

The use of open–top chambers to assess the effects of elevated temperatures on subtropical grassland vegetation *in situ*: A case study on KwaZulu–Natal Sandstone Sourveld

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ABSTRACT

The predicted increase (1.4–5.8 °C) in Earth's surface temperature within this century, and ± 3 °C increase in southern Africa by 2060, motivated the present study. This rise in temperature, particularly within the subtropics and tropics, are expected to have a major impact on floristic biodiversity, which are likely to be exacerbated by increased urbanisation and poor management practices. The subtropical grasslands of South Africa, specifically KwaZulu–Natal Sandstone Sourveld (KZNSS), are of particular concern due to their high biodiversity and endemism.

This dissertation presents an assessment of, (1) the utility of open–top chambers (OTCs) in simulating *in situ* elevated temperatures within a subtropical grassland (KZNSS), and (2) the productivity and floristic responses of KZNSS vegetation to elevated temperatures. Soil and air temperature, other abiotic variables (relative humidity [RH], soil moisture content and light intensity) as well as plant productivity, density and species composition were compared between OTC and control plots located within a remnant patch of KZNSS. The plots ($n = 5$ for OTC and control plots) were monitored for two months in each of four seasons, after which all vegetation within them was harvested for identification and biomass measurements. Floristic and above ground productivity (AGP) data were compared between OTC and control plots during the low (autumn and winter) and high (spring and summer) productivity period (LPP and HPP, respectively) and for all seasons combined (annually) at the life form, family and species levels. Unweighted pair group method with arithmetic mean analysis (UPGMA) and non–parametric multidimensional scaling (NMDS) ordination were used to compare floristic composition and structure between OTC and control plots.

The principal findings of this research were as follows:

- The OTCs were capable of raising the average, maximum and minimum day and night, air and soil temperatures across all seasons to levels within the range predicted for southern Africa in the next century.
- This increase in temperature was accompanied by a reduction in RH and soil moisture content, while effects on light intensity were marginal.
- Elevated temperatures significantly increased graminoid, shrub and combined AGP but decreased forb density, within certain seasons.
- Elevated temperatures increased species richness slightly, had no effect on patterns of family dominance, and decreased species evenness during both productivity periods and annually.

It can be concluded that OTCs are able to elevate *in situ* temperatures within subtropical grasslands to levels within the range predicted for southern Africa. Poaceae species benefited most from these elevated temperatures in terms of AGP and this increase in graminoid dominance appears to have compromised forb AGP and densities, while shrubs were largely unaffected. Elevated temperatures had little effect on community composition and diversity but did alter floristic structure and productivity patterns. With an increase in Poaceae dominance at elevated temperatures, plant competitive interactions could be intensified within KZNSS, subsequently altering community composition and structure. The results of the present study highlight the need for more research on the responses of subtropical grasslands to different climate change scenarios.

PREFACE

The experimental work described in this dissertation was carried out in the School of Life Sciences, University of KwaZulu–Natal, Durban, from February 2014 to December 2016, under the supervision of Dr Sershen Naidoo.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

DECLARATION 1 – PLAGIARISM

I, Rowan David Buhrmann, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
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DECLARATION 2 – PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis (include publications in preparation, submitted, *in press* and published and give details of the contributions of each author to the experiment work and writing of each publication)

Publication 1: Buhrmann, R.D., Ramdhani, S., Pammenter, N.W. & Naidoo, S., 2016, ‘Grasslands feeling the heat: The effects of elevated temperatures on a subtropical grassland’, *Bothalia* 46, a2122. doi: 10.4102/abc.v46i2.2122

Signed:

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LIST OF ABBREVIATIONS

AGP	Above-ground biomass production
BGP	Below-ground biomass production
C	Carbon
c.	Approximately
CO ₂	Carbon dioxide
DEA	Department of Environmental Affairs
GCMs	General Circulation Models
HPP	High Productivity Period
IAPs	Invasive alien plants
IPCC	Intergovernmental Panel on Climate Change
ITEX	International Tundra Experiment
IUCN	International Union for Conservation of Nature
KZN	KwaZulu-Natal
KZNSS	KwaZulu-Natal Sandstone Sourveld
LPP	Low Productivity Period
NMDS	Non-metric multidimensional scaling
NPP	Net primary productivity
OTCs	Open-top chambers
PAR	Photosynthetically active radiation
POSA	Plants of southern Africa
RH	Relative humidity
SANBI	South African National Biodiversity Institute
SD	Standard Deviation
TWNR	Tanglewood Nature Reserve
UPGMA	Unweighted Pair Group Method with Arithmetic mean
USA	United States of America
viz.	Namely
VPD	Vapour pressure deficit
[CO ₂]	Carbon dioxide concentration

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Plants in a changing climate

Various climate change scenarios are predicted to influence plant growth and performance, and in turn floristic composition and structure (Bellard *et al.* 2012; Zavaleta *et al.* 2003). These effects are predicted to occur at various scales and may differ across vegetation types (Bellard *et al.* 2012; Cramer *et al.* 2001; Gonzalez *et al.* 2010; Laurance *et al.* 2011; Melillo *et al.* 1993). Over the last few decades, a wealth of research on climate change has focused on predicting how biodiversity specifically will respond to this global phenomenon (Bellard *et al.* 2012; Dillon, Wang & Huey 2010; Laurance *et al.* 2011). The ability to predict these impacts on plant biodiversity is crucial for designing current and future conservation and management plans. Predictions at local and global scales require *in situ* research that is based on realistic climate change scenarios, as well as reproducible methods that allow for comparative studies (e.g. northern versus southern hemisphere). However, climate change studies on *in situ* plant responses have proven to be challenging to accomplish, particularly within subtropical and tropical regions.

Nevertheless, various elements of climate change have been predicted to affect biodiversity at all levels, from organisms to biomes (Parmesan 2006). There are currently very few cases of extinctions that can be directly attributed to climate change, but it has been suggested that climate change will be the greatest threat to biodiversity in the future, even surpassing habitat destruction (Leadley *et al.* 2010). Generally, terrestrial biomes of the world are delimited by climate, with temperature and precipitation being the main predictors of the locations of these biomes (Donoghue & Edwards 2014; Whittaker 1975; Woodward, Lomas & Kelly 2004). Thus a change in climate is likely to result in changes in the delimitation and spatial distribution of current biomes. The shift in species ranges have been well-documented, with general consensus being that species will shift towards the poles or increase in altitude to maintain their current climatic conditions (Bellard *et al.* 2012; Grimm *et al.* 2013;

Parmesan & Yohe 2003). Thomas *et al.* (2004) suggested that global warming will be the greatest threat to biodiversity, and changes in regional temperatures are likely to result in many species shifting their habitable range (Intergovernmental Panel on Climate Change [IPCC] 2007).

Numerous studies have focused on the effects of climate change within areas deemed to be at high risk (e.g. the tundra boreal regions [Marion *et al.* 1997; Molau 1997]), or largely intact areas that are crucial for carbon sequestration (e.g. tropical rainforests [Stephens *et al.* 2007]). However relatively little research has focused on grasslands, probably because they are often viewed as ‘mundane’ and not as species rich as other biomes, with a lower rank in terms of conservation importance in the context of climate change (Parr *et al.* 2014). This is a serious misconception though as grasslands can be as diverse as some tropical rain forests (Murphy, Andersen & Parr 2016) and in playing an important role as carbon sinks, are critically important in climate change mitigation (Boval & Dixon 2012). In addition, grasslands are not just at risk from the effects of climate change, but are also threatened by a range of anthropogenic factors globally.

1.2 Grasslands around the world

Grasslands cover around 40% of the Earth’s surface (excluding the ice covered regions of Greenland and Antarctica) and serve as home to over one billion people globally (Reyers *et al.* 2005). Of the 234 Centres of Plant Diversity around the world (Davis, Heywood & Hamilton 1995), over 110 are either located in, or contain some areas of grassland. Additionally, 35 of the 142 terrestrial ecoregions (Olson *et al.* 2001) as well as 11% of the world’s endemic bird areas are found within grasslands (White, Murray & Rohweder 2000). Besides their high biodiversity, grasslands play host to a number of services that humans utilize, ranging from agriculture to tourism (Egoh *et al.* 2011; Reyers *et al.* 2005; White, Murray & Rohweder 2000).

Approximately 20% of the Earth’s land surface is covered by tropical grasslands, which accounts for about 15% of the planet’s carbon and 30% of the terrestrial net primary productivity (Parr *et al.* 2014). However, the high biodiversity and carbon sequestration capacity of this biome has not increased its global conservation and protection status

dramatically, with close to 23.6% of grasslands having been transformed through anthropogenic activities and only 11.9% being under protection (Hoekstra *et al.* 2005).

South Africa occupies less than 2% of the Earth's terrestrial area, yet is well known for its high biodiversity. It plays host to nearly 10% of the plant, and 7% of the mammal, reptile and bird species found globally, and contains three of the 35 globally recognised biodiversity hotspots (Driver *et al.* 2004). With only 4.8% of its total land area under formal protection, South Africa falls short of the IUCN's recommended 10% protected land area coverage. Floristically, South Africa ranks in the top five countries containing the highest number of plant species; with the most recognised being the Cape Floristic Region (Reyers *et al.* 2005). Apart from the Cape Floristic Region, grasslands within the Maputaland–Pondoland–Albany biodiversity hotspot have also been reported to harbour high levels of plant biodiversity and represent centres of endemism (Mucina & Rutherford 2006).

The grasslands of South Africa cover 16.5% of the terrestrial land or an area of 349.174 km², where climate, fire and grazing are the major determinants of the grassland biome boundary (Neke & Du Plessis 2004). These grasslands have been said to be analogous to the southern Australian (C₄ grasslands), North American (tallgrass prairies), South American (pampas) and south–east Asian (steppe) grasslands (Uys, Bond & Everson 2004). They host almost half of the 34 endemic mammals found in South Africa, 52 of the 122 'Important Bird Areas' found in the country, and 10 of the 14 globally threatened bird species. Additionally, a third of the butterflies and 22% of the reptiles endemic to South Africa are also found in grasslands (Reyers *et al.* 2005). However, it is not just the high levels of biodiversity within South African grasslands that needs to be highlighted; these grasslands, like others elsewhere in the world, also play a major role in carbon sequestration (Boval & Dixon 2012) and contribute to the hydrological cycle by reducing the amount of runoff and erosion, while also storing water underground or in wetlands (Egoh *et al.* 2011).

Unfortunately, the South African grassland biome is under great pressure from transformation and fragmentation, which has already resulted in a loss of 120 175 km² or approximately 33.3% of this grassland biome (Carbutt *et al.* 2011). These pressures are in the form of afforestation, cultivation, overgrazing, mining and urbanisation (Bond

& Parr 2010; Carbutt *et al.* 2011; Egoh *et al.* 2011; Neke & Du Plessis 2004; Reyers *et al.* 2005). Anthropogenic activities contribute to the transformation of these grasslands both directly and indirectly through climate change which is considered to be driven by anthropogenic activities (Dhillon & Wuehlisch 2013). On this note, climate change has been predicted to have major impacts on plant biodiversity around the world (Bellard *et al.* 2012; Trisurat, Shrestha & Kjelgren 2011), and these impacts are likely to extend to grasslands (Boval & Dixon 2012; Grimm *et al.* 2013).

1.3 Climate change scenarios likely to affect plants

The potential effects of climate change on vegetation have been well-documented in terms of predictive models (Cramer *et al.* 2001; Gonzalez *et al.* 2010; Moncrieff *et al.* 2015; Scheiter, Langan & Higgins 2013). Data on *in situ* responses of plants to climate change scenarios such as elevated temperature (Flanagan & Johnson 2005; Godfree *et al.* 2011; Ojima *et al.* 1993; Zavaleta *et al.* 2003) and atmospheric carbon dioxide (CO₂) levels (Mikkelsen *et al.* 2008; Mueller *et al.* 2016; Norby *et al.* 2005; O’Leary *et al.* 2015; Zavaleta *et al.* 2003) are rare, particularly for subtropical and tropical regions. Elevated levels of CO₂, changes in precipitation, and elevated temperatures are three of the most widely researched consequences of climate change. A detailed assessment of all the impacts of each of these climate change scenarios on plants is beyond the scope of this dissertation but have been extensively reviewed elsewhere (IPCC 2007) and for the sake of contextualising the chapters that follow, are only briefly discussed below.

1.3.1 Elevated CO₂

The carbon cycle has been classified as one of the most important cycles to occur on Earth (Dhillon & Wuehlisch 2013). Without carbon, there would simply be no life. However, due to anthropogenic activities, the level of CO₂ within the atmosphere has increased dramatically (IPCC 2007), and has been directly related to the rise in global temperatures and irregular precipitation events (Zavaleta *et al.* 2003). This increase in CO₂ is primarily caused by fossil fuel emissions from anthropogenic activities, and secondarily from land use change (IPCC 2013). Plant responses to elevated CO₂ concentrations ([CO₂]) vary according to the photosynthetic pathway utilised by each plant. Under elevated [CO₂], plants using the C₃ photosynthetic pathway may

experience enhanced growth responses through increased leaf photosynthesis, relative to plants using the C₄ pathway (Prior *et al.* 2011; Zhu *et al.* 2012). These responses however vary under different environmental factors, including temperature (Ainsworth & Long 2005; Reddy, Rasineni & Raghavendra 2010). There is an increasing body of knowledge supporting the fact that rising [CO₂] will have a negative impact on terrestrial biomes around the world, with implications for plant community and structure (Leadley *et al.* 1999).

1.3.2 Changes in precipitation

It is anticipated that climate change will result in a redistribution of various climatic conditions around the Earth (Williams, Jackson & Kutzbach 2007). There has been an increase in evidence suggesting that anthropogenic climate change underlies current fluctuations in the global hydrological cycle (i.e. evaporation and precipitation) (Trenberth 2011). The general consensus is that under various climate change scenarios, regions that are currently wet will get wetter, while dry regions will become drier (IPCC 2007; O’Gorman & Schneider 2009; Sun *et al.* 2007). The increase in extreme weather and precipitation events has been noted by numerous authors (see Dhillon & Wuehlisch 2013; O’Gorman & Schneider 2009) and there are suggestions that these extremes are directly linked to climate change–induced changes in temperature (Allan & Soden 2008; Held & Soden 2006).

1.3.3 Elevated temperatures

The continued increase in the emission of greenhouse gases, caused by anthropogenic activities, has and is predicted to result in a rise in global temperatures (Huntingford & Mercado 2016; IPCC 2007; Parmesan *et al.* 2013). Increasing levels of CO₂ will further enhance the infrared radiation warming the Earth’s surface (Loik & Harte 1997; Shaver *et al.* 2000), causing a rise in temperature of between 1.4 and 5.8 °C within the next century (IPCC 2007). It has been projected that future extreme temperature events, i.e. temperatures reaching >5 °C above the average, will tend to last longer, become more frequent, and be more intense than current events (IPCC 2007; Hatfield & Prueger 2015). By studying historical records and working with predictive models, such as the General Circulation Models (GCMs), it is predicted that there will be a greater warming effect during winter than summer, and a larger increase in the daily air minimum than

maximum air temperatures (IPCC 2007; Wan, Luo & Wallace 2002). Global warming has been referred to as one of the greatest threats to biodiversity (Malcolm *et al.* 2006). Grassland vegetation is predicted to be significantly affected by rising temperature (Alward, Detling & Milchunas 1999; De Boeck *et al.* 2007; Wan *et al.* 2005; White, Murray & Rohweder 2000; Zavaleta *et al.* 2003); this forms the focus of the present study.

1.4 Effect of elevated temperatures on grasslands

Numerous global studies on the effects of elevated temperatures have shown that most vegetation types will experience an increase in productivity, with the most pronounced effects occurring within cooler climates (Alward, Detling & Milchunas 1999; Nemani *et al.* 2003; Rustad *et al.* 2001). Boval and Dixon (2012) assert that highly diverse grasslands of the world deserve special consideration under the effects of global warming. It has been shown that grass production is triggered by increases in soil temperature when water is not a limiting factor, suggesting that temperature might be the limiting factor in grassland productivity (specifically in montane grasslands of KwaZulu–Natal [Everson & Everson, 1987]). However, the effects of elevated temperatures are not limited to productivity; elevated temperatures can alter the composition and structure of grasslands through changes in plant phenology (Körner & Basler 2010; Wan *et al.* 2005), possibly altering plant–pollinator interactions (Hegland *et al.* 2009), as well as reducing yield and seed quality (Hampton *et al.* 2013; Thuzar *et al.* 2010). Additionally, seed germination is driven by temperature and the availability of water (Mondoni *et al.* 2012; Walck *et al.* 2011); where water determines initiation of root extension, and temperature is required for the activation of numerous enzymes (Long *et al.* 2015). A number of grassland species also exhibit seed dormancy, the development and breaking of which is controlled largely by temperature (Gorgone–Barbosa *et al.* 2016; Santana, Baeza & Blanes 2013).

Most studies on climate change have focused on temperate grasslands (Flanagan & Johnson 2005; Godfree *et al.* 2011; Ojima *et al.* 1993; Zavaleta *et al.* 2003), with very little research done on the effects of global warming on the tropical and subtropical grasslands (Aronson & McNulty 2009). This is worrying, since tropical (and subtropical) vegetation types are likely to experience the greatest loss in biodiversity

with global warming (Perez *et al.* 2016) since many of the species they house have developed thermal specialisation, limiting their habitable range (Laurance *et al.* 2011). Many of these species may therefore have ‘nowhere to go’ in a changing climate (Loarie *et al.* 2009).

This motivated the present study on the effects of elevated temperatures on subtropical grassland vegetation, more specifically KwaZulu–Natal Sandstone Sourveld (KZNSS).

1.5 This study

This dissertation reports on a study that investigated the effects of simulated elevated temperatures on subtropical grassland vegetation. The study used open–top chambers (OTCs) to simulate these elevated temperatures *in situ* and measured plant responses across different seasons.

1.5.1 Aims and objectives

The two aims of the study were as follows: (1) to assess the utility of OTCs in elevating *in situ* temperatures when installed in subtropical grassland vegetation (specifically KZNSS); (2) to investigate the effects of elevated temperatures on subtropical grassland productivity and floristics (specifically KZNSS). These aims were addressed via the following objectives:

- i. To compare air and soil temperatures and other abiotic variables (relative humidity, soil moisture content and light intensity) between OTC (elevated temperature) and control (ambient temperature) plots seasonally.
- ii. To compare plant productivity (above– and below– ground) and density between ambient and elevated temperature plots seasonally.
- iii. To compare floristic composition, diversity and structure between ambient and elevated temperature plots seasonally. To relate air and soil temperature and other abiotic parameters, within ambient and elevated temperature plots to plant productivity and floristic characteristics.

1.5.2 Study site

This study was conducted on a remnant patch of KZNSS located within Tanglewood Nature Reserve, in the Kloof district of the eThekweni Municipal Area (Durban), South Africa (29°48'37"S, 30°49'18"E) (Figure 1). It is a privately owned National Heritage Site (No. 10) managed by the Walker Family Trust, that is securely fenced with controlled access. The site has an altitudinal range of 407–503 m above sea level, and contains ±18 ha of KZNSS which is under a triennial burning regime, with regular alien vegetation removal.

KwaZulu–Natal Sandstone Sourveld, endemic to the province of KwaZulu–Natal, South Africa, is a species rich grassland scattered with low shrubs. This grassland includes many endemic species and has been characterised by flat or rolling plateaus with steep slopes (Rutherford *et al.* 2006). It experiences summer, and occasional winter rainfall with a mean annual precipitation of between 550–1000 mm. It has been classified as an endangered grassland vegetation type and is listed as having a significant irreversible loss of natural habitat (Department of Environmental Affairs [DEA] 2011; Rutherford *et al.* 2006). However, at a provincial level, KZNSS has been classified as being critically endangered (Jewitt 2011). It has been estimated that KZNSS originally covered 179671 ha, but as of 2008, only 11.4% (20566 ha) remains, with only 0.1% (194.0 ha) under formal protection within the Vernon Crookes and Krantzklouf Nature Reserves (DEA 2011; Jewitt 2011; Rutherford *et al.* 2006). KwaZulu–Natal Sandstone Sourveld faces similar transformation pressures as other South African grasslands and is often utilised for agriculture (sugar cane and timber plantations) and urban development (Rutherford *et al.* 2006).

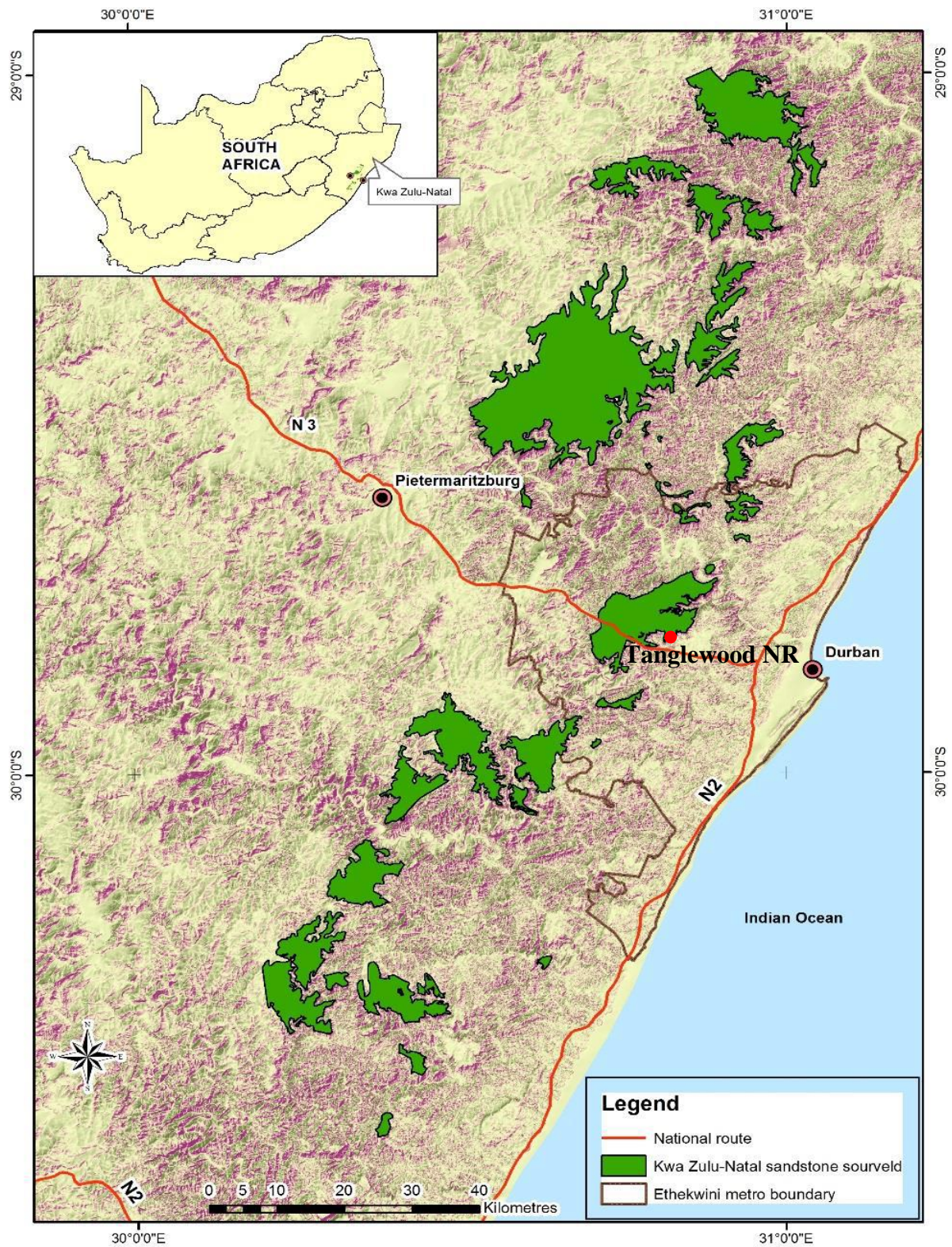


Figure 1. Remnant patches of KwaZulu–Natal Sandstone Sourveld within the KZN Location, and location of study site (Tanglewood nature Reserve) (Source: KwaZulu–Natal Sandstone Sourveld Research Programme – Close–out Report: Phase 1, 2011–2014).

1.5.3 Dissertation outline

This chapter has provided the background and motivation, and set out the aims and objectives for the present study. A brief overview of the literature pertaining to the effects of climate change on plants and grassland vegetation in particular is also provided. The chapter also features information on the KZNSS, the subtropical grassland type within which this study was conducted.

Chapter 2 presents and discusses data on the utility of OTCs in elevating *in situ* air and soil temperatures within a subtropical grassland (KZNSS). Data on the OTC effects on relative humidity, soil moisture content and light intensity are also presented in order to comment on the suitability of OTCs for investigating subtropical grassland responses to elevated temperatures *in situ*. Additionally, seasonal and annual plant productivity and density for the various lifeforms (graminoids, forbs and shrubs) is compared between elevated (OTC) and ambient (control) temperature plots.

Chapter 3 presents and discusses data on the effects of elevated temperatures on plant productivity and density for dominant families and species within a subtropical grassland (KZNSS). In addition, vegetation composition and structure are compared between elevated (OTC) and ambient (control) temperature plots within the high and low productivity periods.

Chapter 4 provides a summary of the key findings in relation to the aims and objectives. Conclusions on the utility of the OTCs in simulating elevated temperatures within a subtropical grassland, and the productivity and floristic responses of KZNSS vegetation to these elevated temperatures are also drawn. This chapter concludes by highlighting the significance and limitations of this study, and offering recommendations for future research.

CHAPTER TWO

ASSESSING ELEVATED TEMPERATURE EFFECTS ON SUBTROPICAL GRASSLAND VEGETATION USING OPEN-TOP CHAMBERS

2.1 Abstract

Background: Tropical and subtropical Africa is predicted to experience a rise in temperature. The effects of rising temperatures on temperate grasslands have been studied using open-top chambers (OTCs) but reports for tropical/subtropical grasslands are scarce. This study used OTCs to investigate the effects of elevated temperatures on a threatened subtropical grassland type, namely KwaZulu-Natal Sandstone Sourveld (KZNSS).

Objectives: To assess the effects of OTCs on selected abiotic parameters and plant productivity within KZNSS.

Methods: Five OTC and control plots were randomly distributed at the same altitude within a patch of KZNSS. Air and soil temperature, relative humidity (RH), soil moisture content and light intensity were monitored in all plots in spring, summer, autumn and winter. Biomass production and plant density were measured in each season, for each life form (graminoid, forb and shrub), separately and combined.

Results: The OTCs resulted in a rise in average, maximum and minimum day and night, air and soil temperatures. This increase, the degree of which differed across seasons, was accompanied by a decline in RH and soil moisture content. Elevated temperatures led to a significant increase in combined, graminoid and shrub above-ground productivity (AGP) and a decrease in forb density, but in certain seasons only. Below-ground biomass production was unaffected by elevated temperatures.

Conclusions: OTCs can simulate realistic increases of air temperature in subtropical grasslands. Graminoids and shrubs appear to benefit from elevated temperatures whilst forbs decrease in abundance, possibly through competition and/or direct physiological effects.

2.2 Introduction

Extreme weather events, changes in precipitation, considerable variation in temperature and sea level rise provide evidence for global climate change (Dhillon & Wuehlisch 2013). Climate change, suggested to be largely a consequence of anthropogenic activities (Dhillon & Wuehlisch 2013), has impacted negatively on plant biodiversity in terms of both distribution and diversity (Trisurat, Shrestha & Kjelgren 2011).

Increasing temperatures, in particular, are likely to have a number of effects on plant communities globally (De Boeck *et al.* 2007). Global surface temperatures have risen by approximately 0.8 °C over the last century and are predicted to increase by 1.4–5.8 °C during the 21st century (Intergovernmental Panel on Climate Change [IPCC] 2007). In Africa, this increase in temperature is predicted to result in longer growing periods, higher fecundity, higher biomass allocation towards roots, and a possible shift towards tree-dominated biomes (Scheiter & Higgins 2009).

Predicting ecosystem responses to climate change is therefore becoming increasingly important, particularly in tropical regions, where species occupy narrow ranges owing to thermal specialisation (Laurance *et al.* 2011). These areas are likely to experience the greatest loss in biodiversity with an increase in temperature (Perez, Stroud & Feeley 2016). The effects of rising temperatures on grasslands is an important consideration given their high biodiversity (Boval & Dixon 2012), particularly in the tropics, where they occupy approximately 20% land cover (Parr *et al.* 2014). Grasslands play a particularly important role in carbon sequestration and nutrient recycling (Boval & Dixon 2012), and are threatened in many parts of the world by land-use change, poor management and climate change (Jewitt 2011; Parr *et al.* 2014; Sala *et al.* 2000).

The grassland biome in South Africa boasts high levels of endemic mammals, reptiles, butterflies, and very high levels of plant species diversity (Reyers *et al.* 2005). However, an estimated 35% of South African grassland has been either transformed or degraded (Egoh *et al.* 2011). An understanding of how grassland vegetation types will respond to, or be impacted on, by increased temperatures can inform their future conservation and management (Thuiller *et al.* 2008). Various climate change models and numerous *in* and *ex situ* experiments have attempted to predict and demonstrate the

effects of rising temperatures within temperate grasslands (Flanagan & Johnson 2005; Godfree *et al.* 2011; Ojima *et al.* 1993), but effects on tropical and subtropical grasslands have received less attention (Godfree *et al.* 2011). Nevertheless, one of the major effects of rising temperatures on grassland and other biomes is predicted to be altered plant productivity (Guoju *et al.* 2005). Plant productivity, or more correctly net primary productivity (NPP), is the net rate of carbon (C) gain incorporated into plant vegetation over a given time period (Girardin *et al.* 2010; Long & Hutchin 1991). It represents a large portion of organic matter consumed by animals and microbes. Changes in NPP can therefore impact the quantity and quality of food available to animals, including humans (Potter, Klooster & Genovese 2012). In regions where plant carbon assimilation is limited because of low temperatures, increased temperatures could increase NPP (De Boeck *et al.* 2007); however, these responses are likely to be species and life form dependent (Lattanzi 2010; Luo *et al.* 2013; Wand *et al.* 1999).

Plants undergo physiological changes such as altered carbon assimilation rates when subjected to increased temperatures (Ahuja *et al.* 2010; Saxe *et al.* 2001). The effects of increased temperature are not universal – for example, C₃ and C₄ plants show different physiological responses to elevated temperatures (Horton & Murchie 2000; Sage 2000). C₄ plants have a carbon fixation pathway that is far less sensitive to an increase in temperature than C₃ types (Gowik & Westhoff 2011). In fact, C₄ crops and grasses are 40% more efficient at converting photosynthetically active radiation (PAR) into biomass than C₃ types in general (Long 1999). In warmer climates, the greater efficiency of C₄ photosynthesis at higher temperatures is likely to result in higher NPP in C₄ relative to C₃ species, particularly in low-latitude grasslands, deserts and coastal zones (Sage 2000). These differences in NPP between C₃ and C₄ species can impact on species composition and richness by altering competitive interactions (Wand *et al.* 1999).

Increased temperatures, in altering the standing biomass, can also impact on fire regimes, which are an essential part of grassland ecology (Trollope, Trollope & Hartnett 2002). Global warming is therefore expected to impact the carbon budget as a consequence of changes to plant photosynthesis and growth, as well as soil respiration (Schindlbacher, Zechmeister-Boltenstern & Jandl 2009; Wan *et al.* 2005). More

specifically, changes in carbon cycling induced by global warming, as a result of changes in NPP and heterotrophic respiration, can influence whether terrestrial ecosystems act as carbon sources or sinks (Wan *et al.* 2005). Increased evaporative cooling in the leaves, another predicted consequence of increased temperature, can also induce water stress in certain plant species (Beerling, Osborne & Chaloner 2001) and impact on ecosystem hydrology (Williams & Scott 2009). An increase in temperature is usually accompanied by a subsequent increase in water deficit, restricting plant growth and photosynthesis, which leads to varied effects in different ecosystems (De Boeck *et al.* 2007; Wan, Luo & Wallace 2002). This implies that both stresses, temperature and water deficit, and their interactive effects need to be considered when assessing plant responses to increased temperature.

The use of open-top chambers (OTCs) for studying the effects of simulated elevated temperatures on grassland (and other) vegetation has recently gained more interest, but predominantly in temperate and polar regions (Flanagan & Johnson 2005; Flanagan, Sharp & Letts 2013; Molau & Mølgaard 1996). However, the utility of these chambers in studying the effects of elevated temperatures on tropical and subtropical vegetation has yet to be established. This, and the likelihood that Africa will experience some of the greatest increases in temperature (IPCC 2007), prompted the present study, which used OTCs to assess the *in situ* growth and community structure responses of subtropical grassland vegetation to elevated temperatures. This study was conducted in a remnant patch of KwaZulu–Natal Sandstone Sourveld (KZNSS) in Durban, South Africa. This grassland type is located along the coastal belt of KwaZulu–Natal and is home to a number of endemic species (Mucina & Rutherford 2006). It is highly transformed and critically endangered (Jewitt 2011). The study assessed whether OTCs can simulate realistic increases (i.e. increases in line with predictions for this century) in temperatures in a subtropical grassland. More importantly, the effects of OTCs on soil and air temperature and a range of abiotic parameters (relative humidity, soil moisture content and light intensity) are related to seasonal and annual plant productivity and density responses across different life forms.

2.3 Methods

2.3.1 Site selection and experimental approach

The study site was a remnant patch of KZNSS located within Tanglewood Nature Reserve, in the eThekweni Municipal Area (Durban), South Africa (29°48'37"S, 30°49'18"E). Five experimental (OTC) and five control, hexagonally shaped plots, approximately 2.012 m², were randomly distributed along a south–east facing slope at approximately the same altitude (425 m above sea level). Each experimental plot was paired with a control plot, located within 2 m of each other. Before installing the chambers each season, the vegetation in all plots was cut to ground–level and the soil was disked to promote seedling recruitment through softening the soil and splitting of grass roots (Farooq *et al.* 2011; van Acker, Bullied & du Croix Sissons 2004). Once the plots were prepared, hexagonal polycarbonate OTCs (described below) were installed in all experimental plots. Many KZNSS grasses grow rapidly and can exceed the height of the OTCs used here within a period of 2 – 3 months, negating the effects of the chambers. For this reason, the OTCs were installed and left in place for two months only during each of the four seasons (spring: 1 September – 30 November; summer: 1 December – 28/29 February; autumn: 1 March – 31 May; winter: 1 June – 31 August; between 2014 and 2015).

Several antelope species (e.g. blesbok [*Damaliscus pygargus phillipsi*], bushbuck [*Tragelaphus sp.*] and zebra [*Equus quagga*]) occur on the reserve, and to prevent the potential confounding effects of herbivory, the control plots were enclosed by 1.5 m high wire fence.

2.3.2 Chamber construction

The OTCs used in this study are based on a design modified from the International Tundra Experiment (ITEX) (Molau & Mølgaard 1996). The chambers were hexagonal in shape (see Figure 2 for dimensions), 505 mm in height, and constructed using clear 2 mm thick Naxel polycarbonate sheeting (Maizey Engineering Plastic Products, New Germany, South Africa), which has a light transmittance of ±90%. The frame supporting the polycarbonate was constructed from 20 mm plastic conduit tubing. The OTCs were secured to the ground using steel pegs.

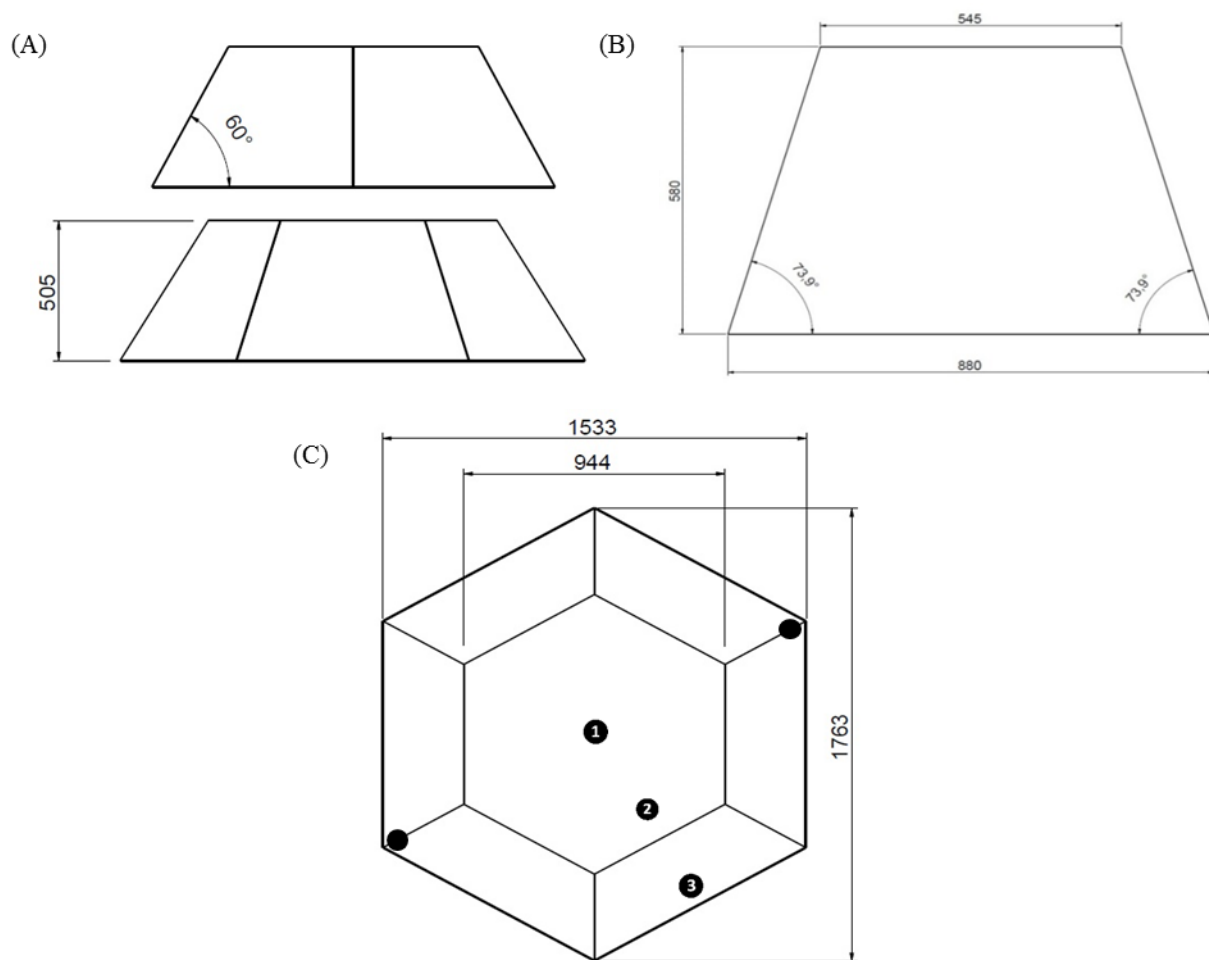


Figure 2. Open-top chamber design modified from Molau and Mølgaard (1996). Side view showing chamber height of 505 mm (A), individual panel of chamber (B), and top view (C) where the black circles indicate locations at which light intensity, relative humidity and temperatures measurements were conducted at midday ($n = 5$). Numbered black circles indicate where digital temperature loggers were positioned.

2.3.3 Measurement of environmental parameters

Air and soil temperatures were monitored across all plots (control and treatment) throughout the experimental period using digital temperature loggers (Thermochron iButton Device – DS1921G, Maxim Integrated™, San Jose, USA), placed 5 cm below ground level for soil temperature and 10 cm above ground level for air temperature. Four temperature loggers were used in each control ($n = 20$) and six ($n = 30$) within each experimental plot. The Thermochrons were programmed to record temperatures on

an hourly basis throughout the experimental period. Their positioning within the control and OTC plots is shown in Figure 1C.

Additionally, air temperature and relative humidity (RH) were measured in all plots 25 cm above ground level using a 4500 Pocket Weather Tracker (Kestrel, Birmingham, UK) weekly (at midday). Soil moisture content and light intensity were also measured weekly (at midday) using a Soil Moisture Meter (HH2 Moisture Meter, Delta-T Devices, Cambridge, UK) and a Digital Light Meter (ESR-1, PP Systems, Amesbury, USA). The positions at which these measurements were carried out within the OTC plots are shown in Figure 1C. Weekly rainfall data were collected using a standard non-recording rain gauge. Seasonal rainfall was calculated by summing the weekly rainfall data.

2.3.4 Plant growth measurements

At the start of each season, immediately before installing the OTCs, soil cores were taken along the immediate periphery of (but external to) all plots, using a soil corer (18 cm long by 4.75 cm wide). A total of three cores were taken for each plot ($n = 15$ for control and OTC) in each season and these were stored at $-18\text{ }^{\circ}\text{C}$. After the 2-month experimental period, soil cores were performed again for each plot ($n = 15$ for control and OTC), but this time within each plot. Soil cores were rinsed under running water with a sieve to free roots of soil and dead roots were removed. The total (indiscriminate) root dry mass was determined by drying the roots collected at $75\text{ }^{\circ}\text{C}$ for 72 h, and then weighing these using a three-place balance (Mettler Toledo, Columbus, USA).

The above-ground biomass of each plant, which was categorised based on life form (graminoid, forb or shrub), within all plots was harvested and placed in separate paper bags. The plant material was dried at $75\text{ }^{\circ}\text{C}$ for 72 h, and then weighed using a three-place balance (Mettler Toledo, Columbus, USA). Biomass production (grams of dry mass [g]) was compared between control and OTC plots, within life forms for above-ground biomass production (AGP). Within life form comparisons were not possible for below-ground biomass production (BGP) as roots could not be separated into individual species/life forms.

2.3.5 Statistical analysis

All data were tested for normality using either the Kolmogorov–Smirnov or Shapiro–Wilk test, depending on sample size. Percentage data were arcsine transformed prior to any analyses. As initial analyses revealed no significant differences in temperature across different locations within individual plots (control and OTC), these data were pooled for all subsequent analyses. Significant differences between control and OTC plots for all environmental parameters (midday temperature, RH, soil moisture content and light intensity) were tested within seasons via a Wilcoxon signed–rank test, as all data were non–parametric. For the Thermochron readings, differences between control and OTC plots were tested for within 3–hour intervals (00:00–03:00; 03:00–06:00; 06:00–09:00; 09:00–12:00; 12:00–15:00; 15:00– 18:00; 18:00–21:00; 21:00–24:00) using a Wilcoxon signed–rank test within each season. The maximum and minimum temperatures for each Thermochron within OTC plots and within control plots were averaged seasonally to determine the average maximum and minimum temperatures. The highest and lowest temperature reading for each season (in OTCs and control plots) was labelled absolute maximum and absolute minimum temperature.

Seasonal plant densities were determined for each life form by dividing the number of individual plants by the plot size (2.012 m²). Above–ground biomass allocation was calculated by expressing the AGP for each life form as a percentage of the total AGP for all life forms combined. Comparisons of AGP, above–ground biomass allocation and plant densities between control and OTC plots were made within and across life forms using a Wilcoxon signed–rank test. Pearson’s correlation was used to determine the relationship between the abiotic parameters (temperature, RH and soil moisture content) and AGP, above–ground biomass allocation and plant density. Where applicable, non–parametric data were tested with a Spearman’s rank correlation. All statistical analyses were performed using IBM SPSS Statistics 21 (SPSS IBM, New York, USA) and differences were considered significant at the 0.05 level.

2.5 Results

The results presented below reflect the effects of open-top chambers (OTCs) on *in situ* temperatures, other abiotic factors, and plant productivity and community structure. Measurements were conducted in each of the four seasons.

2.5.1 Diurnal air and soil temperature in control and OTC plots

Diurnal patterns of variation in temperature were similar in OTC and control plots in all seasons (Figure 3), with the highest temperatures occurring between 11:00 and 12:00 and lowest between 04:00 and 06:00. The air and soil OTC temperatures were significantly ($p < 0.05$ in all cases) higher than the control temperatures for all day and night 3-hour intervals, in all seasons.

Air temperature differences between OTC and control plots were most marked during the day between 12:00 and 14:00, except for summer when differences were at a maximum between 09:00 and 11:00. At night these differences were most marked between 21:00 and 23:00 in autumn, 18:00 and 20:00 in winter and spring, and 03:00 and 05:00 in summer. These differences across time intervals were less apparent for soil temperatures.

Additionally, the OTCs increased the diurnal absolute maximum air and soil temperatures, ranging between 4.4 °C and 5.7 °C and 0.9 °C and 1.8 °C, respectively, and also increased absolute minimum air and soil temperatures, ranging between 0.3 °C and 2.0 °C and 0.3 °C and 0.8 °C, respectively (Table 1). The OTCs had significantly ($p < 0.05$) higher average day and night, air and soil maximums in all seasons, except for winter night air temperature, and had significantly higher average day and night, air and soil minimums in all seasons, except for winter day air temperature (data not shown). When data on the degree of temperature increase induced by OTCs were compared within seasons, the OTCs significantly ($p < 0.05$) increased the day air (by 1.7 °C–2.4 °C; Figure 3A) and soil (by 0.7 °C–1.0 °C; Figure 3A) temperatures, and night air (by 0.3 °C–0.6 °C; Figure 3B) and soil (by 0.5 °C–1.2 °C; Figure 3B) temperatures in all seasons relative to the control, with the exception of night air temperature in winter.

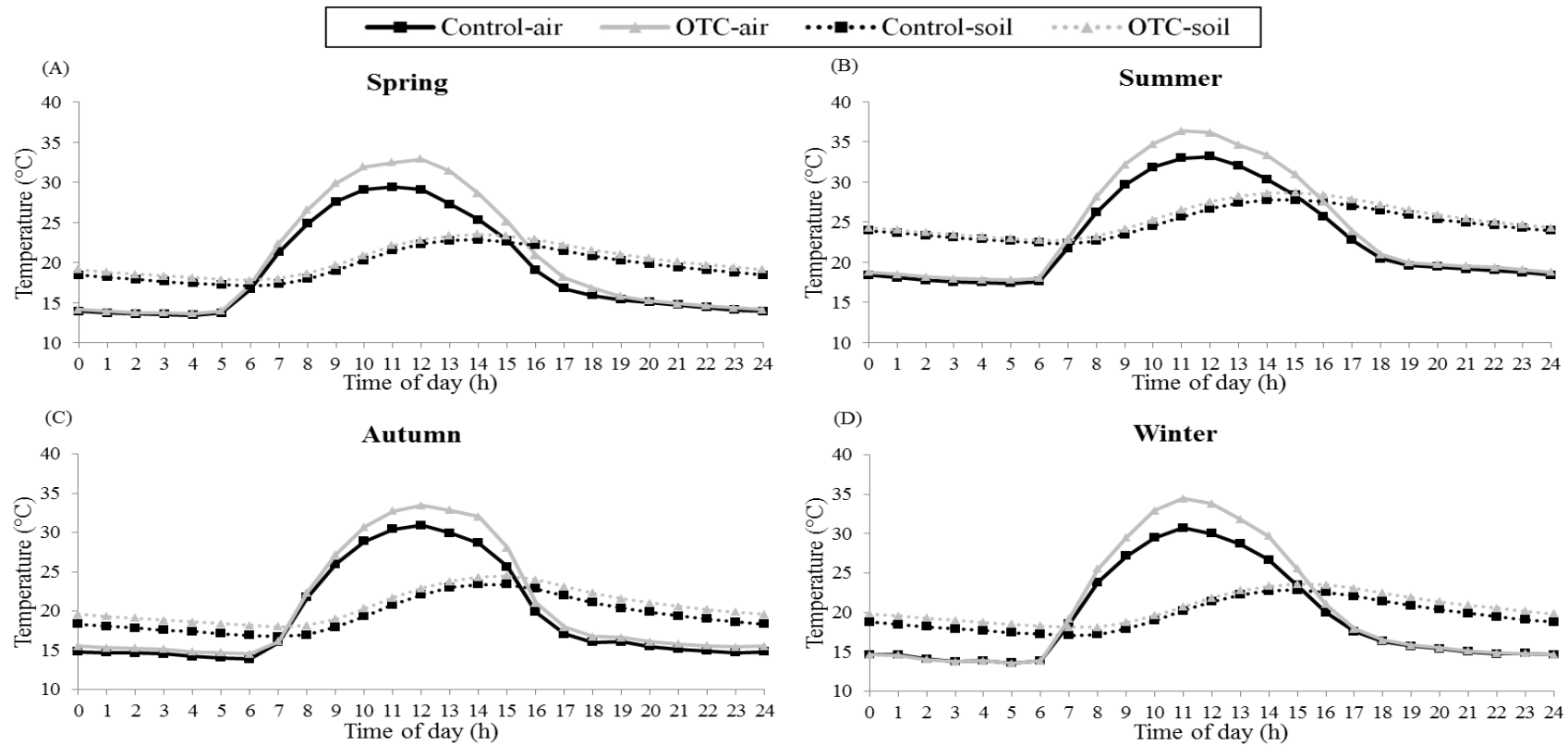


Figure 3. Diurnal variation in air and soil temperature in OTC and control plots in spring (A), summer (B), autumn (C) and winter (D). Air temperature values represent means, with $n = 10240$ for control and OTC in spring, summer and autumn; and $n = 9665$ for control and OTC in winter. For soil temperature values also represent means, with $n = 10240$ for control and OTC in spring, summer and autumn; $n = 9665$ for control and OTC in winter. Except for soil temperatures between 18H00–06H00 in winter, $p < 0.05$ when air and soil temperatures were compared for three hour intervals in each the four seasons. Standard deviations ranged from 9.98–2.16 °C for air temperatures and 3.97–2.09 °C for soil temperatures.

Table 1. Seasonal and annual mean, absolute maximum and absolute minimum temperatures for OTC and control plots.

		Annual		Spring		Summer		Autumn		Winter	
		Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
Mean	Air	20.1±7.6	21.3±8.9	19.2±7.6	20.5±9.4	23.2±7	24.5±8.4	18.2±7.5	19.3±8.3	19.5±7.3	20.5±8.7
	Soil	20.8±4.3	21.7±4.3	19.7±3.4	20.4±3.4	24.9±3.3	25.5±3.7	18.2±3.3	19.4±3.2	19.7±3.2	20.6±3.1
Absolute maximum	Air	45.4	51.1	45.2	49.6	44.1	49.5	45.0	50.0	45.4	51.1
	Soil	35.9	37.7	30.7	31.9	35.9	37.7	30.5	31.4	29.9	30.8
Absolute minimum	Air	5.9	6.2	6.8	7.7	10.3	12.3	7.1	8.7	5.9	6.2
	Soil	11.5	12.1	11.8	12.1	17.2	17.6	13.2	14.0	11.5	12.1

Values in second row represent means±SD.

$n = 10240$ in spring, summer and autumn; $n = 9665$ in winter.

$p < 0.05$ (Wilcoxon signed-rank test) when means were compared within seasonal categories between temperature treatments.

In spring, the OTCs experienced the greatest increase in day air temperature and smallest increase in day soil temperature, whilst autumn was characterised by the smallest increase in day air temperature and highest increase in day soil temperature (Figures 4A and B). In autumn, the OTCs experienced the greatest increase in night air and soil temperature, whilst spring and summer had the smallest increase in night air and soil temperature, respectively.

Midday temperature, RH and soil moisture content, differed significantly ($p < 0.05$) between OTC and control plots in all seasons (Table 2). Average midday OTC temperature readings were significantly ($p < 0.05$) higher than those in control plots; however, the OTC plots exhibited significantly ($p < 0.05$) lower RH and soil moisture content in all seasons (Table 2). Light intensity was significantly ($p < 0.05$) lower in OTC plots in spring, summer and winter; however, these differences were minimal, ranging from 70.59–110.39 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Table 2). When control and OTC data for all seasons were pooled for analysis (annual data), there was a strong negative correlation ($r = -0.745$ – -0.910 , $p < 0.05$) between temperature and RH and within seasons there was a strong positive correlation between RH and soil moisture content in spring ($r = 0.818$, $p < 0.05$) and summer ($r = 0.811$, $p < 0.05$).

There was a large variation in the total rainfall across seasons: 313 mm in spring, 198.5 mm in summer, 46.5 mm in autumn and 13.5 mm in winter.

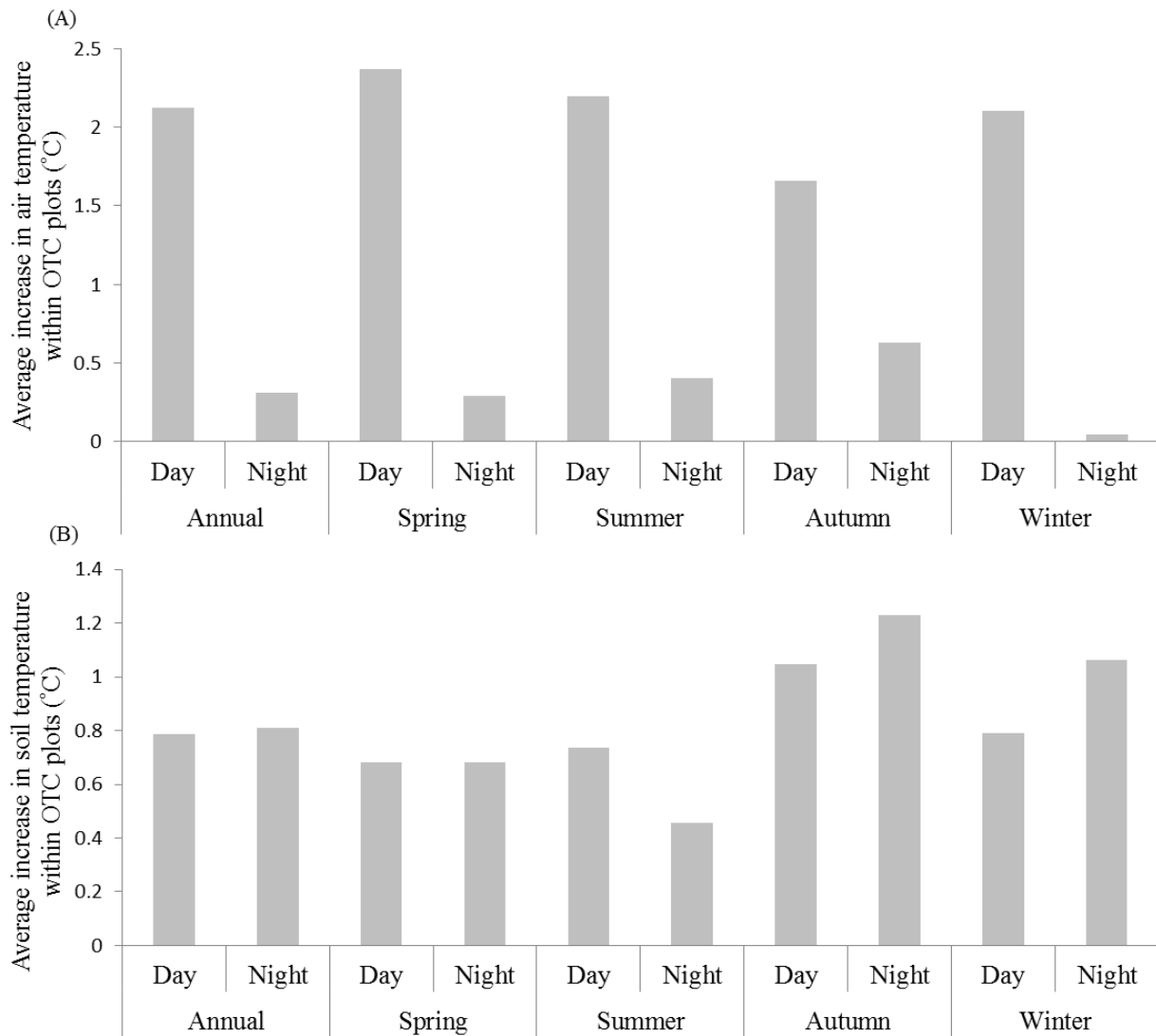


Figure 4. Average increase in temperature (annual and seasonal) within OTC plots, relative to control plots, for air (A) and soil (B). For air and soil measurements $n = 40385$ for annual; $n = 10240$ for spring, summer and autumn; $n = 9665$ for winter. Standard deviations ranged from 1.56–1.82 °C for day, and 0.29–0.53 °C for night air temperature; and from 0.38–0.49 °C for day, and 0.34–0.47 °C for night soil temperature.

Table 2. Seasonal midday air temperature (°C), relative humidity (%), soil moisture content (%), light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and total rainfall (mm) for OTC and control plots.

Season	Elevated/ Ambient	Air Temperature (°C)	Relative Humidity (%)	Soil Moisture Content (%)	Light Intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Total Rainfall (mm)
Spring	Elevated	32.07±4.47	39.2±12.46	7.76±3.34	691.41±165.84	313
	Ambient	28.2±3.69	46.52±14.55	9.85±4.10	762±84.14	
Summer	Elevated	35.3±3.13	49.89±5.02	14.57±4.14	713.67±166.12	198.5
	Ambient	31.45±3.53	54.81±4.99	18.23±3.41	824.05±81.76	
Autumn	Elevated	31.91±4.68	42.71±8.91	6.1±2.71	490.52±171.64	46.5
	Ambient	28.74±4.22	48.3±9.18	8.33±2.63	498.51±89.44	
Winter	Elevated	31.58±3.26	35.45±8.63	3.49±1.45	1120.73±296.73	13.5
	Ambient	28.83±2.88	39.08±9.64	3.81±0.84	1223.98±158.10	

Temperature, relative humidity, soil moisture content and light intensity values represent mean±SD. For air temperature, relative humidity, soil moisture content: $n = 275$ for spring; $n = 125$ for summer and autumn; $n = 175$ for winter. For light intensity: $n = 125$ for spring, autumn and winter; $n = 75$ for summer. Except for autumn light intensity, $p < 0.05$ when values were compared between temperature treatments (Wilcoxon signed-ranked test)

2.5.2 Growth responses

In both OTC and control plots, the highest annual AGP (i.e. all seasons summed) occurred in graminoids, followed by forbs and shrubs (Figure 5). Biomass production for graminoids and all life forms combined was significantly higher in OTC plots ($p < 0.05$; Figure 5). In terms of annual data there was also a trend for shrub productivity to be higher in OTCs, whilst forb productivity was slightly higher in control plots.

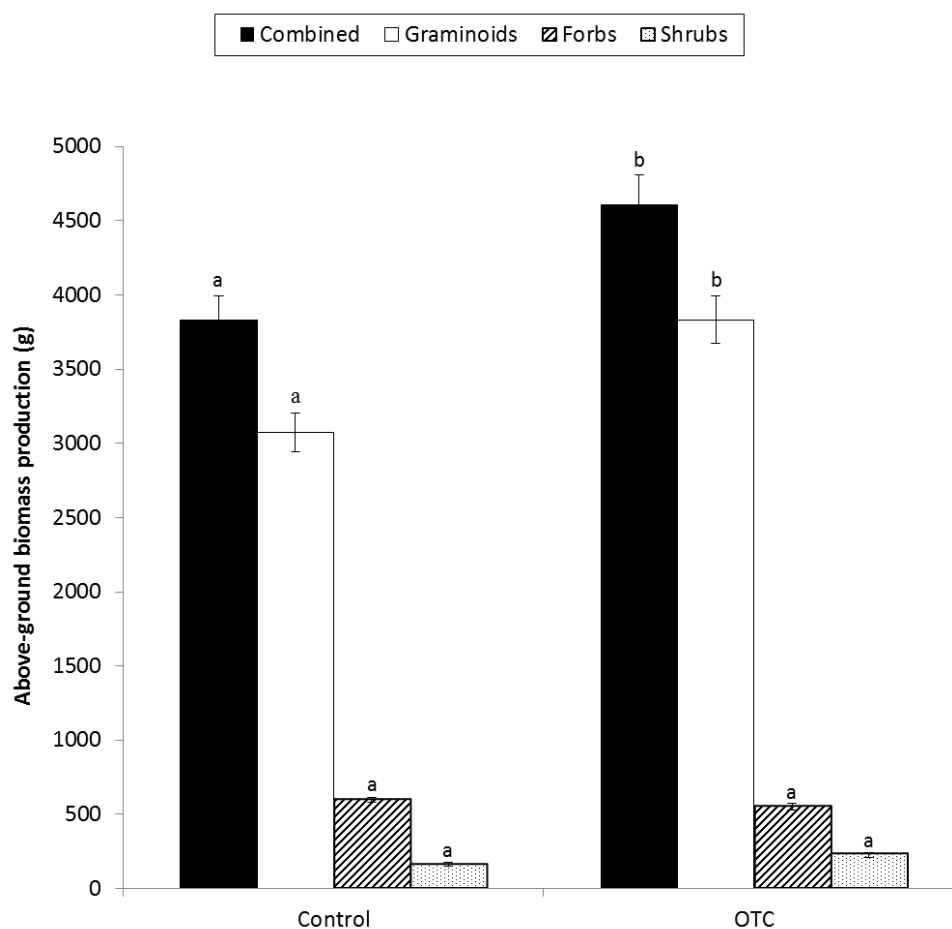


Figure 5. Annual above-ground biomass production (g) for individual and all life forms combined. Values represent mean \pm SD ($n = 20$). Values labelled with different letters are significantly different ($p < 0.05$, Wilcoxon signed-rank test) when compared within life forms between temperature treatments.

When seasonal AGP was compared between OTC and control plots, there were also no significant differences ($p > 0.05$), except in autumn and spring, when graminoid

productivity was higher ($p < 0.05$) in OTCs (Figure 6). Shrub productivity in summer was significantly ($p < 0.05$) higher in OTC plots. Additionally, combined productivity was significantly ($p < 0.05$) higher in OTCs in summer and autumn. Combined productivity was significantly positively correlated ($r = 0.794\text{--}0.956$; $p < 0.05$) with graminoid productivity in all seasons and forb productivity in winter ($r = 0.830$, $p < 0.05$). In summer, there was a strong negative correlation between graminoid and forb productivity ($r = -0.706$, $p < 0.05$). There were no significant differences ($p > 0.05$) in below-ground biomass production (based on soil cores; BGP) between OTC (mean seasonal BGP = $0.004 \pm 0.202\text{g}$) and control (mean seasonal BGP = $0.018 \pm 0.185\text{g}$) plots in individual and combined seasonal scenarios (detailed data not shown).

Maximum temperature was significantly positively correlated with combined AGP (data for all life forms combined) in summer and autumn ($r = 0.818$ and $r = 0.661$, respectively; $p < 0.05$). Maximum temperature was also significantly positively correlated with graminoid AGP in summer and autumn ($r = 0.903$ and $r = 0.661$, respectively; $p < 0.05$). Maximum temperature was also significantly positively correlated with shrub AGP in summer ($r = 0.673$; $p < 0.05$). Minimum temperature was only significantly positively correlated with combined ($r = 0.840$; $p < 0.05$) and graminoid ($r = 0.850$; $p < 0.05$) AGP in autumn.

During autumn, there was a significant negative correlation between combined AGP and both RH ($r = -0.650$; $p < 0.05$) and soil moisture content ($r = -0.840$; $p < 0.05$), as well as between soil moisture content and graminoid AGP ($r = -0.765$; $p < 0.05$). Similarly, in spring there was a strong negative correlation between graminoid AGP and both RH ($r = -0.655$; $p < 0.05$) and soil moisture content ($r = -0.714$; $p < 0.05$).

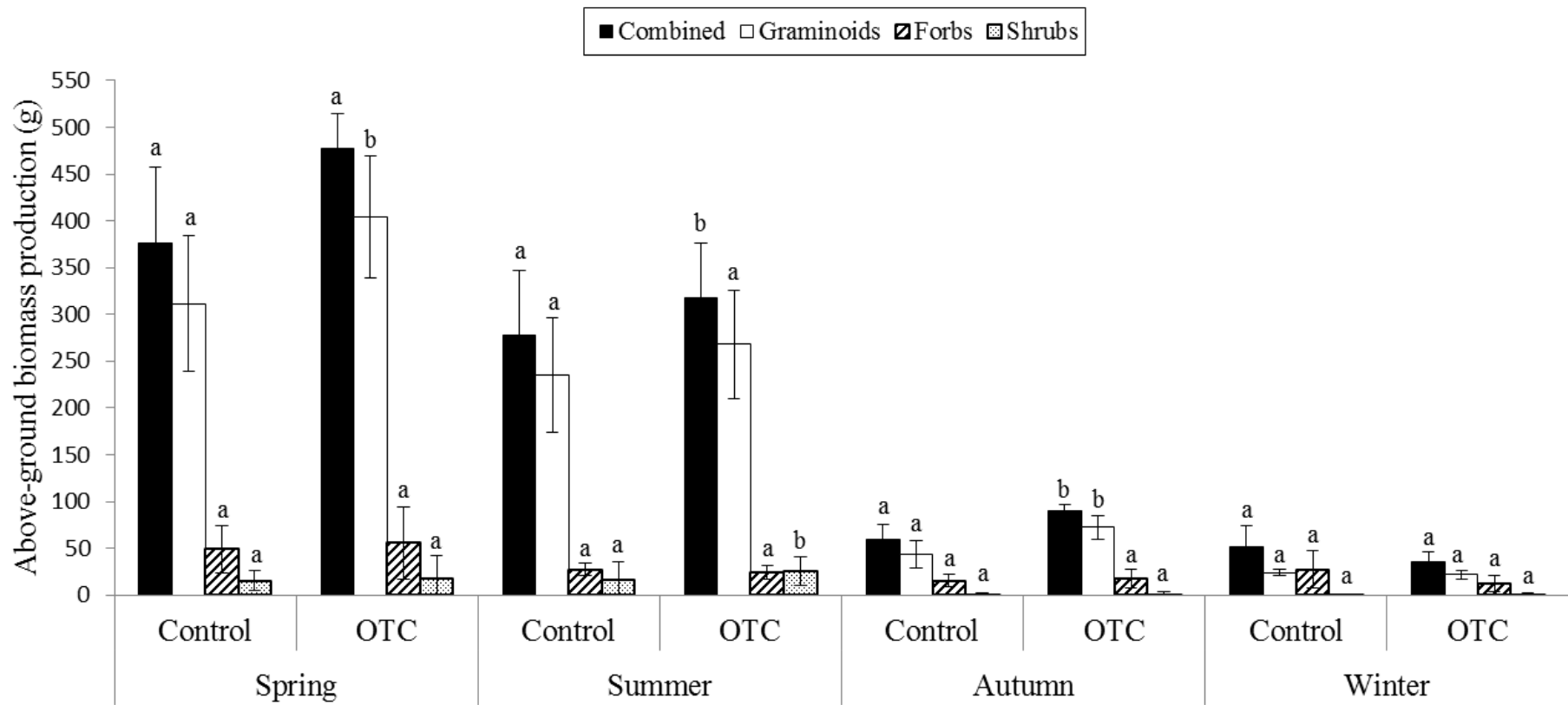


Figure 6. Above-ground biomass production (g) for individual and combined life forms in OTC and control plots. Values represent mean \pm SD ($n = 5$ for individual seasons). Values labelled with different letters are significantly different ($p < 0.05$, Wilcoxon signed-rank test) when compared within life forms between temperature treatments.

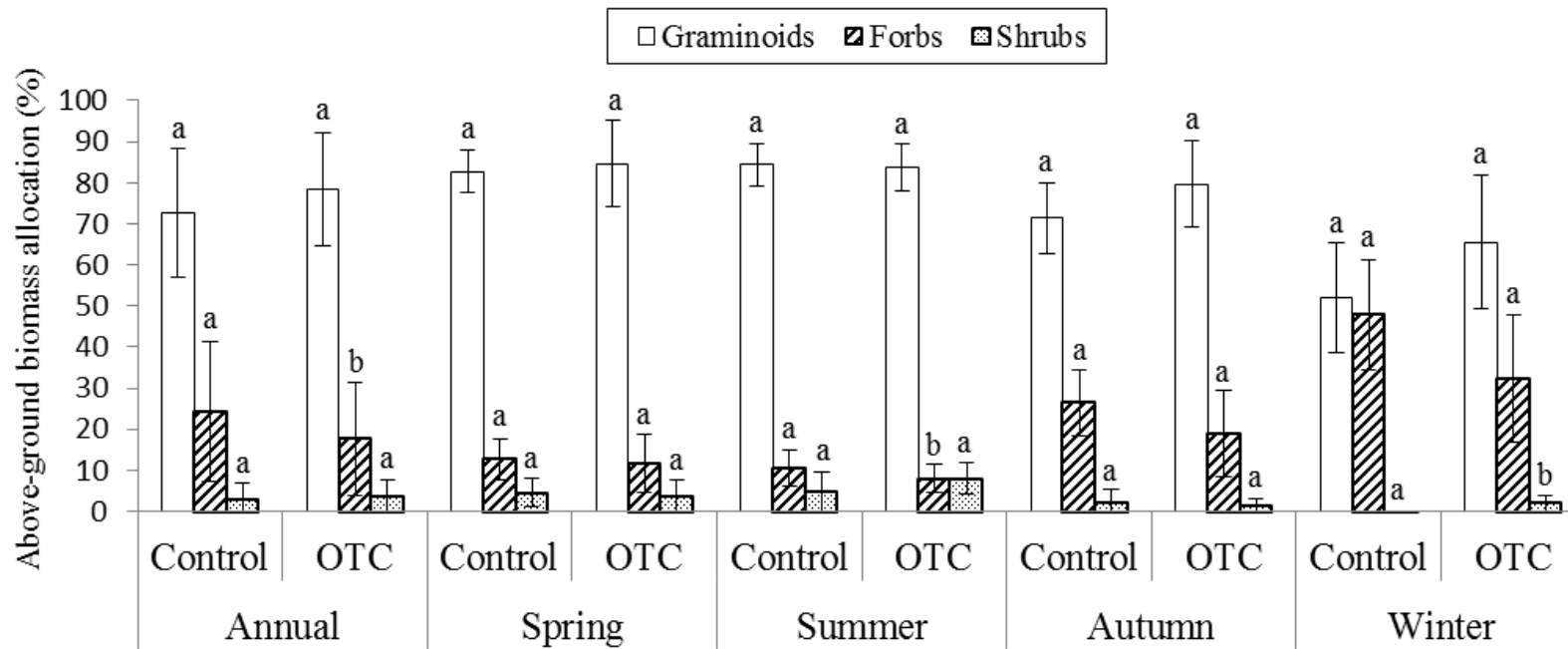


Figure 7. Above-ground biomass allocation (%) for individual life forms in OTC and control plots for each season and for all seasons combined (annual). Values represent mean \pm SD ($n = 5$ for individual seasons and $n = 20$ for all seasons combined) and when labelled with different letters are significantly different ($p < 0.05$, Wilcoxon signed-rank test) when compared within season and life form categories, between temperature treatments.

The clonal propagation strategy adopted by many of the graminoid species sampled prevented the quantification of the graminoid density, thus density was calculated for forbs and shrubs only (Figure 7). Annual and autumn combined density was significantly lower ($p < 0.05$) in OTC plots. Annual, autumn and summer forb densities were also significantly ($p < 0.05$) lower in OTC plots, but shrubs were unaffected (Figure 7). Additionally during autumn, forb density was negatively correlated with maximum ($r = -0.669$; $p < 0.05$) and minimum ($r = -0.674$; $p < 0.05$) temperatures. In summer, there was also a significant negative correlation between maximum temperature and forb density ($r = -0.661$; $p < 0.05$). Additionally, there was a significant positive correlation between forb density and RH in autumn ($r = 0.643$; $p < 0.05$). All other correlations between life forms and abiotic parameters were not significant ($p > 0.05$).

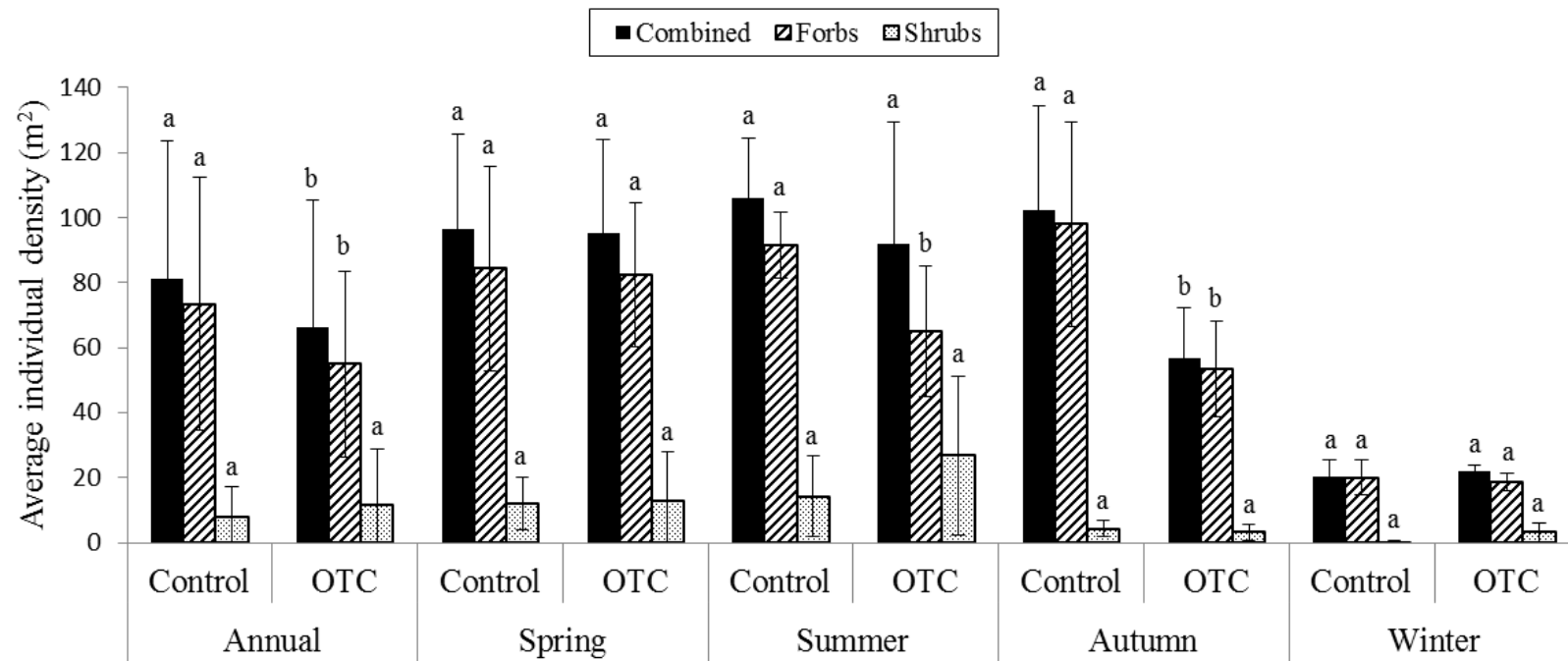


Figure 8. Plant density for forbs and shrubs for each season and for all seasons combined (annual). Values represent mean±SD ($n = 20$ for combined and $n = 5$ for individual seasons). Values labelled with different letters are significantly different ($p < 0.05$, Wilcoxon signed-rank test) when compared within season and life form categories, between temperature treatments.

2.6 Discussion

This study assessed the utility of OTCs in simulating elevated temperatures in a subtropical grassland, and the subsequent vegetation responses to these temperatures in terms of productivity and community structure.

2.6.1 Chamber design and effects on abiotic parameters

The OTCs used in this study are classified as passive systems, as they primarily rely on solar energy being trapped inside the chamber, allowing no direct controlled manipulation of temperature (Marion *et al.* 1997). Studies on elevating temperatures with passive OTCs include many different variations on their design, which in many cases aim to reduce unwanted chamber effects that do not correspond to past or predicted environmental changes associated with global warming (Aronson & McNulty 2009). The effectiveness of OTCs is based on their ability to simulate realistic elevated temperatures, i.e. temperatures that are in line within predictions for the near future. There are other methods that can be employed to elevate temperatures in plant/ecological studies (e.g. infrared heaters [Dukes *et al.* 2005; Sherry *et al.* 2008; Wan *et al.* 2005], in-line heaters when combined with blowers [Norby *et al.* 1997] and thermal inertia (water filled pipes) [Flanagan *et al.* 2013; Godfree *et al.* 2011]). However, these can be costly and cumbersome when installed *in situ*.

The OTC design used in this study, adapted from Molau and Mølgaard (1996), increased air and soil temperatures significantly relative to the control plots, irrespective of the time of day or season (Figure 2). More specifically, mean day temperature increased by ± 2.1 °C for air and ± 0.3 °C for soil, and night temperature by ± 0.8 °C for air and ± 0.8 °C for soil in KZNSS vegetation (when annual data were considered; Figure 3). The degree of air temperature increase relative to the control plots ranged from 1.6 °C to 2.4 °C during the day and 0.0 °C to 0.6 °C during night across the four seasons (Figure 3A). Midday temperatures measured using a precision thermistor (Kestrel 4500 Pocket Weather Tracker) (Table 2) support these findings. Further analysis of the temperature data revealed that the OTCs increased absolute maximum air and soil temperatures (Table 1). For air, this increase ranged between 4.4 °C and 5.7 °C for absolute maximum and between 0.3 °C and 2.0 °C for absolute minimum. Godfree

et al. (2011) experienced similar increases in maximum air temperature in their 70 cm high hexagonal chambers, with a ± 4.1 °C increase in spring–summer, and ± 5.6 °C in summer. Given that temperatures in southern Africa are likely to increase by 3 °C to 4 °C within the century (studies cited in IPCC 2007), these data suggest that the OTC design used in this study can simulate realistic and consistent increases in temperature when installed in subtropical grasslands. Additionally, average, maximum and minimum day and night soil temperatures were significantly ($p < 0.05$) higher in OTCs in all seasons; the absolute maximum was increased by 0.9 °C–1.8 °C whilst the absolute minimum was increased by 0.3 °C–0.8 °C (Table 1). Rustad *et al.* (2001) conducted a meta–analysis on 32 various experimental projects and found that an increase in soil temperature between 0.3 °C and 6.0 °C significantly increased above–ground biomass production and soil respiration. A study in Canada revealed that OTCs with an opening of 1.0 m can increase mean daily soil temperatures (at 3.0 cm below ground level) by 0.58 °C (Marion *et al.* 1997).

Marion *et al.* (1997) noted that the internal air and soil temperatures of OTCs are directly influenced by the ratio of the external chamber surface to the internal volume, together with the ratio of the size of the opening at the top of the chamber to the height of the chamber. A smaller internal volume and top opening of a chamber will result in increased temperatures. Additionally, Godfree *et al.* (2011) examined the increase in temperatures induced by OTCs with a height of 50 cm and 70 cm in a temperate grassland. When compared with the control plot, the 50 cm and 70 cm high OTCs had a mean day temperature increase of 1.2 °C and 3.2 °C, respectively, with a 0.3 °C increase in night temperature. The chamber height used in the present study was 50 cm but the increases in day and night temperatures observed here were both higher than that quoted for 50 cm high OTCs by Godfree *et al.* (2011). So, whilst the chamber design used in the present study may have yielded a realistic increase in temperature for the study site selected, a larger internal volume, larger top opening and lower chamber height may have to be employed when these OTCs are installed in subtropical grasslands characterised by relatively lower present–day mean daily temperatures.

Open–top chambers are prone to numerous ‘chamber effects’, which include, but are not limited to, a reduction in RH, light intensity (Flanagan *et al.* 2013; Godfree *et al.* 2011)

and soil moisture content (Aronson & McNulty 2009). Similar decreases in RH, soil moisture content and light intensity were observed within OTCs in this study, in all seasons. There was also a strong negative correlation between RH and diurnal temperature in all seasons, and a positive correlation between soil moisture content and RH in the warmer seasons (i.e. spring and summer). Flanagan *et al.* (2013) report that in OTCs a reduction in soil moisture content may be a result of an increased vapour pressure deficit (VPD) triggered by the elevated temperatures and decreased RH. Furthermore, Rustad *et al.* (2001) and Wan *et al.* (2002) have shown that with warming there is generally always a reduction in the soil moisture content. Whilst the interaction amongst temperature, RH and soil moisture within OTCs are difficult to disentangle, the results obtained here and elsewhere (Aronson & McNulty 2009; Flanagan *et al.* 2013; Flanagan & Johnson 2005; Godfree *et al.* 2011) suggest that OTCs offer the opportunity to investigate the combined effects of elevated temperatures and reduced soil moisture content in grasslands – both of which have been predicted for subtropical grasslands (IPCC 2007). The decline in light intensity in the OTCs was marginal (70.59–110.39 $\mu\text{mol m}^{-2} \text{s}^{-1}$), given that maximum light intensities measured ranged from 900 to 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Reference to the literature suggests that a significant decline in light intensity can reduce graminoid productivity in grassland vegetation (Olf 1992). In the present study, graminoid productivity increased within the OTCs, implying that the reduction in light intensity within the chambers was not significant enough to override the effects of temperature.

The modifications to the ITEX design made here (e.g. increased chamber size and improved support frame) were to allow the chambers to withstand the heavy summer rains, unpredictable hail storms and strong wind gusts associated with the study area. Even with these modifications, each chamber could be manufactured for less than \$70, making them a very affordable experimental tool for studies focused on grassland responses to climate change.

2.6.2 Plant productivity and community structure

Studies have shown that increased temperature can influence floristics and community structure in temperate grasslands (Saleska *et al.* 2002; Shaver *et al.* 2000). Elevated temperatures can alter recruitment patterns, influencing community composition and

species richness, both of which have a bearing on ecosystem health (De Boeck *et al.* 2007; Laurance *et al.* 2011). Studies in temperate systems have also shown that elevated temperatures can alter productivity in grasslands, which has implications for ecosystem functioning (Dukes *et al.* 2005).

According to Wan *et al.* (2005), elevated temperatures could have both a positive and a negative effect on the carbon sequestration in grasslands, depending on the season, as well as an indirect effect by extending the growing season and altering soil nitrogen mineralisation and availability. This may explain reports of mixed responses of carbon sequestration to elevated temperatures in grasslands (Jones & Donnelly 2004).

In the present study, elevated temperatures increased annual graminoid AGP by $\pm 19.9\%$ and decreased forb AGP by $\pm 9.0\%$ (Figure 4). In terms of annual combined AGP, levels in OTC plots were also $\pm 16.9\%$ higher compared to control plots (Figure 4). Additionally, at elevated temperatures graminoid AGP was significantly higher in spring and autumn, shrub AGP was significantly higher in summer, and combined AGP was significantly higher in summer and autumn (Figure 5). These results are largely understandable as spring and summer represent the major growing seasons in subtropical grasslands. The increase in graminoid and combined AGP within OTCs in autumn (Figure 5) suggests that elevated temperatures may also increase biomass production during periods of low productivity and low rainfall. This study was conducted in a summer rainfall region in southern Africa, where precipitation can vary between 640 and 1800 mm (Neumann, Botha & Scott 2014); the highest precipitation occurs in spring and summer under increased RH and temperature, and rainfall deficit during autumn and winter under increased evaporation (Neumann *et al.* 2014). The potential interactions between elevated temperatures and rainfall deserve further investigation.

Graminoid AGP was positively correlated with diurnal, maximum and minimum temperatures in autumn, and with maximum temperatures in summer when correlated. Furthermore, during summer graminoid AGP was significantly negatively correlated with forb AGP; this was also evidenced by the significant decline in biomass allocation to forbs at elevated temperatures (for annual and summer, Figure 6). This suggests that in KZNSS, elevated temperatures in promoting graminoid productivity may

compromise forb AGP. Interestingly, shrub AGP was significantly enhanced at elevated temperatures in summer (Figure 5). Del-Val and Crawley (2005) observed a reduction in forb and shrub AGP with increased grass biomass. Those authors also showed monocotyledonous species to be stronger competitors within grasslands than forbs or shrubs, and that removal of grasses led to a dramatic increase in forb and shrub AGP. They suggested that competition for light could be the main driver in these competitive interactions. Carlsen, Menke and Pavlik (2000) also suggest that grasses, in forming dense swards, could possibly cause a reduction in the quality and quantity of light reaching forb species below. However, these studies did not manipulate temperature, and at the time of this study there were no recently published data on *in situ* responses of subtropical grassland species to elevated temperature. Nevertheless, Morgan *et al.* (2007) showed that C₄ grass responded positively to elevated temperatures in terms of AGP; however, small population sizes and yearly variation reduced detectable effects on forbs. This may have also contributed to the lack of significant differences observed in the present study.

Typically, C₄ grasses dominate tropical and subtropical grasslands (Still *et al.* 2003). These C₄ species have higher thermal thresholds than C₃ species such as forbs, largely as a consequence of differences in the enzyme they employ for carbon fixation (De Boeck *et al.* 2007; Hatfield & Prueger 2015; Wan *et al.* 2002). The ability of graminoids (mainly C₄ species) to enhance their productivity at elevated temperatures is well documented (Horton & Murchie 2000; Sage 2000), whereas at these temperatures, forbs (mainly C₃ species) could be reaching their thermal maximum temperatures, triggering a reduction in their photosynthetic efficiency and hence, productivity (De Boeck *et al.* 2007; Hatfield & Prueger 2015; Wan *et al.* 2002). Whilst graminoid AGP was enhanced at elevated temperatures in the present study (as described above), forb AGP was not correlated with temperature, RH or soil moisture content. On the other hand, graminoid AGP was negatively correlated within both RH and soil moisture content in spring, the season of maximum growth. This implies that the increase in grass productivity at elevated temperatures in KZNSS may have led to a decrease in soil water availability. This is worrying because forbs and shrubs have an inferior water-use-efficiency to graminoids (Sherry *et al.* 2008; Wan *et al.* 2005).

It is also worth noting that winter biomass allocation percentages in control plots were more comparable between forbs and graminoids than in OTC plots (Figure 6). This difference appears to be the result of a decrease (18.2% relative to control) in forb AGP in the OTC plots in winter and suggests that elevated temperature effects may manifest during times of minimal growth within KZNSS as well. This suggestion is reinforced by the fact that forb density within the OTCs was lower than the control plots for autumn, summer and annual data (Figure 7). Additionally, there was also a strong negative correlation between forb density and both maximum and minimum temperatures in autumn and between forb density and maximum temperature in summer. Alward, Detling & Milchunas (1999), in examining 23 years of correlation data between temperature (minimum, maximum and average), AGP and abundance, showed that native and exotic forbs in cold temperate grasslands exhibited increased net primary productivity (NPP) and density at elevated temperatures.

However, they stated that this result may be a consequence of the decreased annual NPP of a dominant C₄ grass, resulting in increased space, nutrients and water availability, rather than the effects of elevated temperatures. It should be noted that although graminoid densities were not recorded in the present study, they displayed 90%–100% coverage in most control and OTC plots. Carlsen *et al.* (2000) showed that high density grasslands provided a poor habitat for native forbs. Furthermore, Sternberg *et al.* (1999) predicted that an increase in graminoid productivity will result in decreased availability of gaps for forb recruitment or growth, and consequently a reduction in the forb species richness and density. This is very concerning because many of the forb and shrub species endemic to KZNSS are taxa of conservation concern (Mucina & Rutherford 2006).

2.7 Concluding remarks and recommendations

The effects of climate change are becoming increasingly evident and the need to predict ecosystem responses to increased temperatures, in particular, is of paramount importance. This is particularly true for grasslands, which are threatened worldwide. The present study has shown that the effects of elevated temperatures on subtropical grasslands can be successfully investigated *in situ* using OTCs. The elevated temperatures simulated by these OTCs appear to be in line with predictions for the next

century and accompanying ‘chamber effects’, such as decreased soil moisture content, may allow for the simultaneous investigation of different climate change scenarios.

The results suggest that the responses of KZNSS vegetation to elevated temperatures will vary across life forms, with C₄ graminoids appearing to benefit in terms of AGP. There were also signs that this enhanced graminoid productivity at elevated temperatures may lead to the displacement of C₃ forbs, possibly as a result of competition. Shrubs, on the other hand, appear to be unaffected (both directly and indirectly) by elevated temperatures. The effects of elevated temperatures on KZNSS are likely to manifest during periods of maximum and minimum growth. This has implications for KZNSS conservation planning efforts.

We, therefore, recommend the use of OTCs in future investigations of the effects of elevated temperatures on subtropical grasslands. Apart from productivity, such studies should investigate how elevated temperatures are likely to influence species composition and abundance, which will yield more informed recommendations for the conservation of threatened grassland types like KZNSS.

CHAPTER THREE

THE EFFECTS OF ELEVATED TEMPERATURES ON PRODUCTIVITY AND DENSITY WITHIN A SUBTROPICAL GRASSLAND: A FLORISTIC APPROACH

3.1 Abstract

Background: Climate change, and more importantly the rise in temperature, has been suggested to have various effects on grassland vegetation. Unfortunately studies on the floristic responses to elevated temperature in tropical/subtropical grasslands are limited. This study investigated the floristic responses of KwaZulu–Natal Sandstone Sourveld (KZNSS), a threatened subtropical grassland, to elevated temperatures.

Objectives: To assess the effects of elevated temperatures on floristic composition and diversity, and community structure within KZNSS. To compare productivity and density of dominant KZNSS families and species at ambient and elevated temperatures.

Methods: Open–top chambers (OTCs) were used to simulate elevated temperatures in a remnant patch of KZNSS. Floristic, above–ground productivity (AGP) and density data were collected within OTC and control plots in all four seasons. Floristic composition, alpha diversity, AGP and density were compared between OTC and control plots during low (autumn and winter) and high (spring and summer) productivity periods, and based on annual data (all seasons combined). Unweighted pair group method with arithmetic mean analysis (UPGMA) and non–parametric multidimensional scaling (NMDS) ordination were used to compare OTC and control plots.

Results: Collectively, 74 species (from 22 families) were found within control and OTC plots. Elevated temperatures increased species richness slightly but had no significant effects on family dominance patterns. However, species evenness decreased during both productivity periods and in terms of annual data. At elevated temperatures Poaceae species exhibited enhanced AGP; some forb species declined in terms of AGP and density; and shrub species were relatively unaffected. UPGMA analyses clustered species based on temperature treatment, but this was dependent on season. The ordination analyses (which excluded grasses) appeared to group plots based on seasonality, rather than temperature treatment.

Conclusions: Elevated temperatures impacted more on subtropical grassland structure and productivity, than on community composition and diversity. Increased temperatures favoured Poaceae species in terms of AGP and density while forbs declined in terms of both these variables. Evenness declined at elevated temperatures, possibly as a consequence of increased graminoid productivity. In subtropical grasslands, elevated temperatures may reinforce existing family dominance patterns rather than altering them, particularly in relation to the Poaceae.

3.2 Introduction

Rising temperatures and extreme variability in weather patterns have sparked increased research into how vegetation will respond to climate change. Studies have shown that the consequences of climate change, such as elevated temperature, altered precipitation and nitrogen deposition can impact on floral biodiversity (Bellard *et al.* 2012; Corlett & Westcott 2013; Zavaleta *et al.* 2003). These negative impacts are largely due to climate change induced habitat fragmentation and altered species distributions (Trisurat, Shrestha & Kjelgren 2011). These negative impacts of climate change on plant biodiversity are variably exacerbated by urbanisation, poor conservation and agricultural practices (Concepción *et al.* 2015; McKinney 2002).

Rising temperatures are predicted to have dire consequences for species in tropical and subtropical regions in particular, as their thermal specialization limits their natural range (Laurance *et al.* 2011). These threats apply to both tropical/subtropical forests and species rich grasslands (Bond & Parr 2010; Boval & Dixon 2012). Southern Africa, which houses some of the most species rich grasslands in the world (Bond & Parr 2010), has been predicted to experience a ± 3 °C increase in temperature by approximately 2060, with a large number of days exceeding 35 °C (Davis 2011). These predictions are supported by the fact that from 1961 to 2000, the number of days with extreme heat (those that exceeded the 90th percentile) has increased by 8.2 days, with a general trend of increased minimum temperature across southern Africa (New *et al.* 2006).

Given the above predictions, subtropical grasslands are of particular conservation concern within southern Africa, due to their high biodiversity and endemism (Reyers *et al.* 2005). In South Africa, one such subtropical grassland type, viz. KwaZulu–Natal

Sandstone Sourveld (KZNSS), has been classified as critically endangered (Jewitt 2011). Land transformation and invasive alien plants (IAPs) have been shown to compromise both the extent and integrity of KZNSS (Jewitt, 2011), as in other subtropical grassland types in southern Africa (Jonas *et al.* 2006; Henderson 2007; Richardson & van Wilgen 2004) and the world (Westphal *et al.* 2008). However, as in other subtropical grassland types the effects of climate change, such as increased temperature on KZNSS, have been poorly studied (but see Buhrmann *et al.* 2016 upon which Chapter 2 is based). Using open-top chambers (OTCs) Buhrmann *et al.* (2016) exposed KZNSS vegetation to elevated temperatures *in situ* and showed that a daytime air and soil increase of ± 2.1 °C and ± 0.3 °C benefited graminoids and shrubs in terms of productivity, whilst forbs decreased in density, possibly through competition and/or direct physiological effects. However, Buhrmann *et al.* (2016) did not investigate floristic responses of KZNSS vegetation (e.g. species richness, composition and diversity) to elevated temperatures, which forms the focus of the present study.

As alluded to above, the effects of elevated temperatures on subtropical and tropical grasslands are under-studied unlike temperate grasslands which have received much more attention (see Flanagan & Johnson 2005; Godfree *et al.* 2011; Ojima *et al.* 1993; Saleska *et al.* 2002; Shaver *et al.* 2000; Wan *et al.* 2005). Collectively, these studies have shown that there could be a shift in species dominance (from forb to shrub species), possible declines in soil organic carbon, and an overall trend of increased above-ground productivity (AGP) until plant thermal thresholds are reached, upon which there is a decreased AGP. Furthermore, vegetation models have predicted climate change to bring about large changes in grassland floristics, such as shifts towards a more tree dominated biome (Scheiter & Higgins 2009). The floristic changes can be variably attributed to elevated temperature-induced changes in plant productivity (Alward, Detling & Milchunas 1999; Guoju *et al.* 2005), phenology (Körner & Basler 2010; Norby, Hartz-Rubin & Verbrugge 2003; Walther 2003; Wan *et al.* 2005), reproductive success (Hatfield & Prueger 2015; Hedhly, Hormaza & Herreo 2009), decreased diversity and evenness (Sala *et al.* 2000; Walker *et al.* 2006), a shift in species composition (Alward, Detling & Milchunas 1999; Brown, Valone & Curtin 1997), as well as changes in community structure and functioning (Alward, Detling & Milchunas 1999; Grabherr, Gottfried & Pauli 1994; Walther *et al.* 2002).

The changes in plant productivity and physiology induced by elevated temperatures within grasslands impact on floristics by altering plant–plant interactions, e.g. competition (Alward, Detling & Milchunas 1999). Some grassland graminoids for example, are intolerant to shading brought about by an increase in their standing biomass (Bond & Parr 2010; Everson, Everson & Tainton 1988). *Themeda triandra*, a common subtropical grass species, experienced a large decline in cover due to increased self–shading brought about by reduced burning frequency (Bond & Parr 2010). Similarly, some grassland forb species also display intolerance to shading (Bond & Parr 2010; Uys, Bond & Everson 2004).

Recruitment of new individuals is usually highly dependent on the availability of water and temperature (Walck *et al.* 2011). It has been suggested that the early developmental stages of plants (i.e. seedlings) are at a higher risk and more sensitive to the various impacts of climate change (Dalglish, Koons & Adler 2010; Walck *et al.* 2011). Species that have a narrow temperature range for germination are likely to be more negatively affected by increased temperatures, and could experience large declines in their distributions (Donohue *et al.* 2010). Additionally, elevated temperatures are usually associated with a decline in soil moisture content (Seneviratne *et al.* 2010) which can also compromise germination and subsequent seedling survival in grassland species (Ludewig *et al.* 2014; Zeiter *et al.* 2016). However perennial grasslands, such as KZNSS, are dominated by grass and forb elements that rely almost exclusively on vegetative reproduction; and revegetation after disturbances in such grasslands may therefore not be heavily dependent on recruitment of seedlings, i.e. the seed bank (Viragh & Gerencser 1988; Benson & Hartnett 2006).

Many grassland species (forbs and shrubs in particular) also have the ability to grow large underground storage organs, allowing for rapid re–sprouting and flowering after the occurrence of a fire (Bond & Parr 2010). This heavy reliance on vegetative reproduction and ability to re–sprout may therefore render grassland species more resilient to elevated temperatures and associated changes in soil moisture content, recruitment patterns and competition.

The present study investigated the floristic responses of KZNSS vegetation to elevated temperatures induced *in situ* using open–top chambers. The design and utility of these

have already been established for tropical/subtropical (Buhrmann *et al.* 2016, see Chapter 2) and temperate (Flanagan & Johnson 2005; Godfree *et al.* 2011; Ojima *et al.* 1993) grasslands. The study aimed to understand how species composition, density and structure are affected by elevated temperatures in relation to elevated temperature-induced changes in productivity. *In situ* studies of this nature can provide invaluable data for future grassland management and conservation strategies such as burning regimes and species reintroduction. The critically endangered status of the KZNSS (Jewitt 2011) made it an ideal subtropical grassland to conduct the present study in. Buhrmann *et al.* (2016) showed that the effects of elevated temperatures can differ across seasons in grasslands like KZNSS. Consequently, family, species and life form responses to elevated temperatures are compared in this study between the low and high productivity periods typically associated tropical/subtropical grasslands.

3.3 Methods

3.3.1 Site selection, experimental approach and chamber design

The site selection, experimental approach and chamber design follows that described in Chapter Two, section 2.3.1.

Henceforth, the control and OTC plots are referred to as ‘ambient’ and ‘elevated temperature’ plots, respectively. As described in Buhrmann *et al.* (2016), the vegetation was cut to ground level and the soil was disked in both plots before installing the OTCs. Vegetation was allowed to grow for two months during each of the four seasons (spring: 1 September–30 November; summer: 1 December–28/29 February; autumn: 1 March – 31 May; winter: 1 June–31 August; between 2014 and 2015) before being surveyed and harvested as described below. Data for these seasons were subsequently combined to generate two productivity periods viz. a Low Productivity Period (LPP; autumn and winter) and High Productivity Period (HPP; spring and summer).

3.3.2 Floristics and biomass measurements

After the two month experimental period (in each of the four seasons), the above-ground biomass of each plant, which was categorised based on life form (graminoid, forb or shrub), within all plots was harvested and placed into separate bags. All taxa collected were identified to species level and flowering material was collected from

outside the ambient and elevated plots for herbarium voucher specimens (deposited in the Ward Herbarium (UDW), University of KwaZulu–Natal, Westville Campus). Abundance and dry mass was then determined for each species as outlined in Chapter 2 (see section 2.3.4). The abundance of graminoid taxa was excluded due to their clonal propagation strategies. Taxa names and authorships follow the South Africa National Biodiversity Institute’s (SANBI) Plants of southern Africa (POSA) website (Leistner 2000). The conservation status of species was based on SANBI’s Red List of South African Plants (SANBI 2016).

3.3.3 Measurement of abiotic parameters

Air and soil temperatures, relative humidity (RH) and soil moisture content were monitored across all plots (ambient and elevated) throughout the experimental period as described in section 2.3.3 of Chapter 2. Data for these parameters are not presented here (presented and discussed in section 2.5.1 (Chapter 2)) but are briefly described as these parameters were used in correlation analyses between selected plant and abiotic parameters.

3.3.4 Statistical analysis

All data was tested for normality using Shapiro–Wilk Test. The annual percentage sampling effort was determined with rarefaction curves using the Jack1 and Chao2 estimators plotted in EstimateS (Version 9.1.0) (Colwell 2013). The Shannon Exponential and Simpson Inverse indices were ‘determined’ annually (LPP and HPP combined), and for the LPP and HPP using EstimateS (Jost 2006).

Only the dominant taxa within each life form were used for species/family–based comparisons of density and above–ground productivity (AGP) (Figures 12–15), and these are defined as ‘dominant’ species/families henceforth.

For comparisons between LPP and HPP, species and family densities were determined for each life form by dividing the number of individuals of each taxon for a given life form by the plot size (2.012 m²). All taxa within a life form were separated. Families and species were ranked from highest to lowest for density/AGP. The largest families/species in terms of density/AGP were then successively combined until a 50% cut–off was reached for either density or AGP. However in several instances, inclusion

of a family/species resulted in a marginal >50% density/AGP representation. These dominant families/species were compared in terms of density and AGP within productivity periods and life forms, between ambient and elevated plots.

An Unweighted Pair Group Method with Arithmetic mean (UPGMA) accompanied by a Cophenetic Correlation was performed on the presence–absence data of species found within ambient and elevated plots ($n = 5$) within each season ($n = 4$). The UPGMA cluster was performed using the statistical software R (R Core team 2015). A non–metric multidimensional scaling (NMDS) based on the Bray–Curtis Index was performed on the abundance data of forb and shrub taxa found within the ambient and elevated plots within the two productivity periods ($n = 40$). The NMDS analyses were run using Paleontological Statistics Version 3 (PAST3) (Hammer *et al.* 2001).

Comparisons of species composition, AGP and densities between ambient and elevated plots were made within and across taxa using a Wilcoxon signed ranks test for the productivity periods. Pearson’s correlation analyses were used to determine the relationship between abiotic parameters (average temperature, maximum temperature, minimum temperature, RH, and soil moisture content) and plant productivity and density. Where applicable, non–parametric data were related with a Spearman’s rank correlation test. All correlation and mean separation analyses were performed using IBM SPSS Statistics 22 (SPSS IBM, New York, USA) at the 0.05 level of significance.

3.4 Results

3.4.1 Sampling effort

Sampling effort for both the ambient and elevated plots which were calculated for annual data (all seasons combined) and based on the Jack 1 and Chao 2 estimates, were 70.3 and 82.6%, respectively (data not shown).

3.4.2 Abiotic parameters

As mentioned earlier, data for the abiotic parameters measured in this study are not presented here (see section 2.5.1 in Chapter 2), however, in order to contextualise the results that follow, it is necessary to discuss some of the major trends observed in terms

of differences in air and soil temperature, soil moisture content and relative humidity between ambient and elevated plots.

The OTCs elevated day air (by 1.7 °C–2.4 °C) and soil (by 0.7 °C–1.0 °C) temperatures, as well as night air (0.3 °C–0.6 °C) and soil (by 0.5 °C–1.2 °C) temperatures, relative to the control (no OTCs) plots. The degree of this increase differed across seasons. In spring, OTC plots experienced the greatest increase in day air temperature and smallest increase in day soil temperature. Whereas autumn OTC plots were characterised by the smallest increase in day air temperature and highest increase in day soil temperature; additionally in autumn, OTC plots experienced the greatest increase in night air and soil temperature. Spring and summer however had the smallest increase in night air and soil temperature, respectively. Additionally, the OTCs brought about a slight reduction in RH and soil moisture content in all seasons but the effects on light intensity were negligible (see section 2.5.1, Chapter 2 for details).

3.4.3 Floristics

In total, the 74 species from 22 families were found in this study (Appendix 1) in all plots across all four seasons. These species were placed in life forms, which eventually resulted in two graminoid, 18 forb and three shrub families. The Fabaceae was the only family with forb and shrub species. With 61 species being common to both ambient and elevated plots, four being unique to ambient plots, and nine being unique to elevated plots (Appendix 1). One species of conservation concern, *Alepidea amatymbica* Eckl. & Zeyh., and two naturalised exotics (*Lactuca indica* L. and *Paspalum notatum* Flüggé) were found within the ambient and elevated plots.

During the LPP, 55 species were recorded in both ambient and elevated plots; this included 37 forbs, 13 graminoids and five shrubs, where 30 forbs, seven graminoids and three shrubs were shared between both ambient and elevated plots. The ambient and elevated plots hosting five and 10 unique species, respectively (Figure 9). In the HPP, 67 species were found in ambient and elevated plots; this included 42 forbs, 19 graminoids and six shrubs, where 36 forbs, 12 graminoids and four shrubs were shared between both ambient and elevated plots. The ambient and elevated plots hosting eight and seven unique species, respectively (Figure 10). The 12 additional species in the HPP (compared to LPP) consisted of five forbs, six graminoids and one shrub.

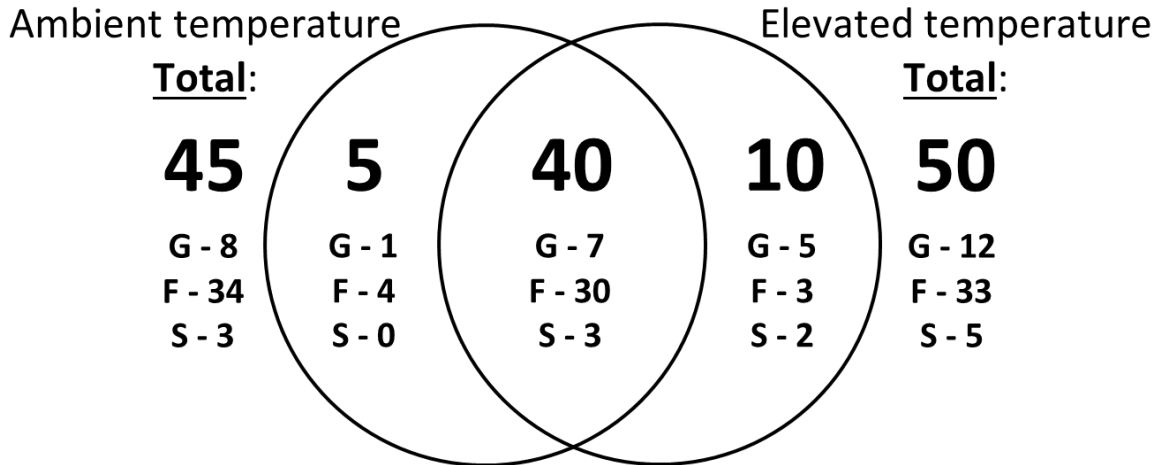


Figure 9. Venn diagram representing the total, shared and unique species that emerged within ambient and elevated temperature plots during the low productivity period ($n_{\text{ambient}} = 10$, $n_{\text{elevated}} = 10$). A total of 55 species were found (G = graminoids, F = forbs, S = shrubs).

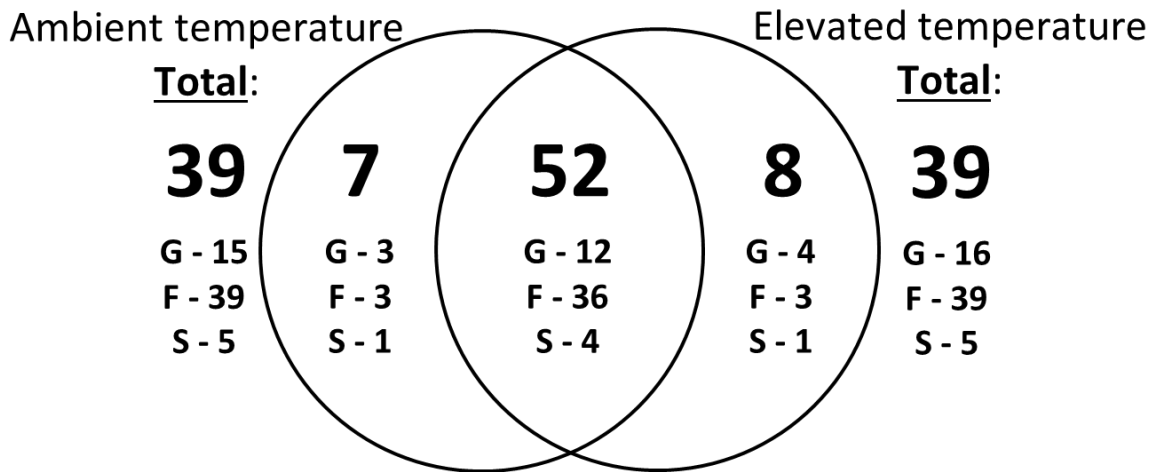


Figure 10. Venn diagram representing total common and unique species that emerged within ambient and elevated temperature plots during the high productivity period ($n_{\text{ambient}} = 10$, $n_{\text{elevated}} = 10$). A total of 67 species were found (G = graminoids, F = forbs, S = shrubs).

Based on annual data and irrespective of temperature treatment the five most speciose families were as follows: Asteraceae > Poaceae > Fabaceae > Iridaceae and Commelinaceae (Figure 11). The Asteraceae had the highest number of species, all of which were present in both the ambient and elevated plots. The elevated plots had one more Poaceae, and one less Fabaceae species compared with the ambient plots, where both Iridaceae and Commelinaceae remained the same throughout. During the LPP, 19 families were found within both ambient and elevated plots, with ambient plots having 16 families while the elevated plots had 18. A total of 20 families were found in both the ambient and elevated plots during the HPP. Of the five most speciose families in the LPP, four had an equal number of species in the ambient and elevated plots, while the Poaceae had two more species in the elevated plots. During the HPP an equal number of Asteraceae, Iridaceae and Commelinaceae species were present in both the ambient and elevated plots, but the Poaceae and Fabaceae had one less species in the elevated plots.

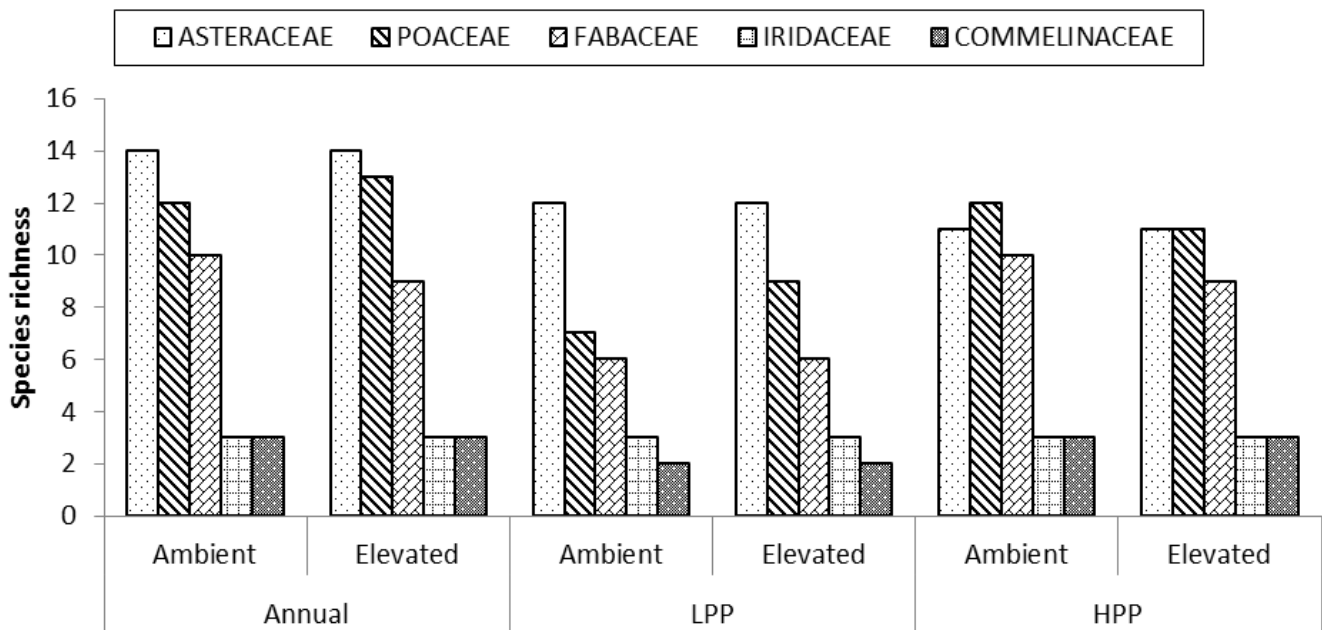


Figure 11. The most species rich families that emerged within ambient and elevated temperature plots. Annual: data for all four seasons combined, LPP: low productivity period, and HPP: high productivity period. $n = 20$ for annual and $n = 10$ for LPP and HPP.

In terms of alpha diversity, the Shannon Exponential Index was slightly higher at elevated temperatures when indices were calculated based on annual data, while the Simpson Index was almost similar. Both alpha diversity measures were markedly higher in the HPP than the LPP, irrespective of temperature treatment. The Shannon Exponential Index was slightly higher, whereas the Simpson Inverse Index was slightly lower during both productivity periods at elevated temperatures (Table 3).

Table 3. Shannon’s Exponential and Simpson’s Inverse indices for ambient and elevated plots.

	Annual		LPP		HPP	
	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
Shannon Exponential	48.54	49.37	37.05	37.82	46.72	47.07
Simpson Inverse	40.99	40.86	31.95	31.58	41.25	40.47

Annual: data for all four seasons combined, LPP: low productivity period and HPP: high productivity period. $n = 20$ for annual and $n = 10$ for LPP and HPP.

3.4.4 Plant density

Figures 12 and 13 represent the dominant forb and shrub families and species in each life form that collectively make up $\geq 50\%$ of the density in either the ambient or elevated plots (as defined earlier). The Poaceae was the overall dominant family in terms of cover and biomass (see section 3.5.4, Figures 14A & 15A), but had to be excluded from density comparisons because of their clonal propagation strategies. The large standard deviations (in results below) are a consequence of some families or species being absent from some plots.

During the LPP there were two forb families, Asteraceae and Commelinaceae, that when combined made up $\geq 50\%$ of the total density in both ambient and elevated plots, where the remaining c. 50% density in the ambient and elevated plots was composed of 14 and 13 families, respectively. Densities of both these families declined in the elevated temperature plots (Figure 12A). Asteraceae constituted 42.46% and 31.46% and Commelinaceae 20.78% and 20.82% of the total density in the ambient and elevated plots, respectively (data not shown). Densities of both Asteraceae and Commelinaceae were negatively correlated with daily mean temperature ($p < 0.05$; $r = -0.661$ and $r = -0.549$, respectively; Appendix 2).

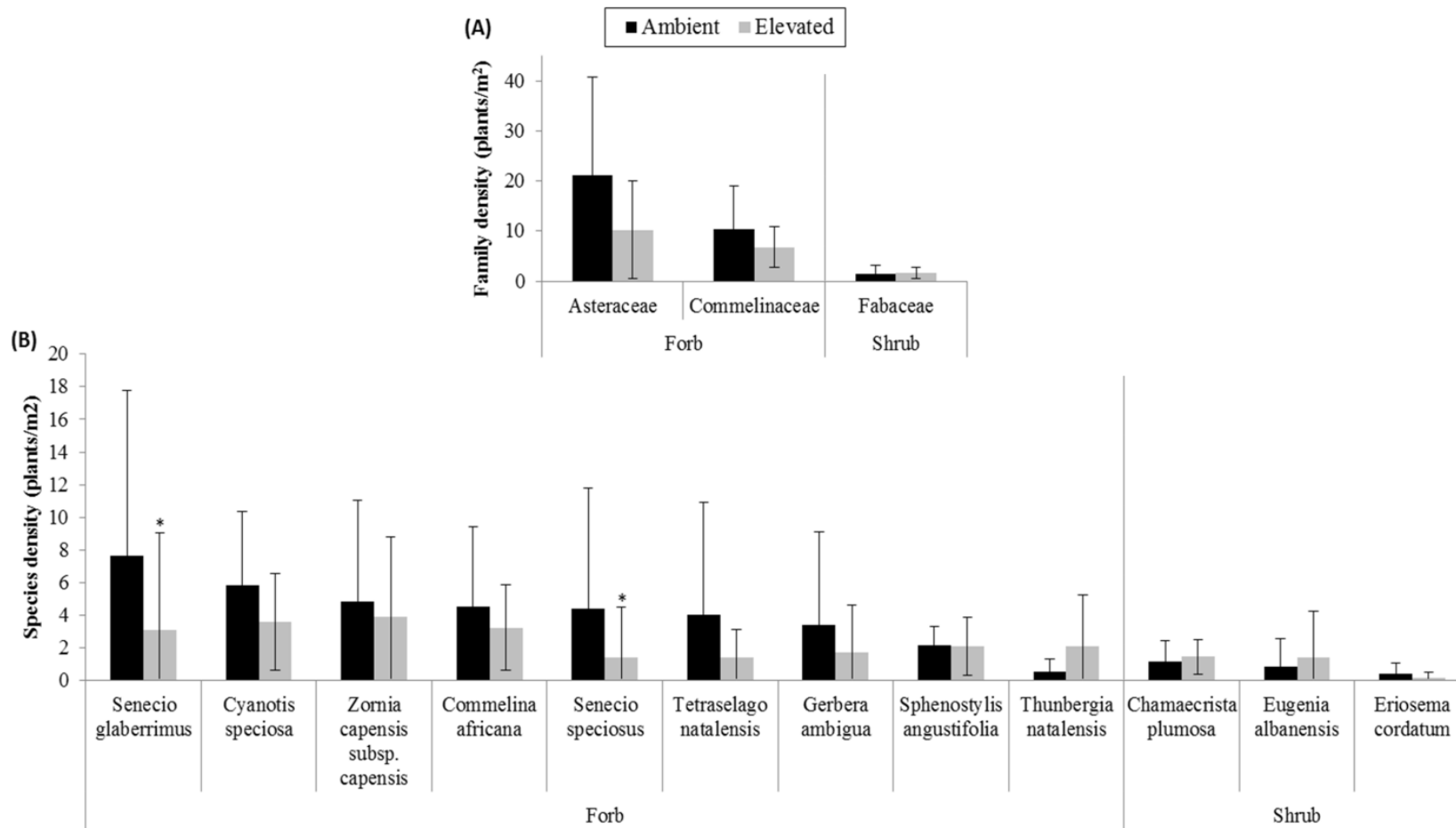


Figure 12. Densities for dominant families (A) and species (B) that emerged within ambient and elevated temperature plots during the low productivity period (autumn–winter). Values represent mean±SD ($n = 10$). * represent significant difference ($p < 0.05$, Mann–Whitney U test) when compared within family/species, between temperature treatments.

There were nine forb species that collectively accounted for $\geq 50\%$ of the total density in both ambient and elevated plots during the LPP, with 25 and 24 species contributing towards the remaining 50% density in the ambient and elevated plots, respectively. Seven species declined, one (*Thunbergia natalensis* Hook.) increased, and one (*Sphenostylis angustifolia* Sond.) remained the same density in the elevated compared to the ambient temperature plots (Figure 12B). *Senecio speciosus* Willd. and *Senecio glaberrimus* DC. showed a significant decrease in density in the elevated plots. Average temperature was significantly negatively correlated with *S. speciosus*, *S. glaberrimus*, *Cyanotis speciosa* (L.f.) Hassk. and *Zornia capensis* Pers. subspecies *capensis* densities ($p < 0.05$; $r = -0.670, -0.856, -0.567$ and -0.775 , respectively; Appendix 2). *Gerbera ambugua* (Cass.) Sch.Bip. was significantly positively correlated with soil moisture content ($p < 0.05$; $r = 0.584$), while *T. natalensis* was significantly negatively correlated with soil moisture content ($p < 0.05$; $r = -0.775$). *Commelina africana* and *S. angustifolia* showed no correlations.

The dominant shrub family (viz. Fabaceae) which contributed 64.58% and 50.79% of the total shrub density during the LPP within either the ambient and elevated plots respectively, was not significantly affected by elevated temperatures (Figure 12A). The remaining shrub families during the LPP consisted of Myrtaceae in only the ambient plots, and both Myrtaceae and Malvaceae in the elevated plots. Of the three shrub species that accounted for $\geq 50\%$ of the total shrub density found during the LPP, *Chamaecrista plumosa* E.Mey. and *Eugenia albanensis* Sond. increased, and *Eriosema cordatum* E.Mey. showed a slight decline in density and was significantly negatively correlated with average temperature ($p < 0.05$; $r = -0.668$). Only *C. plumosa*, *E. albanensis*, and *E. cordatum* were found in the ambient plots, whereas in the elevated plots *Triumfetta pilosa* Roth and Shrub 2 (an unidentified species) were found, but were excluded due to their lack of dominance. No correlation was observed in the family Fabaceae, or species *Chamaecrista plumosa* E.Mey. and *Eugenia albanensis* Sond. (Figure 12B).

In the HPP there were two families that accounted for $\geq 50\%$ of the total forb density in both the ambient and elevated plots, viz. Asteraceae which was also dominant during the LPP, and Fabaceae (which replaced Commelinaceae which dominated during the LPP). Collectively, 15 forb families made up the remaining c. 50% density in both the

ambient and elevated plots during the HPP. Similarly to the LPP, Asteraceae density during the HPP declined in the elevated temperature plots, whereas Fabaceae density during the HPP increased slightly within the elevated compared to the ambient plots (Figure 13A). No correlations between the abiotic factors and Asteraceae or Fabaceae were found (Appendix 2).

Six forb species accounted for $\geq 50\%$ of the total forb density in both ambient and elevated plots during the HPP. Four species (*Senecio glaberrimus*, *Zornia capensis* subsp. *capensis*, *Sphenostylis angustifolia* and significantly so in *Cyanotis speciosa*) declined and two increased (*Tetraselago natalensis* and *Clutia cordata* Bernh.) in density at elevated temperatures (Figure 13B). The remaining forb species during the HPP consisted of 33 species in the ambient plots, and 34 species in the elevated plots. Interestingly, the density of *Senecio madagascariensis* Poir., which did not fall within the $\geq 50\%$ forb species dominance, was also significantly lower in the elevated plots (data not shown). *Cyanotis speciosa* was significantly negatively correlated with both average and minimum temperature ($p < 0.05$; $r = -0.562$ and -0.710 , respectively). However, there was a significantly positive correlation between *S. glaberrimus* and RH ($p < 0.05$; $r = 0.652$), with no significant correlations in the other forb species (Appendix 2).

Similar to the LPP (Figure 12A), only one shrub family, Malvaceae, constituted 61.28% and 71.65% of the total shrub density in the ambient and elevated plots, respectively, during the HPP. The remaining $< 50\%$ density was from the shrub family Fabaceae. During this period Malvaceae density in the elevated plots increased relative to the ambient plots (Figure 13A). Malvaceae also demonstrated a significantly positive correlation with both average temperature and maximum temperature ($p < 0.05$; $r = 0.559$ and 0.487 , respectively). Two shrub species accounted for $\geq 50\%$ of the total shrub density in the ambient and elevated plots during the HPP, with three shrub species constituting the remaining c. 50%. *Triumfetta pilosa* experienced a large increase while *C. plumosa* exhibited a slight increase in density within the elevated plots (Figure 13B). *Triumfetta pilosa* density was significantly positively correlated with average temperature ($p < 0.05$; $r = 0.559$; Appendix 2), with *C. plumosa* showing no correlations.

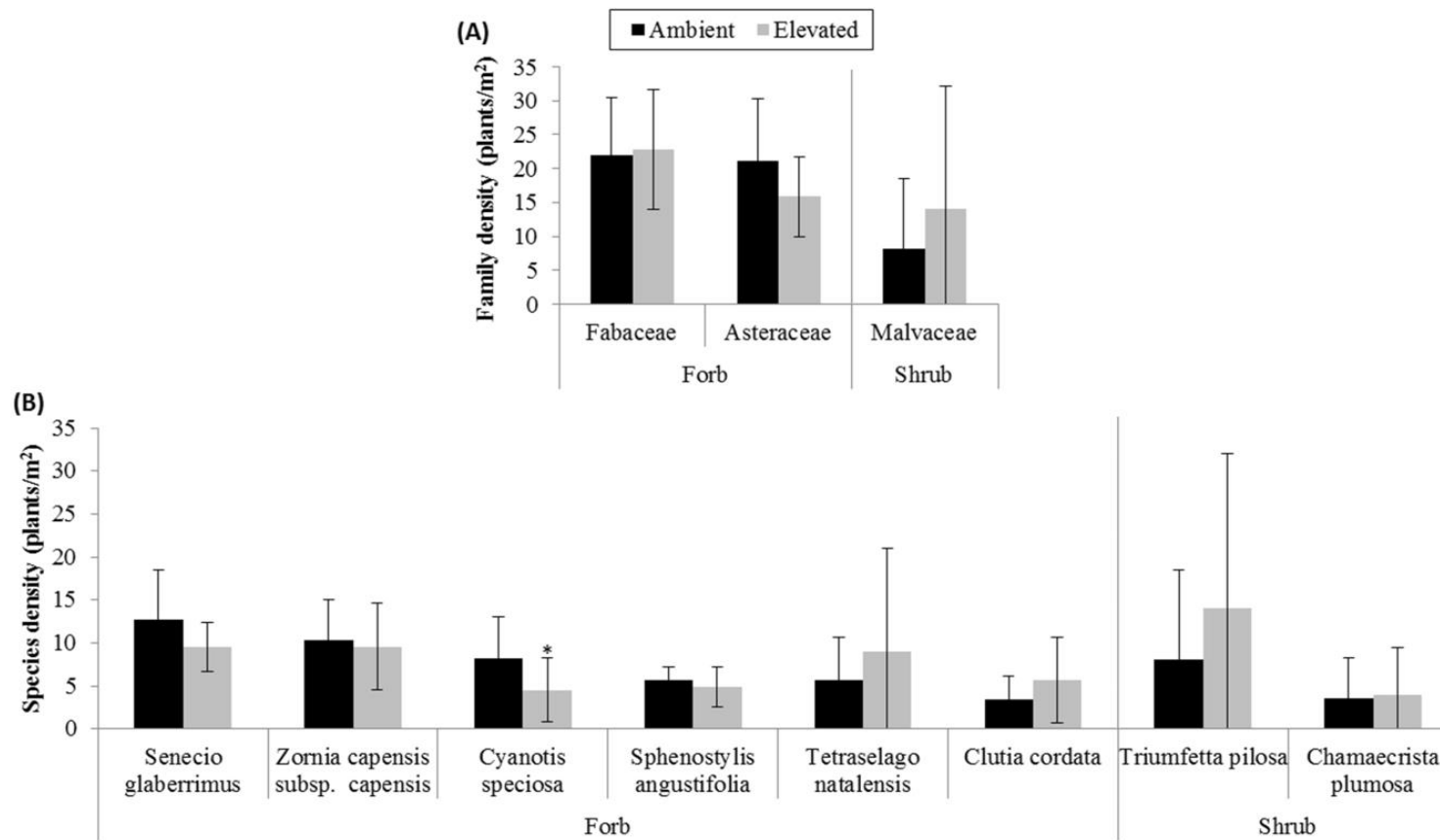


Figure 13. Densities for dominant families (A) and species (B) that emerged within ambient and elevated temperature plots during the high productivity period (spring–summer). Values represent mean±SD ($n = 10$). * represent significant difference ($p < 0.05$, Mann–Whitney U test) when compared within family/species, between temperature treatments.

3.4.5 Plant productivity

Figures 14 and 15 represent the dominant forb, shrub and graminoid families and species within each life form that collectively make up $\geq 50\%$ of the AGP in either the ambient or elevated plots. The large standard deviations (in results below) are a consequence of some families or species being absent in certain plots.

During the LPP there were four forb families that made up $\geq 50\%$ of the total forb AGP in both the ambient and elevated plots (Figure 14A). Two of these families, Thymelaeaceae and Asteraceae, experienced declines in AGP while the other two, Fabaceae and Lamiaceae, experienced increased AGP at elevated temperatures. The remaining 50% AGP in the ambient and elevated plots consisted of nine and 10 forb families, respectively. Asteraceae was significantly positively correlated with soil moisture content ($p < 0.05$; $r = 0.496$; Appendix 3), with no correlations occurring with Thymelaeaceae, Fabaceae and Lamiaceae.

There were four forb species that accounted for $\geq 50\%$ of the total forb AGP in both the ambient and elevated plots during the HPP (Figure 14B), with the remaining forb species during the LPP consisting of 30 species in the ambient plots, and 29 species in the elevated plots. The dominant species *Lasiosiphon kraussianus* (Meisn.) Burt Davy varied largely in terms of productivity as it was absent in many plots. Both *S. angustifolia* and *Ocimum filamentosum* Forssk. exhibited increased AGP in the elevated plots, while *Tetraselago natalensis* (Rolfe) Junell AGP remained comparable between ambient and elevated plots. Interestingly, *O. filamentosum* had very similar densities in both the ambient and elevated plots (0.60 and 0.65 plants/m², respectively) but exhibited a large increase in AGP at elevated temperatures (data not shown). *Ocimum filamentosum* was significantly negatively correlated with soil moisture content ($p < 0.05$; $r = -0.683$), while the other three forb species had no correlation.

The shrub family Myrtaceae constituted $\geq 50\%$ of the total shrub density within both the ambient and elevated plots, and displayed a slight increase in AGP at elevated temperatures (Figure 14A). The remaining AGP of the shrub families during the HPP consisted of Fabaceae in the ambient plots, and both Fabaceae and Malvaceae in the elevated plots. The dominant shrub species *Eugenia albanensis* Sond., contributing $\geq 50\%$ of the total shrub density within both the ambient and elevated plots, was the only

species representing Myrtaceae. The remaining shrub species during the LPP consisted of two species in the ambient plots, and four species in the elevated plots. The shrub species *C. plumosa* (Fabaceae), that contributed <50% of the total shrub density, was the only shrub species in the LPP to exhibit a significant increase in AGP at elevated temperatures (data not shown).

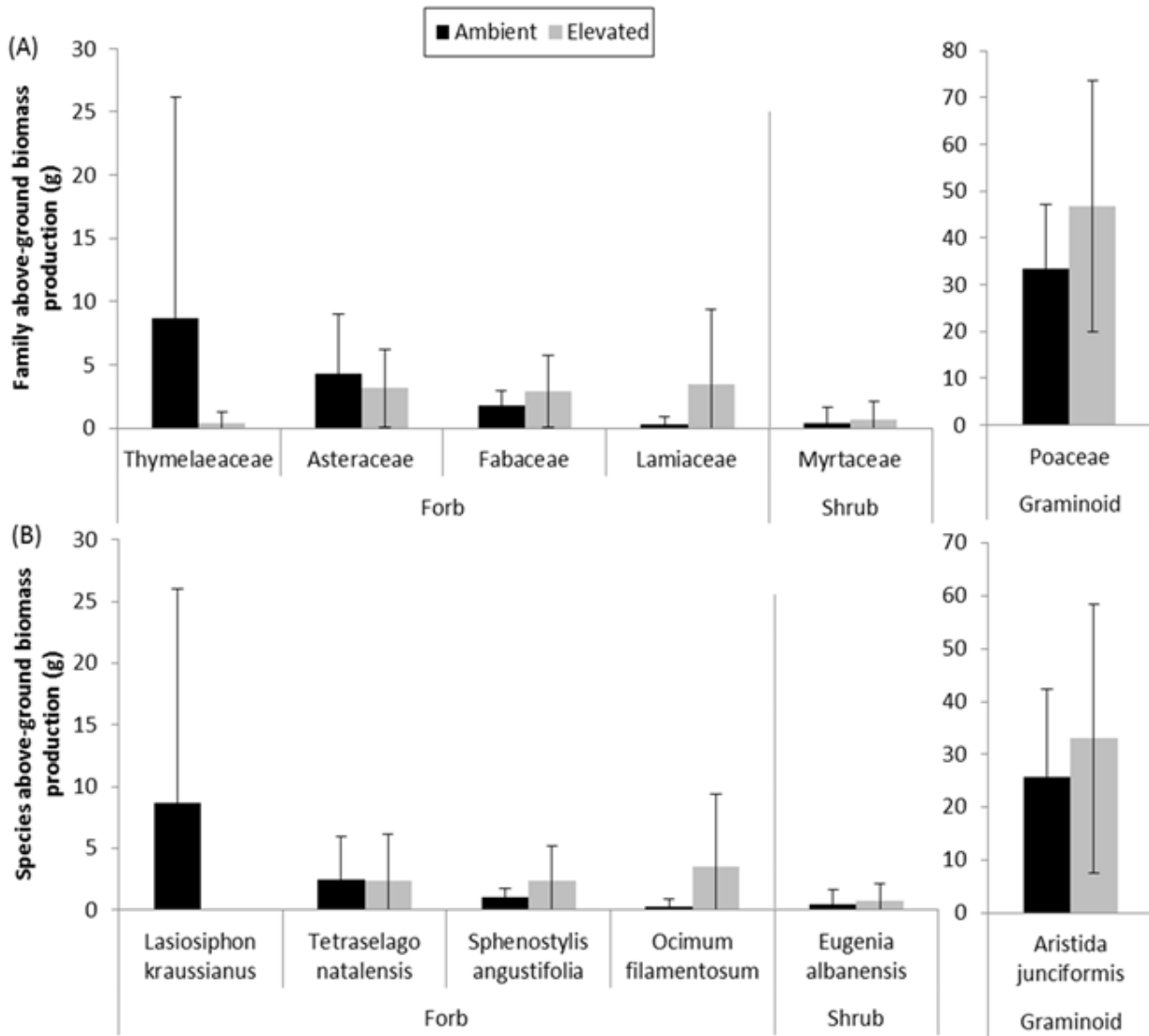


Figure 14. Above-ground biomass production (g) for families (A) and species (B) that emerged within ambient and elevated temperature plots during the low productivity period (autumn–winter). Values represent mean±SD ($n = 10$). Values labelled with * are significantly different ($p < 0.05$, Mann–Whitney U test) when compared within life form categories, between temperature treatments.

The family Poaceae constituted $\geq 50\%$ of the total graminoid AGP within both the ambient and elevated plots and showed a large increase at elevated temperatures (Figure 14A). The only remaining graminoid family making up $< 50\%$ of the AGP in both the ambient and elevated plots was Cyperaceae. Poaceae AGP was significantly positively correlated with average, maximum and minimum temperature ($p < 0.05$ and $r = 0.830$, 0.903 and 0.903 , respectively), and negatively correlated with both RH and soil moisture content ($p < 0.05$ and $r = -0.648$ and -0.758 , respectively). The AGP of only one graminoid species, *Aristida junciformis* Trin. & Rupr., accounted for $\geq 50\%$ of the total graminoid AGP, and exhibited a large increase in AGP in the elevated plots (Figure 14B). The remaining graminoid species during the LPP consisted of seven species in the ambient plots, and 11 species in the elevated plots. *Aristida junciformis* AGP was also significantly positively correlated with minimum temperature ($p < 0.05$; $r = 0.636$), and significantly negatively correlated with soil moisture content ($p < 0.05$; $r = -0.770$).

In the HPP, two forb families, Fabaceae and Asteraceae, were within $\geq 50\%$ of the total family AGP in both the ambient and elevated plots, where 15 forb families constituted to the remaining c. 50% AGP in both the ambient and elevated plots. Similar to the AGP results for the LPP, Asteraceae AGP declined in the elevated plots, whereas Fabaceae experienced an increase in AGP (Figure 15A). Neither forb family correlated with the abiotic factors.

Four forb species accounted for $\geq 50\%$ of the total forb AGP in both the ambient and elevated plots during the HPP, with the remaining forbs consisting of 35 species in both the ambient and elevated plots. Only the forb species *T. natalensis* was dominant (had $\geq 50\%$ total AGP in either the ambient and elevated plots) within both the LPP and HPP. However, during the HPP this species experienced a decline in AGP in the elevated plots (Figure 15B), whereas it exhibited no response to elevated temperatures during the LPP (Figure 14B).

At elevated temperatures an increase in the AGP of *Indigofera hiliaris* Eckl. & Zeyh. was shown, whereas *S. glaberrimus* declined significantly (Figure 15B). Additionally, *S. angustifolia* remaining unchanged, while *S. madagascariensis* (data not shown, contributed $< 50\%$ of the total forb species AGP during the HPP) experienced a

significant decrease ($p < 0.05$) in the elevated plots during the HPP. Interestingly, the increased *I. hiliaris* AGP at elevated temperatures was also accompanied by a large increase (24 plants/m²) in density during the HPP (data not shown). *Tetraselago natalensis* AGP was significantly negatively correlated with maximum temperature ($p < 0.05$; $r = -0.481$), with no correlations observed in the remaining species.

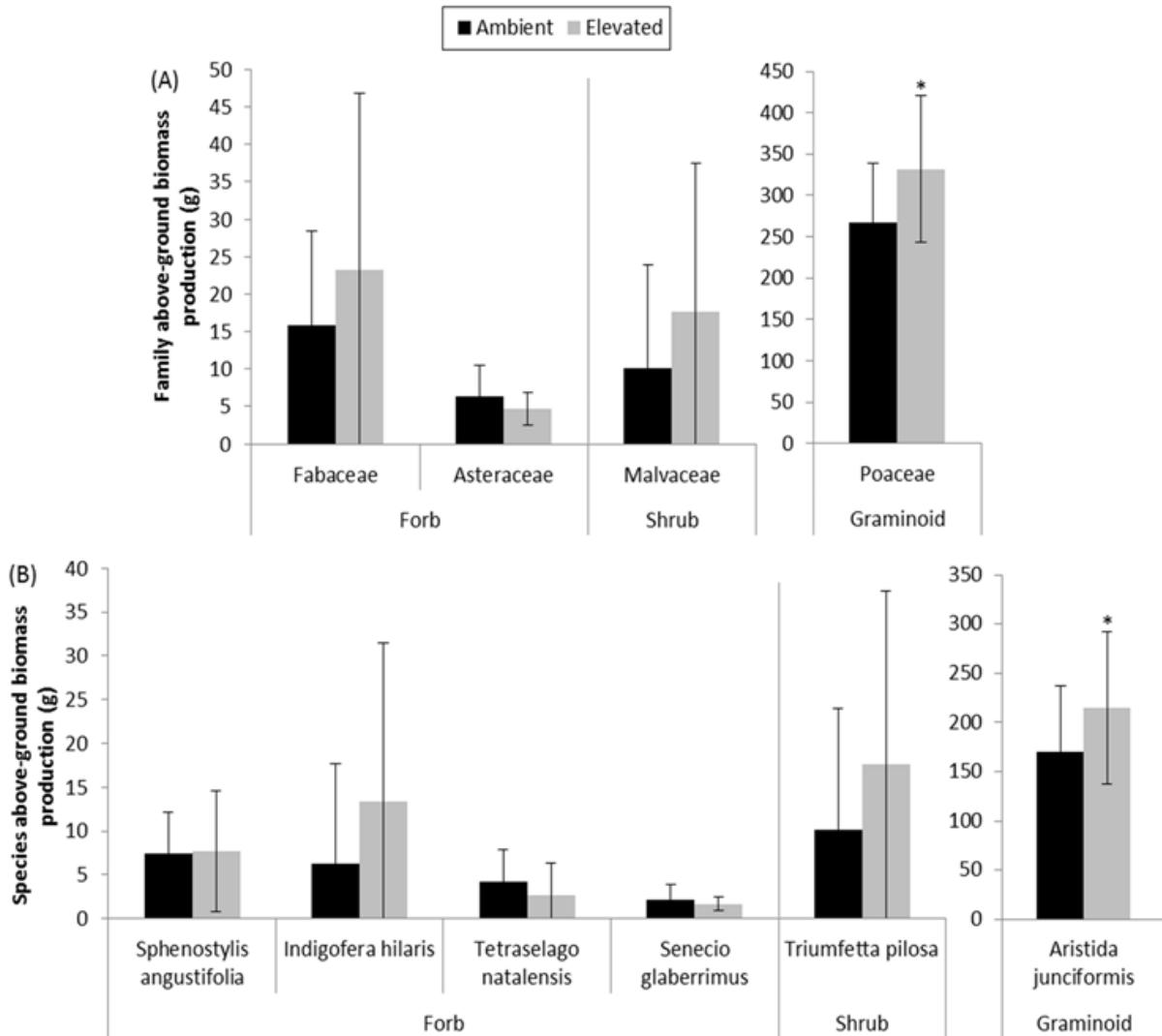


Figure 15. Above-ground biomass production (g) for families (A) and species (B) that emerged within ambient and elevated temperature plots during the high productivity period (spring–summer). Values represent mean±SD ($n = 10$). Values labelled with * are significantly different ($p < 0.05$, Mann–Whitney U test) when compared within life form categories, between temperature treatments.

The shrub family Malvaceae, represented by the species *T. pilosa*, constituted $\geq 50\%$ of the total shrub density within both the ambient and elevated plots, and exhibited a large increase in AGP at elevated temperatures during the HPP (Figures 15A & B). The remaining $< 50\%$ family and species density was from the forb family Fabaceae, with four forb species occurring within both the ambient and elevated plots. Malvaceae AGP was significantly positively correlated with average temperature ($p < 0.05$; $r = 0.479$). Similar to the forb species *I. hilaris*, the increase in *T. pilosa* AGP was accompanied by an increase (5.96 plants/m^2) in density relative to the ambient plots during the HPP (data not shown). *T. pilosa* AGP was also significantly positively correlated with average temperature ($p < 0.05$; $r = 0.479$).

The Poaceae family was again the only dominant graminoid family constituting $\geq 50\%$ of the total AGP within both the ambient and elevated plots during the HPP, and exhibited significantly higher AGP in the elevated plots (Figure 15A). The only remaining graminoid family making up $< 50\%$ of the AGP in both the ambient and elevated plots was Cyperaceae. Poaceae AGP was significantly negatively correlated with both RH and soil moisture content ($p < 0.05$ and $r = -0.725$ and -0.728 , respectively). Similar to the LPP, *A. junciformis* was the only graminoid species to constitute $\geq 50\%$ of the total graminoid AGP within both the ambient and elevated plots. The remaining graminoid species during the HPP consisted of 14 species in the ambient plots, and 15 species in the elevated plots. *Aristida junciformis* had significantly higher AGP in the elevated plots (Figure 15B). The AGP of two other graminoids, *Alloteropsis semialata* (R.Br.) Hitchc. and *Andropogon appendiculatus* Nees, were also significantly ($p < 0.05$) higher in the elevated plots (data not shown, contributed $< 50\%$ of the total graminoid species AGP during the HPP). *Aristida junciformis* did not correlate with the abiotic factors outlined in section 3.3.3.

3.4.6 Cluster analyses

The UPGMA analyses, which included all species found within each plot (ambient and elevated) across all seasons, identified three distinct clusters (A–C; Figure 16). Clusters B and C grouped together (i.e. are more similar to each other than to cluster A). Clusters A and B are composed of winter and autumn (ambient and elevated) plots respectively,

while cluster C contained a mixture of both spring and summer (ambient and elevated) plots.

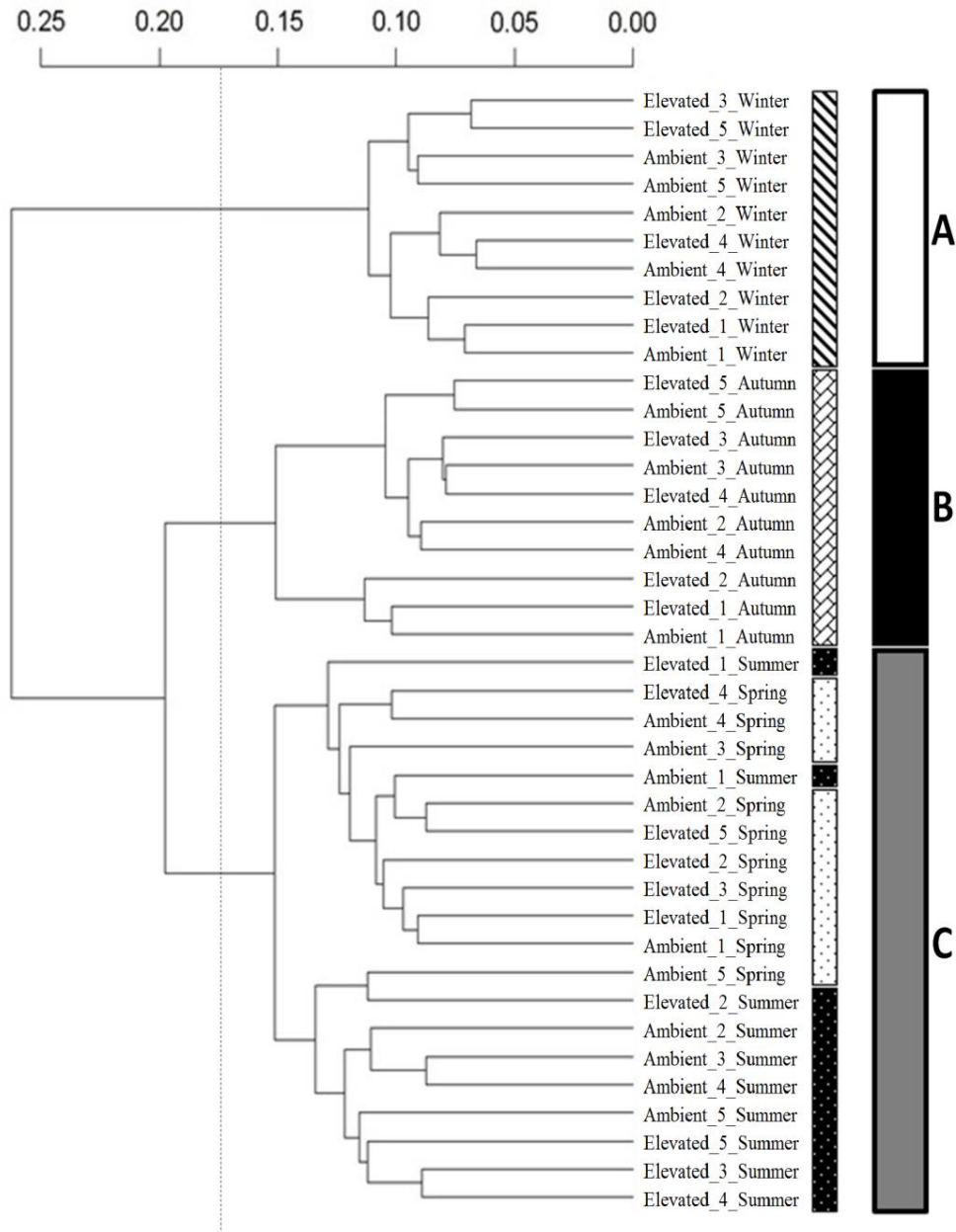


Figure 16. Phenogram of the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) analysis based on the Jaccard Similarity Index on the presence–absence values for ambient and elevated plots in all seasons (Cophenetic correlation: Mantel statistic $r = 0.9379$, $p = 0.009901$). Letters represent clusters defined by phenon line.

3.4.7 Ordination analyses

There were two main groupings in the ordination space separated along Coordinate 1 (-0.10) (Figure 17). The first group consisted of 10 ambient plots only ($n = 5$ autumn and $n = 5$ winter plots). The second group consisted of 30 plots from all four seasons (both ambient and elevated) and separated at c. -0.05 along Coordinate 2. This second group consisted of two sub-groupings: one consisting of eight winter ($n = 5$) and autumn ($n = 3$) elevated plots, and the second consisting of 22 plots which belonged predominately to spring ($n = 10$) and summer ($n = 10$) (both ambient and elevated), with two elevated autumn plots.

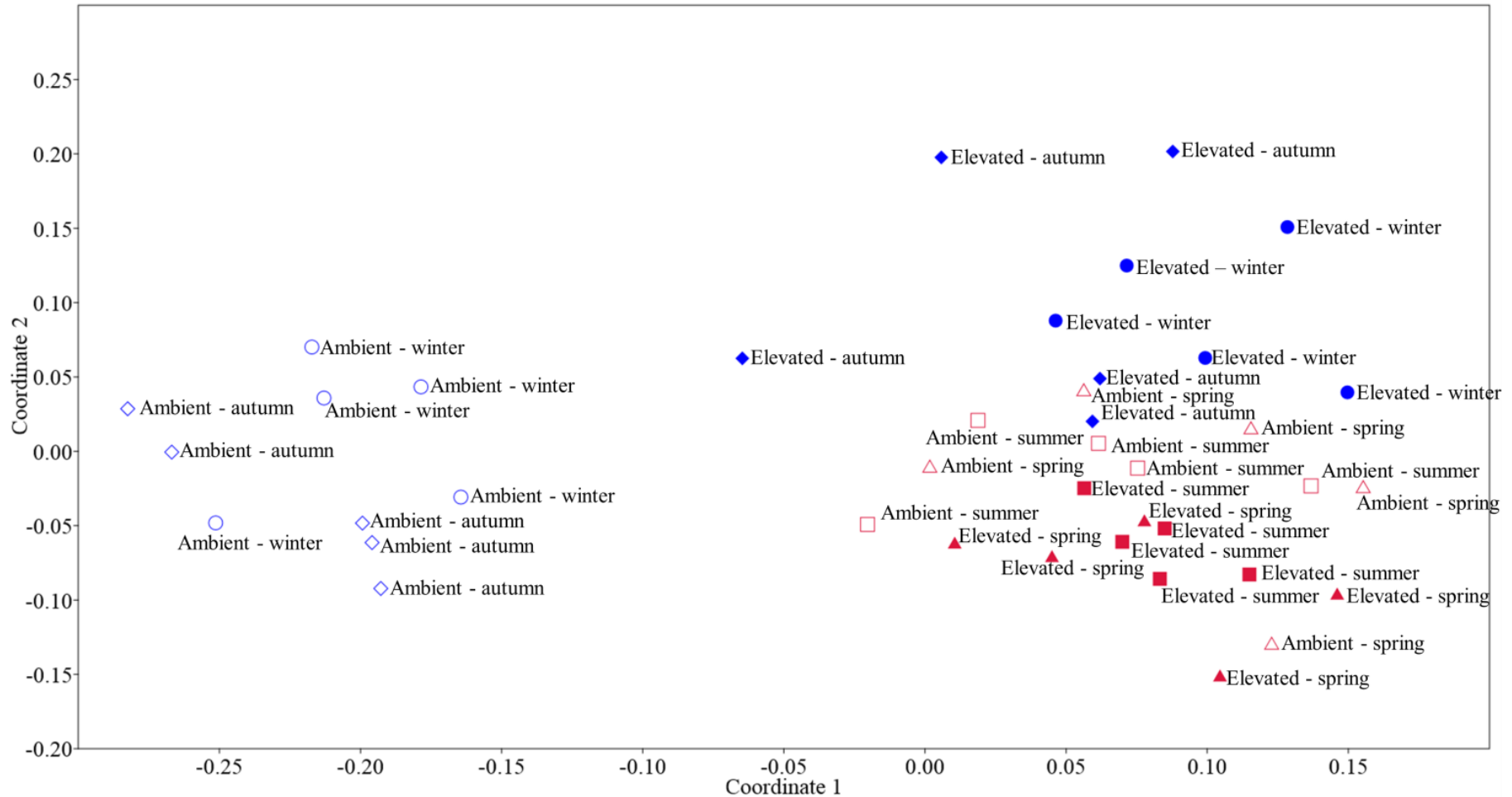


Figure 17. Non-parametric Multidimensional Scaling (NMDS) ordination using the Bray-Curtis similarity index based on forb and shrub species abundance data for ambient and elevated plots in all seasons ($n = 40$; stress = 0.1692). Seasons are represented by a shaped point within the ordination (triangle = spring, square = summer, diamond = autumn, circle = winter). Ambient and elevated plots are represented by hollow and filled points, respectively.

3.5 Discussion

This study was conducted to assess subtropical grassland, specifically KZNSS, vegetation responses to simulated elevated temperatures *in situ*. The study focussed on floristic responses at the family and species levels and made comparisons across life forms (forbs, shrubs and graminoids) and high and low productivity periods typically associated with perennial grasslands. The dynamic relationships among grassland plant community composition, diversity and productivity have been well researched in a number of grassland systems across the world (Tilman *et al.* 1997; Kahmen *et al.* 2005; Zuo *et al.* 2012). However, the potential for different climate change scenarios to influence these relationships are relatively poorly understood, particularly within subtropical/tropical grasslands.

3.5.1 Floristic composition

Despite the limited sampling and small coverage of this study site, sampling effort was high for this study (70.3% and 82.6% for Jack 1 and Chao 2, respectively). The cumulative species richness (ambient and elevated plots combined) of 74 species (see Figures 9 & 10), was however well under the 128 species richness recorded by Drury (2016), for exactly the same grassland site used in this study. However, this author sampled quadrats throughout the grassland, at different altitudes and aspects, with a far higher coverage ($n = 15, 25 \text{ m}^2$ quadrats) than this study and supplemented this data with diagonal transects which were conducted monthly for a year. Coverage in the present study equated to only 21.5% of the total quadrat area sampled by Drury (2016), yet contained 74.6% of the taxa sampled, providing a substantial dataset to draw conclusions from. Additionally, the present study was not designed to determine the species richness of the entire grassland, but rather to compare floristics and productivity between plots exposed to ambient and elevated temperature within at a relatively consistent altitude and aspect.

The most speciose families were Asteraceae, Poaceae and Fabaceae. This is consistent with other grassland studies which have shown that Asteraceae and Poaceae are usually the top two most speciose families. Brand, Brown & du Preez (2010) showed that these families make up 29.7% of the total flora, with Fabaceae (fourth largest family), contributing only 4.9% at their study site. Additionally, similar to other

subtropical/tropical grasslands (Drury *et al.* 2016; Brand, Brown & du Preez 2010), the study site hosted a large number of perennial grass species (Figures 9 & 10; Appendix 1). Typical of subtropical/tropical grasslands (Drury *et al.* 2016; Brand, Brown & du Preez 2010), forbs were the most speciose life form, accounting for 62.2% of the total number of species found (see Figure 11, data not shown). Whereas shrub species are usually only found scattered throughout C₄ grasslands (*i.e.* subtropical/tropical grasslands) (Bond & Parr 2010).

Interestingly, Drury *et al.* (2016) found very few diagnostic and species of conservation concern within remnant patches of KZNSS. Furthermore, only one species, *Alepidea amatymbica*, of 'vulnerable' conservation status (SANBI 2016) was found. Grasslands in southern Africa have been reported to host large number of IAPs (Henderson 2007; Richardson & van Wilgen 2004), however, in the present study only two naturalised exotic species, *L. indica* and *P. notatum* (a graminoid), were present. These findings are similar to Drury (2016), but only *L. indica* was found within the Tanglewood Nature Reserve (TWNR), but not in high abundances. There is active clearing of aliens at the TWNR which may explain the low number of aliens in the reserve in general.

3.5.2 Floristic responses to elevated temperatures

The composition of plant communities can be strongly altered by anthropogenic manipulations of abiotic conditions as well as biogeochemical cycles (Hillebrand, Bennett & Cadotte 2008). Before considering the results obtained here it is instructive for us to note that the effect of spatial variation between plots cannot be ruled out as having an effect on species composition. Numerous studies have demonstrated that even with a small spatial variance, there may be variation in the species composition (Whittaker 1960; Tuomisto 2010; Legendre, Borcard & Peres-Neto 2005). To alleviate the potential effects of small spatial scale and species heterogeneity in this study, sampling was performed over multiple seasons, with replication in each of these, in order to maximise the number of observations made for any particular parameter.

With the above in place, there was little difference in the ranking of the top five dominant families between the ambient and elevated plots in terms of annual data, with the elevated plots having one extra graminoid (a Poaceae), and one less forb (an Asteraceae) (Figure 11). Similarly, the top five dominant families in both the LPP and

HPP were comparable between the ambient and elevated temperatures. Asteraceae, Poaceae and Fabaceae appear to be the most speciose families in a number of subtropical grasslands (Brand, Brown & du Preez 2010; Carbutt & Edwards 2004; Drury *et al.* 2016; Ferreira *et al.* 2010).

It was evident that Asteraceae was the most/one of the most speciose family/families, irrespective of the productivity period (Figure 11). The dominance of this family in South African grasslands, at various ecological levels, is well documented (Gibbs Russell 1987; Brand, Brown & du Preez 2010). The species richness within each of these top five dominant families differed slightly between temperature treatments. For example, only two extra Poaceae species were present in the elevated plots during the LPP while during the HPP elevated plots exhibited one less Poaceae and Fabaceae species (Figure 11; Appendix 1). These results suggest no drastic changes in dominant families in the short term; however this does not exclude the possibility of more drastic long-term change, especially if temperature continues to increase and ecological interactions change.

Gorgone-Barbosa *et al.* (2016) demonstrated that under fluctuating temperatures and disturbances leading to canopy gaps, African grasses could experience a break in dormancy, increasing their chance of establishment. This may explain the results obtained for the LPP described above (Figures 14A & B). Increased productivity in graminoids, for example *A. junciformis*, may have increased competition for resources during the HPP at elevated temperatures. This could hamper the germination and/or seedling growth in the species that were absent from elevated plots during the HPP. This increased AGP could have also greatly reduced the available light for other species. Many grassland species (including many C₄ grasses) are shade intolerant and could have resulted in a reduction in forbs and shrubs (Bond & Parr 2010; Everson, Everson & Tainton 1988; Uys, Bond & Everson 2004).

The Shannon Exponential and Simpson Inverse indices, used to assess both richness and evenness (Nagendra 2002), showed small differences between the ambient and elevated plots. This suggests that even very short periods (2–3 months in this case) of exposure to elevated temperatures can lead a slight increase in diversity and evenness. For example, the Shannon Exponential Index was slightly higher at elevated temperatures,

whereas the Simpson Inverse Index was slightly lower in value at elevated temperatures annually and during both the LPP and HPP (Table 3). The Shannon Exponential Index favours richness, while the Simpson Inverse index has a greater emphasis on evenness (Nagendra 2002). Overall the elevated temperature plots (annually and during both productivity periods) had increased species richness but decreased in evenness compared to the ambient plots. Stirling & Wilsey (2001) found that in plants there was a weak negative correlation between richness and evenness. Elevated temperature in the tundra biome revealed that warming increases dominance (Walker *et al.* 2006), and thus decreased evenness. In this study grasses are shown to increase in dominance in terms of AGP, consequently reducing evenness in the KZNSS vegetation, which has long-term implications. A habitat that has been highly fragmented (like KZNSS) with increased graminoid dominance, should prove a greater risk to species extinction (Hillebrand, Bennett & Cadotte 2008).

3.5.3 Plant density responses to elevated temperatures

The Asteraceae, and Commelinaceae and Fabaceae were found to be the most dominant forb families in terms of density at both ambient and elevated temperatures (in the LPP and HPP) (Figures 12 & 13). However, Asteraceae densities declined during both the LPP and HPP in elevated compared with ambient plots (Figures 12 & 13). During the LPP this decline was mainly due to the significant decline in *S. glaberrimus*, *S. speciosus* and *G. amigua* in elevated plots (Figure 12B), while during the HPP this decline was attributed to a reduced density in *S. glaberrimus* (Figure 13B) and *S. madagascariensis* (not shown).

Additionally, *C. speciosa* and *C. africana* densities declined at elevated temperatures during the LPP (Figure 12B), leading to a reduction in the density of the forb family Commelinaceae (Figure 12A). The density of *C. speciosa* also significantly decreased in the elevated plots during the HPP (Figure 13B). Furthermore, *C. speciosa* density was significantly negatively correlated with both average and minimum temperatures. There were no published reports on the responses of the genus *Cyanotis* to elevated temperatures; however, since some members of the genus are shade tolerant (Sheng & Liu 1995) it is unlikely that their decline in density was related to shading by some

grasses that experienced an increase in productivity at elevated temperatures (see Figures 12A & 13A; discussed below).

Two members of the shrub family Fabaceae, *Z. capensis* subsp. *capensis* and *S. angustifolia*, also exhibited a slight decrease in density during the LPP and HPP within the elevated plots (Figures 12B & 13B). Many members of Fabaceae exhibit seed dormancy which is under the fine control of large fluctuations in temperature (Van Assche, Debucquoy & Rommens 2003). The increase in day and night temperatures within the OTCs may have interfered with these requirements for germination in the Fabaceae species mentioned above. On the other hand, the shrub *Triumfetta pilosa* (Malvaceae) exhibited increased densities in a few plots at elevated temperatures during the HPP (Figure 5B). Fabaceae and Malvaceae were the only dominant shrub families within the LPP (Figure 12A) and HPP (Figure 13A), respectively, that showed a shift from Fabaceae dominance in the LPP to Malvaceae dominance in the HPP (in terms of density).

Since the Asteraceae and Fabaceae are dominant families in grasslands (Drury *et al.* 2016; Brand, Brown & du Preez 2010), this decline in density of some of their representative species at elevated temperatures could have a significant long-term impact on vegetation composition and structure of subtropical grasslands like KZNSS.

3.5.4 Effects of elevated temperatures on above-ground biomass production (AGP)

The AGP data reflect the differences in productivity between the LPP and HPP (Chapter 2, Figure 6). The Poaceae, and to a large extent Cyperaceae dominated the graminoid life form in this study. A single grass species in particular, *A. junciformis*, dominated in terms of AGP, accounting for $\geq 50\%$ of the total AGP in the LPP and HPP.

This grass also exhibited increased AGP within the elevated plots during the LPP (Figure 14B) and HPP (significant in the latter period only; Figure 15B). Other Poaceae species like *Alloteropsis semialata* and *Andropogon appendiculatus* also increased their AGP significantly at elevated temperatures in the HPP. This collectively led to increased Poaceae AGP in the elevated plots during both productivity periods (significant for HPP only; Figures 14B & 15B, respectively). *Aristida junciformis* AGP was significantly positively correlated with minimum temperature in the LPP, and Poaceae AGP was significantly positively correlated with average, maximum and

minimum temperature during the LPP (Figures 14A & B). This, together with the fact that Poaceae AGP, in both the LPP and HPP, was significantly negatively correlated with both RH and soil moisture content, and *A. junciformis* AGP was also significantly negatively correlated with soil moisture content (in the LPP), suggests that the stimulatory effects of elevated temperatures on the Poaceae were strong enough to overcome the negative effects of reduced water availability at elevated temperatures.

It is well documented that the productivity of graminoids (specifically C₄ species) is enhanced at elevated temperatures (Horton & Murchie 2000; Morgan *et al.* 2007; Sage 2000). This, combined with the high water-use-efficiency of graminoids (Sherry *et al.* 2008; Wan *et al.* 2005), suggests that temperature will be the driving force behind increased productivity in grasslands.

This is further supported by *A. junciformis* (and other grasses) using the C₄ carbon fixation pathway (Cerros-Tlatilpa, Columbus & Barker 2011), which would increase the efficiency of this species at elevated temperatures, resulting in increased AGP (Sage 2000). The Poaceae species *Andropogon appendiculatus* also utilises the C₄ pathway, while *Alloteropsis semialata* has been known to use a C₃, C₃-C₄ or C₄ pathway, depending on its locality and climatic conditions (Lundgren *et al.* 2016).

The decline in Asteraceae (forb) density described earlier was accompanied by a decrease in AGP at elevated temperatures during both the LPP and HPP (Figures 14A & 15A, respectively). Fabaceae on the other hand had an overall increase in AGP for both productivity periods, despite the decline in densities noted for some members of this family (Figures 12 & 13). The legume species *Sphenostylis angustifolia* and *I. hiliaris* accounted for this increase in Fabaceae AGP (Figures 14B & 15B, respectively), where *S. angustifolia* experienced no change in density within the ambient or elevated plots during the LPP (Figure 12B), whereas *I. hiliaris* had a slightly increased density within the elevated plots during the HPP (not illustrated). Little, to no research has been done on the effects of elevated temperature on the genera *Sphenostylis* and *Indigofera* within subtropical grasslands, while research on prairie grassland (in the USA) showed mixed results, with some legume species experiencing increased AGP with elevated temperatures and others a decline (Whittington, Tilman & Powers 2013). Whittington,

Tilman & Powers (2013) suggested that the increased AGP in legume species at elevated temperatures could be a consequence of a delayed senescence.

Lasiosiphon kraussianus (Thymelaeaceae) only appeared in some of the ambient plots during the LPP, and did not appear in the elevated plots. This is not however, an indication that this species is in fact negatively affected by the elevated temperatures. *Lasiosiphon kraussiana* is widely distributed in southern Africa (Borris & Cordell 1984), occurring in different climatic regions, biomes and vegetation types, suggesting that it has a great tolerance to climatic variations. This wide range suggests that *L. kraussiana* has high phenotypic plasticity. Its absence is possibly due to the lack of seed in the seed bank or pre-existing rootstocks.

In contrast, *O. filamentosum* (Lamiaceae) exhibited similar densities within the ambient and elevated plots during the LPP but exhibited a significant increase in AGP at elevated temperatures during this period. At the time of this study there were no published studies on the effects of temperatures on *O. filamentosum*, however, *Ocimum basilicum*, has been shown to have increased growth at elevated temperatures (Chang, Alderson & Wright 2005; Caliskan, Odabas & Cirak 2009).

Differences in shrub AGP between the ambient and elevated plots was minimal during the LPP (Figures 14A & B, respectively). However, *T. pilosa* (Malvaceae), which made up $\geq 50\%$ of the total shrub AGP in both ambient or elevated plots during the HPP, exhibited an increase in AGP at elevated temperatures (Figure 15B). This was accompanied by an increased density during the HPP within the elevated plots (Figure 13B). Both AGP and density were significantly positively correlated with average temperature (Appendix 2).

3.5.5 Clustering

The clustering of the ambient and elevated plots showed a distinct ‘winter’ cluster (Cluster A, Figure 16). The second clustering consisted of a distinct ‘autumn’ cluster (B) and a mixed ‘spring + summer’ cluster (C). These clusters and sub-clusters, irrespective of ambient or elevated plot, appear to be determined by seasonal responses, rather than temperature treatments.

The clustering of ‘autumn’ (B) and mixed ‘spring + summer’ clusters (C) suggests that autumn plots are more similar to spring and summer plots. This may be due to the mild and less dramatic changes experienced in South Africa and more specifically coastal subtropical KwaZulu–Natal. Annual and geophytic taxa are still present and observed at the end of summer and the start of autumn. This may explain why ‘autumn’ clustered with spring and summer, and not with winter, despite autumn being a ‘lower’ productivity period. In fact the concept of autumn being a low productivity period may not apply in a strict sense to coastal KZN. Rather it may be viewed as a period/ season of decrease in productivity, but still contributing to above ground biomass and density attained at the end of summer. It is only at the beginning of winter that productivity is comparatively low.

Additionally, within both winter and autumn there was no distinct separation (*i.e.* clustering) between ambient and elevated plots. This suggests that the floral composition in both ambient and elevated plots for these seasons is largely similar. However, in the ‘spring + summer’ sub–cluster there was no distinct summer and spring plot clustering nor were there any clustering between ambient and elevated plots. This suggests that temperature has little effect on species composition (and possibly richness), whereas the seasonal conditions appear to have more influence on grassland species composition. The time frame for this study was possibly too short for any meaningful floristic changes to have occurred and manifested themselves.

3.5.6 Ordination

There were more or less two distinct groups in the ordination space (Figure 17). The first group is composed of ten winter and autumn ambient plots (five in each season), whereas the second group contained the other 30 ambient and elevated plots. Some winter and some autumn elevated plots separated from summer and spring ambient and elevated plots (with two autumn elevated plots grouped with the summer and spring plots). This suggests that temperature is affecting the structure of this grassland, where there is a clear separation between the ambient and elevated plots during the LPP (autumn and winter). However, no clear separation is seen between the ambient and elevated plots during the HPP (spring and summer). Interestingly, the autumn and winter elevated plots grouped closely with the plots in the high productivity period

(spring and summer), suggesting possible similarities between these two sub-groups. This partly supports the notion that autumn is a ‘winding down’ season rather than a strictly ‘low’ productivity period like winter. Therefore it is suggested that elevated temperature has the greatest effect on grassland structure during the cooler, less productive periods.

It should be noted that graminoids were not included in the ordination due to their clonal nature (it was not possible to determine their abundances with any confidence). So this may have influenced the ordination results and would possibly have resolved the grouping further.

3.6 Concluding remarks and recommendations

Knowledge on how different vegetation types, especially endangered ones with limited spatial extent, and under urban and immediate anthropogenic pressure, will respond to the ever pressing effects of climate change will aid future conservation and management plans, ensuring their persistence. Thus the present study will assist with understanding in how elevated temperatures may change plant community composition and structure within subtropical grasslands. Special management practices need to be put in place to prevent further habitat loss of the already highly fragmented endangered KwaZulu-Natal Sandstone Sourveld (Naicker, Rouget & Mutanga 2016). It has been shown that OTCs were capable of successfully elevating temperature with the selected subtropical grassland (Chapter 2). Although this present study (Chapter 3) only sampled a small area within each season, the total number of species identified within each treatment represented a large portion of the species within this grassland. Additionally with elevated temperatures, graminoids showed enhanced productivity, forbs appeared to be negatively affected and shrubs remained unaffected.

The results suggested that elevated temperatures led to minimal changes in community composition at the family level, but richness within these families were altered at elevated temperatures when assessed at each productivity period. Additionally, the elevated temperatures did not affect the ranking of the dominant families. There are however indications of initial increased richness, although elevated temperatures may have negative long-term implications, especially with increased graminoid AGP and dominance.

Graminoid species (e.g. Poaceae) responded positively to elevated temperatures in terms of productivity, which appeared to have been to the detriment of common grassland forbs (typically Asteraceae), which were variably compromised in terms of density and/or productivity. At elevated temperatures, the forb taxa in Asteraceae appeared to show declines in both density and AGP all year around, with the species in the genus *Senecio* experiencing large declines in both density and AGP. The Fabaceae taxa experienced increased AGP within the warmer HPP, with little to no effect occurring on species densities. The shrub taxa appeared to utilise the warmer, wetter conditions in the HPP well, with increased AGP, whereas very little change was observed in the LPP. Where elevated temperatures enhanced taxa density and/or productivity, its effects were significant enough to overcome the potential growth limiting effects of reduced water availability. This was predominately seen in the graminoids across both productivity periods.

Throughout the duration of this study, numerous taxa of interest were identified in terms of their notable response to elevated temperature. This is the first study in South Africa to identify specific coastal grassland taxa that will respond to elevated temperatures, rather than typically preselecting taxa and studying responses thereafter. These included the graminoids (*A. junciformis*, *A. semialata* and *A. appendiculatus*); the forbs (*C. speciosa*, *T. natalensis*, *S. angustifolia*, *I. hilaris*, and especially the species in the genus *Senecio* (*S. glaberrimus*, *S. speciosus* and *S. madagascariensis*)); and shrubs (*E. cordatum*, and *T. pilosa*). All these taxa need further investigation to determine the physiological basis of their responses to elevated temperatures. It is therefore suggested that subtropical grasslands may experience increased growth in graminoids, likely causing greater competition for resources between graminoids and forbs and amongst forb species.

In KZNSS, elevated temperatures will more likely impact on vegetation structure and plant productivity than community composition and diversity. However, the shift in dominance (and hence evenness) over the long-term within the KZNSS, and subtropical grasslands in general, may increase their vulnerability to elevated temperature-induced changes in community composition and diversity. Possibly increasing alien plant invasion and eliminating narrowly distributed red-listed taxa in KZNSS.

The effects of elevated temperature are only part of a more complex scenario, where temperature is only one variable and interactions between other biotic variables need further investigation. These biotic variables, such as competition, mutualisms (Fabaceae), pollination, reproduction and seedbanks need to be considered.

A short term study conducted over four seasons may not be enough to fully understand the responses of subtropical grassland communities to climate change. It is therefore suggested that long-term studies, with the use of OTCs, may provide more comprehensive results regarding the responses of plant communities and individual taxa. Such studies will provide the best data available on maintaining biodiversity through improved conservation and management planning of subtropical grasslands.

CHAPTER FOUR

CONCLUDING REMARKS AND RECOMMENDATIONS

4.1 Major findings

This research has shown that open-top chambers (OTCs) are capable of simulating a realistic increase in temperature within subtropical grasslands. More specifically the OTCs resulted in a ± 2.1 °C increase in day air and a ± 0.3 °C increase in day soil temperatures which is in line with 2050 predictions for the region. The data on ambient air and soil temperatures within the subtropical grassland is also a first for this region, particularly in terms of its level of accuracy and frequency of collection. The OTC effects on other abiotic parameters such as light were negligible, however, there were indications of a slight decrease in soil moisture content which should be investigated using more long-term experiments.

Elevated temperatures impacted more on structure and productivity, than on community composition and diversity. Graminoids, specifically Poaceae, appeared to benefit most in terms of AGP and density, while forbs exhibited a decline in both these variables. This decline in forbs is possibly a consequence of increased graminoid productivity and/or direct physiological effects caused by elevated temperatures. Grassland evenness declined at elevated temperatures, which was possibly also a consequence of increased graminoid AGP. Collectively, the data suggests that elevated temperatures will not alter the existing family dominance patterns within subtropical grasslands such as KZNSS, rather, they are likely to benefit families that presently dominate subtropical grasslands such as the Poaceae. Interestingly, there were also indications that elevated temperatures may increase species richness and later community structure within KZNSS; however, studies of a longer duration are required to confirm these potential changes.

4.2 Challenges and shortcomings

The first challenge faced by this study involved the use of five OTC and control plots during each season only, and a single study site. This was unavoidable due to time and logistical constraints; a pilot study indicated that it was not possible to sample more

than ten chambers in one season. The fact that the site selected for the study had to be securely fenced off to avoid the OTCs being tampered with (stolen or damaged by fire), limited the choice of study sites to one remnant patch of KZNSS grassland, which is fenced along its periphery due to private ownership and being subject to controlled burning and restricted access. Greater levels of replication, in different subtropical grasslands types would have yielded more robust conclusions on the effects of elevated temperatures on subtropical grasslands.

Due to the rapid growth of some of the grasses, particularly in the high productivity periods, the vegetation had to be harvested after two months in any one season. This could not be avoided as the grasses would have outgrown the chambers if the trials were run beyond two months. This prevented the investigation of some of the long-term effects of elevated temperatures (Sherry *et al.* 2008).

This study provided data on vegetation change and variation under ambient and elevated temperature scenarios. By including an ambient (control) scenario, this study has also captured natural variation over a short temporal and spatial scale. Consequently, this provided baseline data on variation for both ambient (natural) and elevated temperature. This baseline data is important for long term monitoring of change in KZNSS.

Additionally, due to the clonal and tufted nature of graminoids, cover and density data were not collected for members of this life form. This limited a holistic assessment of how these species are responding to elevated temperature and influenced the results of the NMDS analyses as these excluded graminoid densities.

Whilst this study did not aim to investigate the effects of elevated temperatures on the phenology of grassland plants, data on these aspects (even for selected species) would have provided additional data on the possible mechanisms governing the productivity and floristic responses observed.

4.3 Recommendations for future studies

The research findings provide motivation for making the effects of elevated temperatures (and other climate change scenarios) on subtropical grasslands a research priority. Furthermore, the study has clearly shown that these effects, as in temperate grasslands, can be investigated *in situ* using OTCs. Increasing the number of replicate

plots (both OTC and control), as well as the number of study sites, beyond the numbers used in this study, will increase the strength and validity of the findings in such studies. Study sites that lie along environmental gradients (e.g. altitudinal, wetness or disturbance) should also be included in future studies on elevated temperature effects on subtropical grasslands.

The duration of each growth season (productivity period) should also be increased as alluded to above. However, this will require an increase in chamber height (to at least 70 cm [Godfree *et al.* 2011]); the effects of this increase in chamber height will have to be investigated for subtropical grasslands. These taller chambers may also provide future studies with opportunities to assess the dual effects of elevated temperatures and reduced soil moisture content and/or relative humidity on subtropical grassland vegetation.

Floristically, determination of graminoid cover and density data should be collected in future studies of this nature and potential methods for this do exist in the literature (e.g. Heelemann *et al.* 2010; Richardson, Karcher & Purcell 2001; Sorrells & Glenn 1991). Similarly, the effects of elevated temperatures on phenology of dominant species (at the very least) should also be explored. Future work should also determine the physiological basis of the productivity and floristic responses observed in the present study. Finally, more research effort must be devoted towards the effects of climate change on subtropical African grasslands which, based on the results of the present study, will provide motivation for their future conservation.

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Appendix 1

Summary of species presence-absence within ambient ($n = 20$) and elevated ($n = 20$) plots. Alien and red list status of indigenous species are provided (according to the South African Biodiversity Institute [SANBI, <http://www.redlist.sanbi.org>]).

Family	Species Name	Invasive and Alien Status	Redlist Status	Ambient	Elevated	Life form
Acanthaceae	<i>Barleria obtusa</i> Nees	Indigenous	LC	x	y	Forb
Acanthaceae	<i>Thunbergia natalensis</i> Hook.	Indigenous	LC	x	y	Forb
Apiaceae	<i>Alepidea amatymbica</i> Eckl. & Zeyh.	Indigenous	VU	x	y	Forb
Apocynaceae	<i>Asclepias albens</i> (E.Mey.) Schltr.	Indigenous	LC	x	y	Forb
Apocynaceae	<i>Cryptolepis oblongifolia</i> (Meisn.) Schltr.	Indigenous	LC	x	y	Forb
Asteraceae	<i>Aster bakerianus</i> Burt Davy ex C.A.Sm.	Indigenous	LC	x	y	Forb
Asteraceae	<i>Berkheya echinacea</i> (Harv.) O.Hoffm. ex Burt Davy	Indigenous	LC	x	y	Forb
Asteraceae	<i>Berkheya setifera</i> DC.	Indigenous	LC	x	y	Forb
Asteraceae	<i>Berkheya speciosa</i> (DC.) O.Hoffm.	Indigenous	LC	x	y	Forb
Asteraceae	<i>Gerbera ambigua</i> (Cass.) Sch.Bip.	Indigenous	LC	x	y	Forb
Asteraceae	<i>Helichrysum aureum</i> (Houtt.) Merr.	Indigenous	LC	x	y	Forb
Asteraceae	<i>Helichrysum auriceps</i> Hilliard	Indigenous	LC	x	y	Forb
Asteraceae	<i>Helichrysum herbaceum</i> (Andrews) Sweet	Indigenous	LC	x	y	Forb
Asteraceae	<i>Helichrysum nudifolium</i> (L.) Less.	Indigenous	LC	x	y	Forb
Asteraceae	<i>Lactuca indica</i> L.	Alien	NE	x	y	Forb
Asteraceae	<i>Senecio brachypodus</i> DC.	Indigenous	LC	x	y	Forb
Asteraceae	<i>Senecio glaberrimus</i> DC.	Indigenous	NE	x	y	Forb
Asteraceae	<i>Senecio madagascariensis</i> Poir.	Indigenous	LC	x	y	Forb

NE = not evaluated; LC = least concern; VU = vulnerable; x = present in ambient plots; y = present in elevated plots

Family	Species Name	Invasive and Alien Status	Redlist Status	Ambient	Elevated	Life form
Asteraceae	<i>Senecio speciosus</i> Willd.	Indigenous	LC	x	y	Forb
Caryophyllaceae	<i>Dianthus zeyheri</i> Sond.	Indigenous	LC	x	y	Forb
Commelinaceae	<i>Commelina africana</i> L.	Indigenous	LC	x	y	Forb
Commelinaceae	<i>Commelina benghalensis</i> L.	Indigenous	LC	x	y	Forb
Commelinaceae	<i>Cyanotis speciosa</i> (L.f.) Hassk.	Indigenous	LC	x	y	Forb
Cyperaceae	<i>Bulbostylis hispidula</i> (Vahl) R.W.Haines subsp. <i>pyriformis</i> (Lye) R.W.Haines	Indigenous	LC	x	y	Graminoid
Cyperaceae	<i>Cyperus obtusiflorus</i> Vahl var. <i>obtusiflorus</i>	Indigenous	LC	x	y	Graminoid
Eriospermaceae	<i>Eriospermum mackenii</i> (Hook.f.) Baker	Indigenous	LC	x	y	Forb
Euphorbiaceae	<i>Acalypha peduncularis</i> E.Mey. ex Meisn.	Indigenous	VU	x	-	Forb
Euphorbiaceae	<i>Clutia cordata</i> Bernh.	Indigenous	LC	x	y	Forb
Fabaceae	<i>Albizia</i> sp.	-	-	x	-	Shrub
Fabaceae	<i>Chamaecrista plumosa</i> E.Mey.	Indigenous	LC	x	y	Shrub
Fabaceae	<i>Crotalaria</i> sp.	-	-	x	y	Forb
Fabaceae	<i>Eriosema cordatum</i> E.Mey.	Indigenous	LC	x	y	Shrub
Fabaceae	<i>Indigofera dimidiata</i> Vogel ex Walp.	Indigenous	LC	x	y	Shrub
Fabaceae	<i>Indigofera hiliaris</i> Eckl. & Zeyh.	Indigenous	LC	x	y	Forb
Fabaceae	<i>Rhynchosia monophylla</i> Schltr.	Indigenous	LC	x	y	Forb
Fabaceae	<i>Sphenostylis angustifolia</i> Sond.	Indigenous	LC	x	y	Forb
Fabaceae	<i>Tephrosia macropoda</i> (E.Mey.) Harv.	Indigenous	LC	x	y	Forb
Fabaceae	<i>Zornia capensis</i> Pers. subsp. <i>capensis</i>	Indigenous	LC	x	y	Forb
Gentianaceae	<i>Sebaea natalensis</i> Schinz	Indigenous	LC	x	y	Forb
Hypoxidaceae	<i>Hypoxis argentea</i> Harv. ex Baker var. <i>argentea</i>	Indigenous	LC	x	y	Forb

NE = not evaluated; LC = least concern; VU = vulnerable; x = present in ambient plots; y = present in elevated plots.

Family	Species Name	Invasive and Alien Status	Redlist Status	Ambient	Elevated	Life form
Hypoxidaceae	<i>Hypoxis rigidula</i> Baker	Indigenous	LC	-	y	Forb
Iridaceae	<i>Aristea abyssinica</i> Pax	Indigenous	LC	x	y	Forb
Iridaceae	<i>Hesperantha lactea</i> Baker	Indigenous	LC	x	y	Forb
Iridaceae	<i>Watsonia densiflora</i> Baker	Indigenous	LC	x	y	Forb
Lamiaceae	<i>Ocimum filamentosum</i> Forssk.	Indigenous	LC	x	y	Forb
Lobeliaceae	<i>Lobelia flaccida</i> (C.Presl) A.DC.	Indigenous	LC	x	y	Forb
Malvaceae	<i>Triumfetta pilosa</i> Roth	Indigenous	LC	x	y	Shrub
Myrtaceae	<i>Eugenia albanensis</i> Sond.	Indigenous	LC	x	y	Shrub
Poaceae	<i>Alloteropsis semialata</i> (R.Br.) Hitchc.	Indigenous	LC	x	y	Graminoid
Poaceae	<i>Andropogon appendiculatus</i> Nees	Indigenous	LC	x	y	Graminoid
Poaceae	<i>Andropogon gayanus</i> Kunth	Indigenous	LC	x	y	Graminoid
Poaceae	<i>Aristida junciformis</i> Trin. & Rupr.	Indigenous	LC	x	y	Graminoid
Poaceae	<i>Ctenium concinnum</i> Nees	Indigenous	LC	x	y	Graminoid
Poaceae	<i>Cynodon dactylon</i> (L.) Pers.	Indigenous	LC	-	y	Graminoid
Poaceae	<i>Digitaria eriantha</i> Steud.	Indigenous	LC	x	y	Graminoid
Poaceae	<i>Eragrostis superba</i> Peyr.	Indigenous	LC	x	y	Graminoid
Poaceae	<i>Monocymbium cerasiiforme</i> (Nees) Stapf	Indigenous	LC	x	y	Graminoid
Poaceae	<i>Panicum natalense</i> Hochst.	Indigenous	LC	x	y	Graminoid
Poaceae	<i>Panicum schinzii</i> Hack.	Indigenous	LC	x	-	Graminoid
Poaceae	<i>Paspalum notatum</i> Flügge	Alien	NE	x	y	Graminoid
Poaceae	<i>Setaria lindenbergiana</i> (Nees) Stapf	Indigenous	LC	x	y	Graminoid
Poaceae	<i>Themeda triandra</i> Forssk.	Indigenous	LC	-	y	Graminoid
Rubiaceae	<i>Pentanisia angustifolia</i> (Hochst.) Hochst.	Indigenous	LC	x	y	Forb

NE = not evaluated; LC = least concern; VU = vulnerable; x = present in ambient plots; y = present in elevated plots.

Family	Species Name	Invasive and Alien Status	Redlist Status	Ambient	Elevated	Life form
Santalaceae	<i>Thesium costatum</i> A.W.Hill	Indigenous	LC	-	y	Forb
Scrophulariaceae	<i>Tetraselago natalensis</i> (Rolfe) Junell	Indigenous	LC	x	y	Forb
Thymelaeaceae	<i>Lasiosiphon capitatus</i> (L.f.) Burtt Davy	Indigenous	LC	x	y	Forb
Thymelaeaceae	<i>Lasiosiphon kraussianus</i> (Meisn.) Burtt Davy var. <i>kraussianus</i>	Indigenous	LC	x	y	Forb
Unknown taxa	Forb 1	-	-	x	y	Forb
Unknown taxa	Grass 1	-	-	-	y	Graminoid
Unknown taxa	Grass 2	-	-	x	-	Graminoid
Unknown taxa	Grass 3	-	-	-	y	Graminoid
Unknown taxa	Grass 4	-	-	-	y	Graminoid
Unknown taxa	Shrub 1	-	-	-	y	Shrub
Unknown taxa	Shrub 2	-	-	-	y	Shrub

NE = not evaluated; LC = least concern; VU = vulnerable; x = present in ambient plots; y = present in elevated plots.

Appendix 2.

Significant correlations of family/ species density with selected abiotic factors. Data for ambient ($n = 20$) and elevated ($n = 20$) plots were pooled for analysis and correlations (Pearson's correlation [parametric] and Spearman's rank correlation [non-parametric]) were performed within each productivity period.

Productivity Period	Life Form	Family	Species	Maximum temperature ¹		Minimum temperature ¹		Average temperature ¹		Relative Humidity		Soil Moisture Content			
				<i>r</i> - value	<i>p</i> - value	<i>r</i> - value	<i>p</i> - value	<i>r</i> - value	<i>p</i> - value	<i>r</i> - value	<i>p</i> - value	<i>r</i> - value	<i>p</i> - value		
LPP	Forb	Acanthaceae	-	-	-	-	0.451	0.046	-	-	-	-	0.500	0.025	
		Acanthaceae	<i>Thunbergia natalensis</i>	-	-	-	-	0.500	0.025	-	-	-	-	-0.775	0.050
		Asteraceae	-	-	-	-	-	-	-0.661	0.002	-	-	0.703	0.001	
		Asteraceae	<i>Gerbera ambigua</i>	-	-	-	-	-	-	-	-	-	-	0.584	0.007
		Asteraceae	<i>Senecio brachypodus</i>	-	-	-	-	-	-	-0.588	0.006	-	-	0.566	0.009
		Asteraceae	<i>Senecio glaberrimus</i>	-	-	-	-	-	-	-0.856	0.000	-	-	0.731	0.000
		Asteraceae	<i>Senecio speciosus</i>	-	-	-	-	-	-	-0.670	0.001	-	-	0.815	0.000
		Commelinaceae	-	-	-	-	-	-	-	-0.549	0.012	-	-	0.541	0.014
		Commelinaceae	<i>Cyanotis speciosa</i>	-	-	-	-	-	-	-0.567	0.009	-	-	0.623	0.003
		Euphorbiaceae	<i>Clutia cordata</i>	-	-	-	-	-	-	-0.544	0.013	-	-	0.521	0.017
		Fabaceae	-	-	-	-	-	-	-	-0.607	0.005	-	-	0.474	0.035
		Fabaceae	<i>Zornia capensis subsp capensis</i>	-	-	-	-	-	-	-0.775	0.000	-	-	0.657	0.002
		Hypoxidaceae	<i>Hypoxis argentea var argentea</i>	-	-	-	-	-	-	0.580	0.007	-	-	-0.777	0.000

¹ Refer to Section 2.3.5 Statistical Analyses, pg 18.

Productivity Period	Life Form	Family	Species	Maximum temperature ¹		Minimum temperature ¹		Average temperature ¹		Relative Humidity		Soil Moisture Content	
				<i>r</i> - value	<i>p</i> - value	<i>r</i> - value	<i>p</i> - value	<i>r</i> - value	<i>p</i> - value	<i>r</i> - value	<i>p</i> - value	<i>r</i> - value	<i>p</i> - value
		Fabaceae	<i>Eriosema cordatum</i>	-	-	-	-	-0.507	0.022	-	-	-	-
HPP	Shrub	Malvaceae	-	0.487	0.029	-	-	0.559	0.010	0.555	0.011	0.608	0.004
		Malvaceae	<i>Triumfetta pilosa</i>	0.487	0.029	-	-	0.559	0.010	0.555	0.011	0.608	0.004
		Scrophulariaceae	-	-0.704	0.001	-	-	-0.580	0.007	-0.540	0.014	-0.501	0.024

¹ Refer to Section 2.3.5 Statistical Analyses, pg 18.

Appendix 3.

Significant correlations of family/ species above-ground biomass production with selected abiotic factors. Data for ambient ($n = 20$) and elevated ($n = 20$) plots were pooled for analysis and correlations (Pearson's correlation [parametric] and Spearman's rank correlation [non-parametric]) were performed within each productivity period.

Productivity Period	Life Form	Family	Species	Maximum temperature ¹		Minimum temperature ¹		Average temperature ¹		Relative Humidity		Soil Moisture Content		
				<i>r</i> - value	<i>p</i> - value	<i>r</i> - value	<i>p</i> - value	<i>r</i> - value	<i>p</i> - value	<i>r</i> - value	<i>p</i> - value	<i>r</i> - value	<i>p</i> - value	
LPP	Forb	Asteraceae	-	-	-	-	-	-	-	-	-	0.496	0.026	
		Asteraceae	<i>Senecio madagascariensis</i>	-	-	-	-	0.580	0.007	-	-	-0.558	0.011	
		Asteraceae	<i>Senecio speciosus</i>	-	-	-	-	-0.616	0.004	-	-	0.759	0.000	
		Commelinaceae	-	-	-	-	-0.501	0.025	-	-	0.529	0.016		
		Lamiaceae	<i>Ocimum filamentosum</i>	-	-	-	-	-	-	-	-	-0.683	0.030	
	Shrub	Fabaceae	<i>Eriosema cordatum</i>	-	-	-	-	-0.617	0.004	-	-	-	-	
	Graminoid	Poaceae	-	0.903	0.000	0.903	0.000	0.830	0.003	-0.648	0.043	-0.758	0.001	
		Poaceae	<i>Aristida junciformis</i>	-	-	0.636	0.048	-	-	-	-	-0.770	0.009	
	HPP	Forb	Apocynaceae	<i>Cryptolepis oblongifolia</i>	-0.460	0.041	-	-	-	-	-	-	-	-
			Commelinaceae	-	-0.639	0.002	-	-	-0.683	0.001	-0.493	0.027	-0.475	0.034
Commelinaceae			<i>Cyanotis speciosa</i>	-0.635	0.003	-0.623	0.003	-0.802	0.000	-0.618	0.004	-0.591	0.006	
Scrophulariaceae			-	-0.481	0.032	-	-	-	-	-	-	-	-	
		Scrophulariaceae	<i>Tetraselago natalensis</i>	-0.481	0.032	-	-	-	-	-	-	-	-	

¹ Refer to Section 2.3.5 Statistical Analyses, pg 18.

Productivity Period	Life Form	Family	Species	Maximum temperature ¹		Minimum temperature ¹		Average temperature ¹		Relative Humidity		Soil Moisture Content	
				<i>r</i> - value	<i>p</i> - value	<i>r</i> - value	<i>p</i> - value	<i>r</i> - value	<i>p</i> - value	<i>r</i> - value	<i>p</i> - value	<i>r</i> - value	<i>p</i> - value
HPP	Shrub	Fabaceae	-	-0.742	0.036	-	-	-0.510	0.022	-0.717	0.000	-0.687	0.001
		Fabaceae	<i>Chamaecrista plumosa</i>	-	-	-0.496	0.026	-	-	-	-	-	-
		Fabaceae	<i>Eriosema cordatum</i>	-0.478	0.033	-	-	-0.585	0.007	-0.606	0.005	-0.579	0.007
		Malvaceae	-	-	-	-	0.479	0.033	-	-	-	-	
		Malvaceae	<i>Triumfetta pilosa</i>	-	-	-	-	0.479	0.033	-	-	-	-
	Graminoid	Poaceae	-	-	-	-	-	-	-0.725	0.000	-0.728	0.000	

¹ Refer to Section 2.3.5 Statistical Analyses, pg 18.