month growing season (March to August).

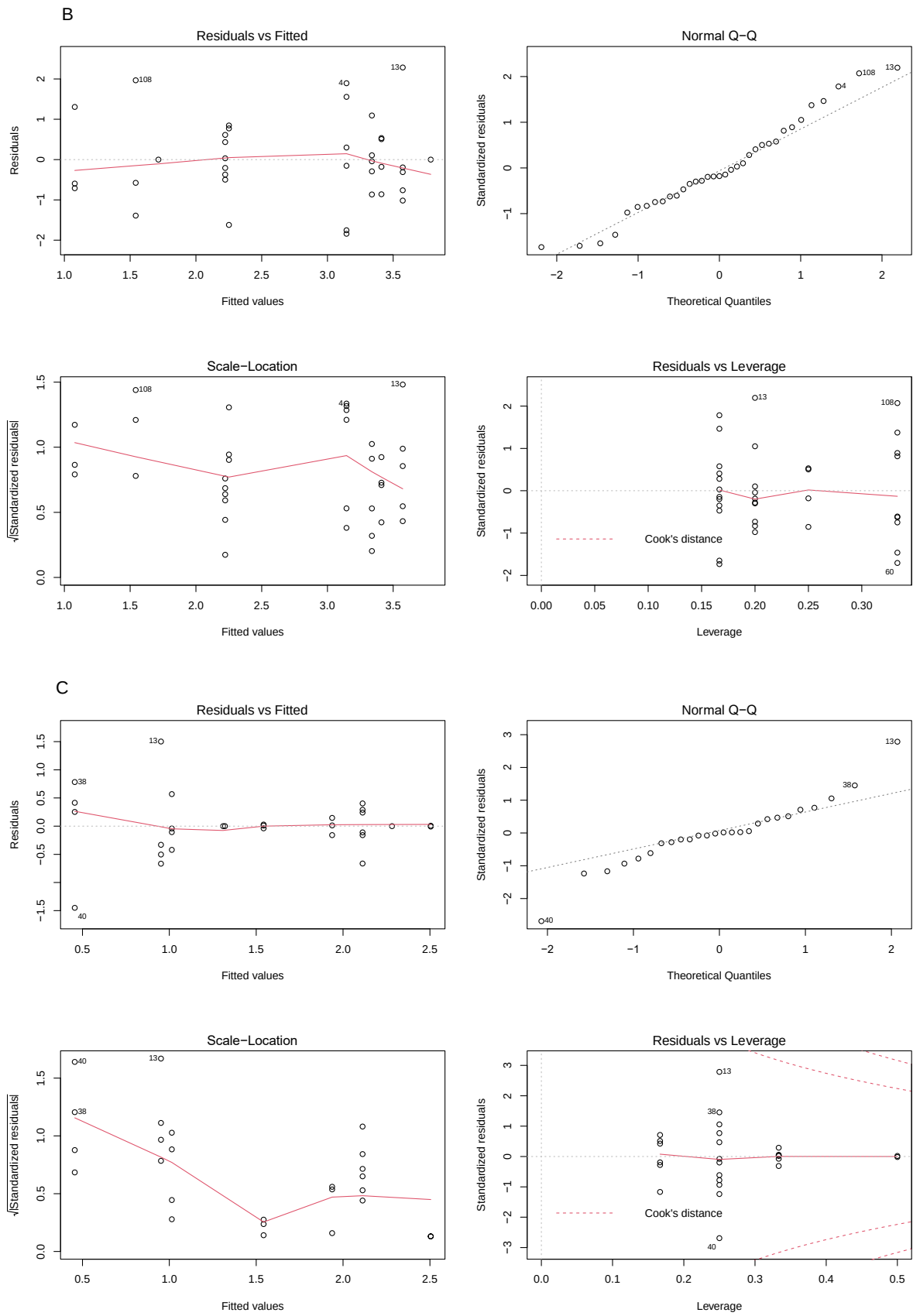
Species	Treatment	BA_mar	BA_apr	BA_may	BA_jun	BA_jul	BA_aug
<i>Leucosidea sericea</i>	WARM.650	0.79	1.09	0.66	0.41	0.11	0.08
<i>Leucosidea sericea</i>	WARM.650	0.50	0.59	0.56	0.53	0.47	0.53
<i>Leucosidea sericea</i>	WARM.650	0.30	0.47	0.49	0.50	0.53	0.77
<i>Leucosidea sericea</i>	WARM.650	0.16	0.17	0.34	0.57	1.22	1.65
<i>Leucosidea sericea</i>	WARM.650	0.54	0.59	0.37	0.40	0.55	1.02
<i>Leucosidea sericea</i>	WARM.650	0.60	0.11	0.14	0.17	0.26	0.39
<i>Leucosidea sericea</i>	WARM.650	0.62	0.70	0.68	0.66	0.63	0.71
<i>Leucosidea sericea</i>	WARM.650	0.63	0.75	0.88	1.03	1.38	1.76
<i>Leucosidea sericea</i>	WARM.650	0.23	0.29	0.33	0.38	0.49	0.70
<i>Leucosidea sericea</i>	WARM.650	0.75	0.88	0.82	0.77	0.73	0.94
<i>Leucosidea sericea</i>	WARM.650	0.36	0.51	0.45	0.39	0.30	0.30
<i>Leucosidea sericea</i>	WARM.650	0.24	0.33	0.34	0.36	0.42	0.69
<i>Leucosidea sericea</i>	AMB.650	0.80	0.79	0.69	0.73	1.24	2.06
<i>Leucosidea sericea</i>	AMB.650	0.39	0.42	0.35	0.37	0.40	0.15
<i>Leucosidea sericea</i>	AMB.650	0.38	0.41	0.44	0.47	0.55	0.10
<i>Leucosidea sericea</i>	AMB.650	0.26	0.28	0.30	0.32	0.37	0.32
<i>Leucosidea sericea</i>	AMB.650	0.12	0.14	0.17	0.20	0.26	0.33
<i>Leucosidea sericea</i>	AMB.650	0.54	0.54	0.41	0.30	0.13	0.07
<i>Leucosidea sericea</i>	AMB.650	0.29	0.57	0.63	0.80	1.44	2.43
<i>Leucosidea sericea</i>	AMB.650	0.31	0.39	0.45	0.51	0.64	0.64
<i>Leucosidea sericea</i>	AMB.650	0.56	0.63	0.73	0.85	1.10	0.95
<i>Leucosidea sericea</i>	AMB.650	0.29	0.39	0.39	0.39	0.41	0.65
<i>Leucosidea sericea</i>	AMB.650	0.24	0.36	0.34	0.32	0.30	0.48
<i>Leucosidea sericea</i>	WARM.400	0.62	0.71	0.59	0.48	0.35	0.04
<i>Leucosidea sericea</i>	WARM.400	0.34	0.44	0.49	0.55	0.72	0.97
<i>Leucosidea sericea</i>	WARM.400	0.19	0.16	0.19	0.23	0.32	0.24
<i>Leucosidea sericea</i>	WARM.400	0.08	0.09	0.09	0.08	0.07	0.08
<i>Leucosidea sericea</i>	WARM.400	0.26	0.30	0.27	0.26	0.32	0.42
<i>Leucosidea sericea</i>	WARM.400	0.20	0.27	0.33	0.40	0.56	0.66
<i>Leucosidea sericea</i>	WARM.400	0.45	0.53	0.60	0.69	0.92	0.70
<i>Leucosidea sericea</i>	WARM.400	0.21	0.26	0.32	0.48	1.10	1.90
<i>Leucosidea sericea</i>	WARM.400	0.19	0.23	0.30	0.39	0.59	0.78
<i>Leucosidea sericea</i>	AMB.400	0.62	0.61	0.55	0.51	0.50	0.04
<i>Leucosidea sericea</i>	AMB.400	0.56	0.69	0.75	0.82	0.98	1.30
<i>Leucosidea sericea</i>	AMB.400	0.36	0.37	0.42	0.57	1.13	1.88
<i>Leucosidea sericea</i>	AMB.400	0.53	0.51	0.71	1.47	3.89	1.25
<i>Leucosidea sericea</i>	AMB.400	0.27	0.36	0.41	0.47	0.61	0.36
<i>Leucosidea sericea</i>	AMB.400	0.67	0.53	0.81	1.32	3.06	3.17
<i>Leucosidea sericea</i>	AMB.400	0.53	0.72	0.68	0.65	0.61	0.54
<i>Leucosidea sericea</i>	AMB.400	0.99	0.76	0.78	0.80	0.85	0.19
<i>Leucosidea sericea</i>	AMB.400	0.67	0.89	0.99	1.10	1.35	1.23
<i>Leucosidea sericea</i>	AMB.400	0.23	0.36	0.39	0.43	0.51	0.95
<i>Festuca costata</i>	WARM.650	1.76	2.77	5.75	5.09	4.48	3.68
<i>Festuca costata</i>	WARM.650	0.62	5.09	13.66	9.80	6.59	2.50

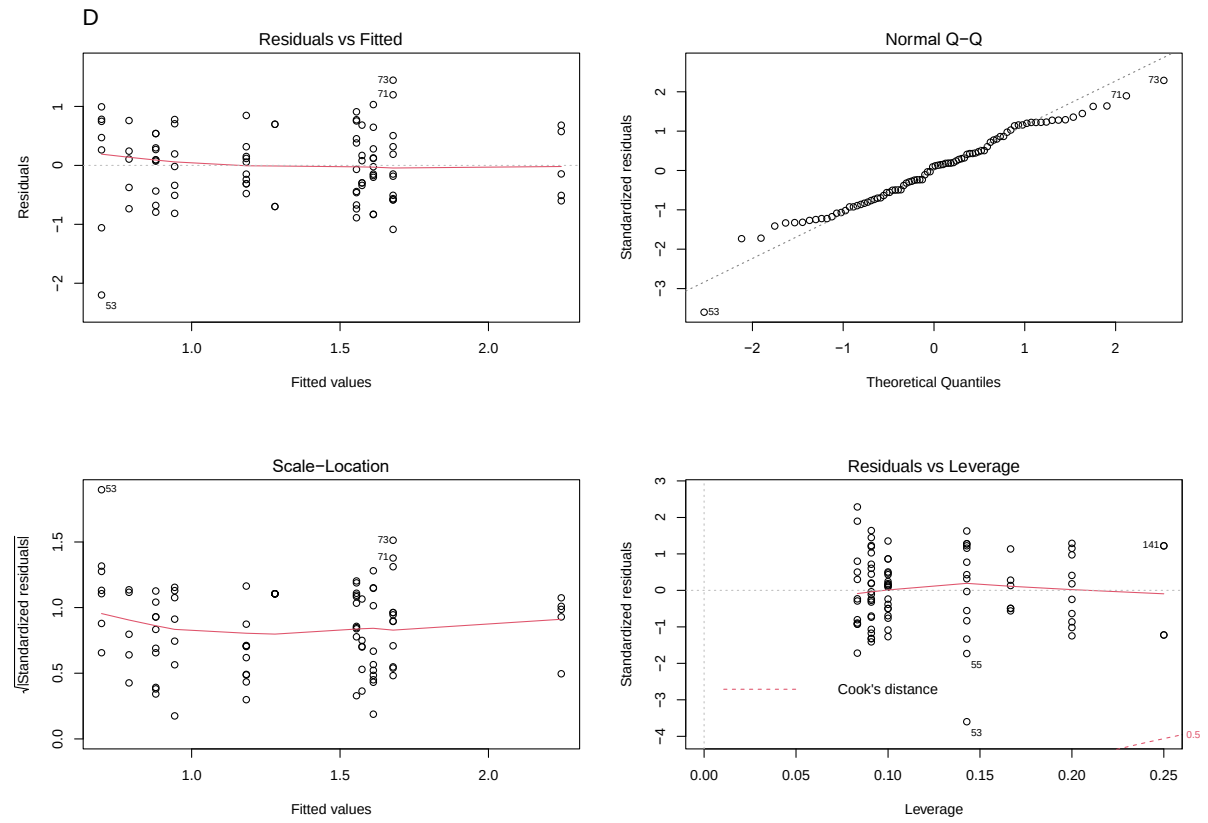
Appendix B continued.

Species	Treatment	BA_mar	BA_apr	BA_may	BA_jun	BA_jul	BA_aug
<i>Festuca costata</i>	WARM.650	2.59	5.75	11.46	12.84	14.29	16.50
<i>Festuca costata</i>	WARM.650	3.90	2.24	3.90	3.57	2.59	2.07
<i>Festuca costata</i>	WARM.650	5.22	4.72	9.28	5.35	2.50	1.27
<i>Festuca costata</i>	WARM.650	4.24	4.97	8.28	7.64	6.74	6.16
<i>Festuca costata</i>	WARM.650	1.76	6.16	9.28	8.28	7.33	6.16
<i>Festuca costata</i>	WARM.650	5.89	8.61	13.45	10.52	8.44	7.03
<i>Festuca costata</i>	AMB.650	1.91	1.40	2.41	2.86	3.36	4.84
<i>Festuca costata</i>	AMB.650	1.83	2.96	3.47	6.88	11.46	12.43
<i>Festuca costata</i>	AMB.650	5.48	1.91	2.32	3.79	5.61	6.30
<i>Festuca costata</i>	AMB.650	7.80	3.05	6.30	5.75	6.16	10.54
<i>Festuca costata</i>	AMB.650	5.09	3.06	9.80	9.80	10.52	21.40
<i>Festuca costata</i>	AMB.650	6.02	3.07	3.06	3.36	4.01	4.00
<i>Festuca costata</i>	AMB.650	4.72	2.99	5.48	5.75	6.30	9.75
<i>Festuca costata</i>	AMB.650	1.91	3.01	5.75	6.02	6.88	10.80
<i>Festuca costata</i>	AMB.650	7.64	3.03	3.68	5.35	7.33	8.33
<i>Festuca costata</i>	WARM.400	2.24	2.15	1.61	3.26	5.75	5.89
<i>Festuca costata</i>	WARM.400	4.13	2.07	1.34	2.41	3.06	5.48
<i>Festuca costata</i>	WARM.400	2.77	4.01	5.35	8.12	10.71	13.04
<i>Festuca costata</i>	WARM.400	5.09	4.84	2.68	3.57	4.60	4.84
<i>Festuca costata</i>	WARM.400	4.01	2.77	3.36	4.48	6.02	7.80
<i>Festuca costata</i>	WARM.400	6.74	1.91	1.76	3.36	5.48	12.63
<i>Festuca costata</i>	WARM.400	3.79	7.18	5.61	10.52	14.72	18.39
<i>Festuca costata</i>	WARM.400	6.88	4.97	4.36	5.35	6.45	8.61
<i>Festuca costata</i>	WARM.400	3.79	7.18	4.48	9.80	15.38	21.66
<i>Festuca costata</i>	WARM.400	5.75	4.48	7.64	7.18	6.88	8.44
<i>Festuca costata</i>	WARM.400	4.01	1.99	2.41	4.48	5.89	9.11
<i>Festuca costata</i>	WARM.400	3.68	5.09	4.24	6.45	8.61	10.71
<i>Festuca costata</i>	AMB.400	2.86	2.86	2.41	2.86	3.57	4.13
<i>Festuca costata</i>	AMB.400	2.96	4.13	4.01	4.48	5.35	6.16
<i>Festuca costata</i>	AMB.400	9.63	5.75	7.03	8.44	10.71	14.50
<i>Festuca costata</i>	AMB.400	8.77	3.90	4.48	5.61	6.88	8.94
<i>Festuca costata</i>	AMB.400	3.90	6.45	7.18	7.18	8.44	9.98
<i>Festuca costata</i>	AMB.400	5.09	2.41	5.35	5.75	9.98	10.89
<i>Festuca costata</i>	AMB.400	8.28	7.18	7.96	9.98	11.27	13.66
<i>Themeda triandra</i>	WARM.650	0.39	0.29	10.89	0.76	17.43	22.73
<i>Themeda triandra</i>	WARM.650	1.54	2.15	1.83	1.76	2.07	1.76
<i>Themeda triandra</i>	WARM.650	1.61	0.81	1.27	0.92	0.67	0.26
<i>Themeda triandra</i>	WARM.650	2.86	2.07	3.79	2.41	1.34	0.72
<i>Themeda triandra</i>	WARM.650	0.32	1.21	2.41	1.61	0.87	0.54
<i>Themeda triandra</i>	AMB.650	1.76	3.68	2.15	2.86	3.68	2.15
<i>Themeda triandra</i>	AMB.650	1.21	2.97	4.48	4.13	3.79	5.47
<i>Themeda triandra</i>	AMB.650	1.68	2.98	1.91	3.79	7.03	5.32
<i>Themeda triandra</i>	AMB.650	1.40	3.02	5.35	7.96	11.65	15.91
<i>Themeda triandra</i>	AMB.650	1.83	3.04	3.16	4.13	5.61	5.78
<i>Themeda triandra</i>	AMB.650	2.41	2.41	2.86	3.06	3.57	4.24
<i>Themeda triandra</i>	AMB.650	3.36	1.27	2.41	2.86	4.01	4.72

Appendix C. The assumptions required for linear modelling (homoscedasticity, normal response variable, and linear relationships) graphs checked by testing the constancy of variance and normality of the residuals for the Stem Length Growth Rate (**A**), Change in Biomass (**B**), Leaf area (**C**) and the total Chlorophyll content (**D**)







Appendix D. The change in biomass accumulation ($\text{Biomass}_{\text{final}} - \text{Biomass}_{\text{initial}}$) over a six-month growing season (March to August) of the target species (i) when competing by itself (thus no competition) or with its neighbour species (j). Species names are abbreviated as LS: *Leucosidea sericea*, FC: *Festuca costata* (FC) and TT: *Themeda triandra* (TT). ‘NA’ represents species that did not survive.

Temperature	CO ₂	Competitive		
		Pairwise interaction	Biomass gain (i)	Biomass gain (j)
AMB	400	LSi-LSj	0.3423	-2.8367
AMB	400	LSi-LSj	-0.4408	-0.9020
AMB	400	LSi	-0.4279	NA
AMB	400	FCi	NA	-0.7475
AMB	400	LSi-FCj	-0.0482	-1.8984
AMB	400	LSi-FCj	-1.4822	-2.8904
AMB	400	LSi-FCj	-0.0063	-0.0996
AMB	400	LSi	0.4741	NA
AMB	400	LSi	-0.3276	NA
AMB	400	LSi	-0.4255	NA
AMB	400	FCi	-3.6072	NA
AMB	400	FCi-TTj	-3.4396	-1.0872
AMB	400	FCi-TTj	-3.1886	-0.8041
AMB	400	FCi-LSj	-1.8984	-0.0482
AMB	400	FCi-LSj	-2.8904	-1.4822
AMB	400	FCi-LSj	-0.0996	-0.0063
AMB	400	LSi	NA	0.4741
AMB	400	LSi	NA	-0.3276
AMB	400	LSi	NA	-0.4255
AMB	400	FCi	NA	-3.6072
AMB	400	TTi-FCj	-1.0872	-3.4396
AMB	400	TTi-FCj	-0.8041	-3.1886
AMB	650	LSi-LSj	-0.7464	0.4681
AMB	650	LSi-LSj	0.1423	0.1640
AMB	650	LSi-LSj	-0.0339	-0.1228
AMB	650	FCi-FCj	-1.7380	-6.2861
AMB	650	FCi	-0.4870	NA
AMB	650	FCi-FCj	-3.0843	-9.9641
AMB	650	TTi	NA	-0.2336
AMB	650	TTi-TTj	-2.5253	-2.1590
AMB	650	LSi-FCj	0.2845	-1.6266
AMB	650	LSi-FCj	-1.2701	-9.6719
AMB	650	LSi-FCj	-0.1958	1.1996
AMB	650	LSi	-0.2331	NA

AMB	650	<i>LSi</i>	-0.2138	NA
AMB	650	<i>LSi</i>	-0.1448	NA
AMB	650	<i>FCi</i>	-2.9850	NA
AMB	650	<i>FCi-TTj</i>	-5.2717	-8.6026
AMB	650	<i>FCi-TTj</i>	-0.4064	-2.3383
AMB	650	<i>FCj-LSi</i>	-1.6266	0.2845
AMB	650	<i>FCj-LSi</i>	-9.6719	-1.2701
AMB	650	<i>FCj-LSi</i>	1.1996	-0.1958
AMB	650	<i>LSi</i>	NA	-0.2331
AMB	650	<i>LSi</i>	NA	-0.2138
AMB	650	<i>LSi</i>	NA	-0.1448
AMB	650	<i>FCi</i>	NA	-2.9850
AMB	650	<i>TTi-FCj</i>	-8.6026	-5.2717
AMB	650	<i>TTi-FCj</i>	-2.3383	-0.4064
WARM	400	<i>LSi-LSj</i>	0.3441	0.4993
WARM	400	<i>LSi-LSj</i>	-0.3748	-0.0283
WARM	400	<i>LSi</i>	0.0006	NA
WARM	400	<i>FCi-FCj</i>	-2.1646	-0.8046
WARM	400	<i>FCi-FCj</i>	-6.0889	0.1491
WARM	400	<i>FCi-FCj</i>	-2.2462	-3.4977
WARM	400	<i>LSi-FCj</i>	0.3033	-8.6560
WARM	400	<i>LSi-FCj</i>	-0.0899	-1.0226
WARM	400	<i>LSi-FCj</i>	-0.2695	-10.6006
WARM	400	<i>LSi</i>	-0.1465	NA
WARM	400	<i>LSi</i>	-0.9993	NA
WARM	400	<i>LSi</i>	-0.3516	NA
WARM	400	<i>FCi</i>	-1.5969	NA
WARM	400	<i>FCi</i>	-3.0239	NA
WARM	400	<i>FCi</i>	-4.1678	NA
WARM	400	<i>FCi-LSj</i>	-8.6560	0.3033
WARM	400	<i>FCi-LSj</i>	-1.0226	-0.0899
WARM	400	<i>FCi-LSj</i>	-10.6006	-0.2695
WARM	400	<i>LSi</i>	NA	-0.1465
WARM	400	<i>LSi</i>	NA	-0.9993
WARM	400	<i>LSi</i>	NA	-0.3516
WARM	400	<i>FCi</i>	NA	-1.5969
WARM	400	<i>FCi</i>	NA	-3.0239
WARM	400	<i>FCi</i>	NA	-4.1678
WARM	650	<i>LSi-LSj</i>	0.4248	-0.0166
WARM	650	<i>LSi-LSj</i>	-0.2787	-0.8828
WARM	650	<i>LSi-LSj</i>	-0.2848	0.1258
WARM	650	<i>FCi</i>	-1.1396	NA
WARM	650	<i>FCi-FCj</i>	-1.1099	-8.2520

WARM	650	<i>TTi</i>	NA	-13.2494
WARM	650	<i>LSi-FCj</i>	-0.0501	1.0849
WARM	650	<i>LSi-FCj</i>	-0.6719	2.3411
WARM	650	<i>LSi-FCj</i>	-0.2814	-1.1396
WARM	650	<i>LSi-TTj</i>	-0.1139	-0.1288
WARM	650	<i>LSi</i>	0.0344	NA
WARM	650	<i>LSi-TTj</i>	-0.2700	0.8027
WARM	650	<i>FCi-TTj</i>	-2.6119	1.2741
WARM	650	<i>FCi-TTj</i>	-0.6795	-0.1302
WARM	650	<i>FCj-LSi</i>	1.0849	-0.0501
WARM	650	<i>FCj-LSi</i>	2.3411	-0.6719
WARM	650	<i>FCj-LSi</i>	-1.1396	-0.2814
WARM	650	<i>TTi-LSj</i>	-0.1288	-0.1139
WARM	650	<i>LSi</i>	NA	0.0344
WARM	650	<i>TTi-LSj</i>	0.8027	-0.2700
WARM	650	<i>TTi-FCj</i>	1.2741	-2.6119
WARM	650	<i>TTi-FCj</i>	-0.1302	-0.6795
