

The hydrological and ecophysiological effects of simulated climate warming on the soil and vegetation of the Nama Karoo

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Abstract

Climate change is increasingly recognised as threat to individual species performance and survival. Manipulative field experiments, such as warming experiments, are crucial to understanding the potential future effects of a warming climate on plant function and survival. In warm, semi-arid ecosystems, increasing temperatures are expected to have detrimental effects on plant growth and survival due to exceedance of optimum temperatures for growth and functional processes such as photosynthesis. However, such detrimental effects may not be felt by all plant functional types. Most notably, C₃ and C₄ species are expected to respond in different ways; C₄ species are expected to show a more positive growth response to increasing temperatures than are C₃ species. Experimental studies globally have found variable effects of warming on plants, but relatively little is known about how warm, semi-arid ecosystems will respond to warming, especially in southern Africa. Many regions of the world are expected to become drier under climate change but the prediction of future rainfall changes has far higher uncertainty than the prediction of future temperatures.

I investigated the effect of warming on plant growth by establishing a simulated climate warming experiment near Middelburg in the Nama Karoo (Eastern Upper Karoo, Mucina and Rutherford 2006), a warm, semi-arid region with vegetation comprised of both C₄ grass and C₃ shrub species. A variety of warming systems have been used for experimental manipulation in the field, but the most commonly used is the open-topped chamber (OTC) system. I designed an experiment using a modified version of an OTC system first employed by Godfree et al (2011) in central NSW Australia, using water-filled PVC pipes, painted black, as a thermal buffer within the chambers.

Six control and six heated plots were set up, and growth of a selected shrub species (*Eriocephalus ericoides* (L.f.) Druce) and grasses was analysed during a single growing season, and up until the end of the dry season. Soil water content and plant water potential measurements were made to determine the effect of warming on the soil and *Eriocephalus ericoides* water balance in treatment plots.

The OTC system successfully raised temperatures during the day and night, with mean daytime increases of 0.89°C and night-time increases of 1.03°C over the treatment period. The addition of top panels during the winter months would likely have attributed to the continued increase of temperatures during these cold months, and the system likely reduced frost incidence and severity in warmed plots. The diurnal warming pattern was more even

during winter than during summer months. There did not appear to be a rain shadow effect due to the chambers, except for one incidence in July.

Warmed *Eriocephalus ericoides* had higher growth rates than control plots when soil moisture content was above 3.55%. Warming had no impact on grass growth, perhaps due to decreased water availability in treatment plots prohibiting the grasses from responding to higher temperatures.

Daily measures of volumetric soil water content revealed significantly lower soil water content in heated plots, likely due to increased evapotranspiration. Values of gravimetric soil water content, measured monthly, differed between treatment and months (water content was lower in warmed plots) while plant water potential sampled at the same times showed no effect of warming treatment but also differed over time. Soil water content and shrub water potential were positively related in a curvilinear relationship best described by a polynomial function that suggested maximum shrub water potential above gravimetric soil moisture levels of 5%.

Overall, both the positive effect of warming on the growth of the C₃ shrub *Eriocephalus ericoides* during the growing season and the lack of response of the C₄ grasses were unexpected. For *Eriocephalus ericoides*, this suggests that the Middelburg site temperatures may be sub-optimal for growth, highlighting the need for better projections regarding the climate change impacts on the woody shrub flora of this region. It is possible that low rainfall prohibited any response to warming in the C₄ grass component, indicating a need for further manipulative experiments including water addition in order to better understand this response. Future warming in this region may well lead to greater ecological success of C₃ shrubs in this region; but if this is combined with increased rainfall in this region, as some models have projected, the response of the C₄ grasses remain uncertain.

The use of a passive OTC system with water-filled PVC pipes as a thermal buffer provided an inexpensive yet effective method of applying warming in the field, with the only suspected experimental artefact due to a reduction in wind speed. Further work in this region could attempt to quantify the effect of this artefact on plant growth, possibly via the use of physical wind-breaks.

Opsomming

Klimaatsverandering is 'n voortdurende bedreiging vir spesie funksie en voortlewing, en eksperimentele studies is 'n belangrike agent om toekomstige gevolge van klimaatsverwarming op die voortlewing en funksionering van plante te verstaan. In warm, droë ekosisteme word dit verwag dat verwarming 'n negatiewe effek op plantegroei sal hê as gevolg van 'n oorskreiding van optimale temperature vir fotosintese en groei. Hierdie effekte sal moontlik nie vir alle plant funksionele tipes realiseer nie. Dit word veral verwag dat C₃ en C₄ spesies nie op dieselfde manier sal reageer nie. Die verwagting is dat C₃ plantegroei nie dieselfde grootte reaksie tot temperatuur stygings as C₄ spesies sal toon nie. Wêreldwye eksperimentele studies vind wisselvallige effekte van verwarming op plante, maar daar is relatief min inligting beskikbaar oor hoe warm, droë ekosisteme sal reageer op klimaatsverwarming, veral in suidelike Afrika. Daar word verwag dat baie streke van die wêreld droër sal word as gevolg van klimaatsverandering, maar die voorspelling van hierdie veranderinge is meer onseker as die voorspelling van toekomstige styging van temperature.

'n Klimaatsverwarming eksperiment is in 'n area van die Nama Karoo met C₄ grasse en C₃ struik opgestel om die effek van verwarming op plantegroei te ondersoek. Vele verwarmingssysteme is al in die verlede gebruik, maar die mees gewilde sisteem is die kas waarvan die bokant nie bedek is nie. Ses verwarnde en ses kontrole gedeeltes is in die veld uitgesit en die groei van die grasse en 'n teiken struik (*Eriocephalus ericoides* (L.f.) Druce) is geanaliseer vir twee maande van die groeiseisoen en vier verdere maande tot aan die einde van die droë seisoen. Grondwaterinhoud- en plantwaterpotensiaalmetings is geneem om die effek van verwarming op die waterbalans van die grond en plante van die eksperimentele terrein te ondersoek. Die omgewing binne die kas is bedags en snags suksesvol verwarm; gemiddelde dag temperature was 0.89°C hoër binne die kaste en gemiddelde nag temperature was 1.03°C hoër binne die kaste regdeur die eksperimentele tydperk. Die bybevoeging van die boonste panele het waarskynlik bygedra tot die aanhoudende verwarming gedurende die kouer maande en het waarskynlik die intensiteit en voorkoms van ryp binne-in die kaste verminder. Die daaglikse patroon van verwarming is meer eweredig gedurende die winter as gedurende die somer, en dit lyk nie asof daar 'n reën-skadu effek is nie, behalwe vir een insident in Julie.

Verwarnde struik het vinniger gegroei wanneer die grondwaterinhoud hoër is as 3.55%, maar die verwarming het geen effek op die groei van die grasse gehad nie, dalk omdat laer

grondwaterinhoud tydens die groeiseisoen die grasse verhinder om op warmer temperature te reageer.

Daaglikse metings van volumetriese grondwaterinhoud het 'n betekenisvolle verskil tussen verwarnde en kontrole gedeeltes getoon, waarskynlik as gevolg van hoër verdamping en transpirasie. Maandelikse metings van grafimetriese grondwaterinhoud het 'n verskil tussen verwarnde en kontrole gedeeltes asook 'n verskil tussen verkillende maande getoon, terwyl maandelikse metings van plantwaterpotensiaal net verskil het tussen maande.

Grondwaterinhoud en plantwaterpotensiaal was positief verwant in 'n kromlynige verhouding wat dui op maksimale plantwaterpotensiaal wanneer grondwaterinhoud waardes hoër is as 5%.

Algeheel was beide die reaksie van die C₃ struik en die gebrek van 'n reaksie van die C₄ grasse onverwags. Die reaksie van *E. ericoides* dui aan dat die groei van hierdie struik gedeeltlik beperk word deur sub-optimale temperature tydens die groeiseisoen en dat meer inligting nodig is om die toekomstige reaksie van houtagtige struik in die Nama Karoo te kan verstaan. Vir die gras komponent dui dit aan dat verdere navorsing nodig is om die verwantskap tussen groei en waterbeskikbaarheid te verstaan.

Toekomstige klimaatsverwarming in hierdie streek kan dalk tot hoër ekologiese sukses van C₃ struik in hierdie omgewing lei, maar as verwarming met verhoogde reënval saamval, is die reaksie van die C₄ grasse 'n belangrike onbekende. Die gebruik van 'n passiewe verwarmingsstelsel met 'n termiese buffer bied 'n lae-tegnologie en goedkoper manier om temperature in die veld te verhoog, met die effek van windskuiling as die enigste vermoede artefak. Verdere studie in hierdie tipe ekosisteem kan lei tot die kwantifisering van hierdie artefak, moontlik deur fisiese windskuilings te gebruik.

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Abbreviations

°C – Degrees Celsius

CO₂ – Carbon Dioxide

IPCC – Intergovernmental Panel on Climate Change

ITEX – International Tundra Experiment

NH – Northern Hemisphere

NPP – Net Primary Productivity

NSW – New South Wales

OTC – Open-topped Chamber

PVC - Polyvinyl Chloride

RCP – Representative Concentration Pathway

SRS – Spectral Reflectance Sensor

WUE – Water Use Efficiency

1. Introduction

1.1 Anthropogenic climate change, projections and uncertainties

Projections of warming due to anthropogenic greenhouse gas emissions point to a rise in global surface temperature of at least 1°C by mid century (even under stringent emissions control and including the implementation of “negative emissions” technology); with a maximum of above 5°C possible over centuries, depending on the emissions pathway followed over the next few decades. This range of scenarios has been most recently been described in so-called Representative Concentration Pathways or RCPs (IPCC 2014; IPCC 2007). Compounding the uncertainty in projected anthropogenic climate change due to emissions pathway, there is also some variation between the main General Circulation Models applied at global and regional scale as to the projected rate of warming, and significant disagreement as to the timing, geographic pattern, and even the sign of rainfall change (IPCC 2014). As a result, anthropogenic climate change continues to be seen as a potentially major threat to the survival of many species, but there is very large uncertainty as to the location, extent and timing of adverse impacts on terrestrial ecosystems (Broennimann, et al. 2006; Cahill et al. 2012; Conway 2008; IPCC 2007; Thomas et al. 2004).

There is almost incontrovertible evidence that the climate of South Africa is changing. Between 1960 and 2003, average yearly temperatures have increased by 0.13°C per decade (Benhin 2006). Analysis of long-term data from the national parks of South Africa has revealed that all of the parks for which sufficient data was available have warmed over the last 50 to 100 years, with only two exceptions (one showing no change and one showing a decrease in temperature) (van Wilgen et al. 2016). While it is clear that warming has already occurred in South Africa, predictions of rainfall patterns are uncertain and there is little evidence of rainfall changes in the historical records (MacKellar et al. 2014). Areas in the south-central regions of the country are expected to see increases between 4 and 5°C by 2100 (van Wilgen et al. 2016).

1.2 Impacts of climate change on natural systems

Climate change has already affected natural systems in measurable ways and impacts are expected to continue and worsen well into the 21st century. Climate change has been shown to have affected plant and animal physiology, phenology, distribution, abundance and

symbiotic interactions between species (Cleland et al. 2007; Hughes 2000; 2002; Luo 2007; Portner & Farrell 2008; Rosenzweig et al. 2008; Turnbull et al.) .

Temperature is one of the major determining factors of plant health and performance, due to its partial control of photosynthesis and respiration and its effect on water use. Temperature is thought to be a strong limiting factor over 33% of the global terrestrial surface (Nemani 2003).

Temperature increases affect the length of the growing season, as has been shown in many areas of the Northern Hemisphere (Hughes 2000; Mccarty 2001; Rötter & Van De Geijn 1999). Increasing night-time temperatures especially have been found to lengthen the growing season (Luo 2007; Turnbull et al. 2002), affecting productivity in many regions.

Models of plant growth show that increased temperature and a longer growing season will lead to greater annual net primary productivity (NPP) (Ollinger et al. 2008). Earlier emergence of spring vegetation is one facet of the increase in growing season length, higher spring temperatures are stimulating earlier bud burst in Northern Hemisphere trees (Bergh et al. 2003; Nemani 2003) and models show that later activity in autumn is expect to further lengthen the growing season (Bergh et al. 2003). This pattern is already apparent, as NPP increased by 6.17% between 1982 and 1999, mainly in the Northern Hemisphere, but also in the tropics (Nemani 2003). Increasing night-time temperatures are leading to a decrease in the diurnal temperature range; as a result, plants are expected to experience greater proportions of time within the range of optimum temperatures for photosynthesis and growth (Kirschbaum 2004; Rötter & Van De Geijn 1999). In ecosystems with a woody component, these two factors may lead to higher annual growth and increased carbon sequestration (Rustad et al., 2001), as has been shown by studies of boreal forests (Piao et al. 2008).

Climate warming affects the distribution and abundance of plants by shifting climatic envelopes, requiring plants to adapt, migrate or become locally extinct. In the Northern Hemisphere, plants have moved, on average, 6.1km North and 6.1m upward in elevation per decade (Thuiller 2007). Abundance of plant species can change locally by shifts in recruitment of seedlings. In arid and semi-arid regions of western United States, seedling survival is strongly determined by their ability to tolerate severe conditions at the soil surface (Loik et al. 2000). Ameliorated conditions can lead to increased recruitment of stronger species, changing local abundance and species composition, as has been found for the only two vascular plant species in Antarctica (Hughes 2000).

Ecosystem warming has had variable effects on photosynthetic rate between studies, ecosystem and species. Increases, decreases and the absence of effect have been reported. In colder systems, warming leads to increased photosynthesis by alleviating cold temperature constraints on various components of photosynthesis (Luo 2007).

C₃ plants show a wide range of optimum temperatures, suggesting that these plants may be able to respond to changes in temperature patterns (Sharkey & Bernacchi 2012), but the effect of warming is greatly dependent on the initial environmental conditions and the local adaptation of the species in question (Luo 2007). Increasing temperatures tend to increase the rate at which oxygenation occurs at the active site of Rubisco in C₃ species (Sharkey & Bernacchi 2012). Increased atmospheric concentration of CO₂ could lessen this effect by increasing the ratio of CO₂:O₂ within the photosynthetic tissue. C₃ plants are also better able to respond to increased CO₂ concentrations at higher temperatures (Chartzoulakis & Psarras 2005) and are thus expected to gain favour from temperature increases and CO₂ fertilisation. C₄ species, on the other hand, are not expected to increase their photosynthetic rates under higher CO₂ concentrations, but will do so under higher temperatures. This response to warming in C₄ species is highly dependent on water availability (Collatz et al. 1998). Warming has been shown to decrease soil water in many studies, and negative responses of photosynthesis are often attributed to inadequate water availability (Rustad et al., 2001).

Plant respiration is also affected by warming, and can increase by a factor of 1.4 to 4.0 under warming of 10°C (Turnbull et al. 2002). Temperature has been shown to increase annual respiration in individual Northern Hemisphere forests, attributed mainly to root and microbial respiration, but no broad-scale pattern was found (Valentini et al. 2000). Photosynthesis and respiration rates will not respond to climate change in equal measures and the carbon balance of ecosystems are expected to change due to this discrepancy (Ryan 1991).

1.3 Experimental efforts to quantify warming impacts

Experimental studies are essential for informing projections of how ecosystems may be affected by, and respond to future climate change (Walker et al. 2006). Hundreds to thousands of experiments have been conducted on plants under both field and laboratory conditions in order to explore the effects of projected warming on individual physiological processes, ecophysiological function, demographic effects, and even the ecosystem impacts (e.g. as summarised in IPCC 2001, IPCC 2007, IPCC 2014). However the vast majority of studies on the risk of climate change are based in the Northern Hemisphere, where low

temperature is perceived to be the primary factor limiting plant growth (Hollister & Webber 2000; Llorens et al. 2002; Llorens et al. 2003; Rustad et al. 2001). This has resulted in a bias in climate change response data, with implications for perceptions of how best to adapt to the changes these may bring to natural systems.

Far fewer studies have explored the effects of warming in southern Hemisphere systems (IPCC 2007), where water is assumed to be the primary limiting factor for plant growth and ecosystem processes, and not temperature (Holmgren et al. 2006; Li et al. 2008). This gap in the literature persists, despite the fact that arid and semi-arid ecosystems comprise a large proportion of global ecosystems, encompassing about one third of the land surface (Schwinning et al. 2004). For arid and semi-arid ecosystems, anthropogenic warming is expected to increase heat and water stress, with likely adverse effects on plant performance and even survival that may or may not be offset by the direct positive effects on leaf-level carbon balance and water-use efficiency due to increasing CO₂ levels. Rather few experimental tests of this hypothesis have been conducted, particularly under field conditions internationally, and almost none in the southern Hemisphere. The results of only a single field warming manipulation in southern Africa has been published (Maphangwa et al. 2012).

There is a common perception that plant species of warm arid and semi-arid ecosystems are pre-adapted to high temperature and drought conditions, and that the effects of increasing temperature on plant growth and survival may be muted (Way & Oren 2010). However, warming may pose a risk to those plants already near their thermal optimum (Cahill et al. 2012; Maphangwa et al. 2012), despite pre-adaptation to high temperatures that are assumed to increase tolerance of increasing temperatures. There is some evidence for southern African deserts to support this view. Maphangwa et al. (2012) found, for example, that miniature leaf succulent species of the Succulent Karoo Biome experienced greatly increased mortality under field warming treatments.

Foden et al (2007) inferred from field observations in the western Succulent and Nama Karoo region, that the observed die-back of *Aloidendron dichotomum* could be attributed to increasing temperatures exceeding thermal limits for individuals in populations near the warm range edge of the species (but see also Jack et al., 2006). Towards the eastern reaches of the Nama-Karoo, Masubelele et al. (2014) have recorded shifts in species composition that include the increase in C4 grass cover and biomass, which could also indicate a response to local warming.

Despite these apparent risks, and the observed trends in climate, there is very little empirical evidence through which the credibility of the projections can be assessed. This research gap must be one of the most striking omissions in research efforts by scientists focusing on projecting anthropogenic climate change impacts in southern Africa.

Due to the difficulty in predicting the effect that climate change will have on precipitation patterns (Bai et al. 2008; IPCC 2014), it is not easy to predict how the water balance of ecosystems will be affected by climate change. However, many authors have predicted overall decreases in water availability due to a warming-induced increase in evaporation from the soil and transpiration from plants. Other authors have found experimental evidence of an increase in water availability under simulated climate change conditions. Theorised explanations for an increase in water availability differ between authors. Zavaleta et al. (2003) proposed that the observed 5-10% increase in spring soil moisture is due to warming-induced acceleration of canopy senescence, which led to a decrease in transpirational water losses. Increases in soil moisture have also been theorised to be due to an increase in plant water use efficiency prompted by higher CO₂ concentrations (Nelson et al. 2004; Reyes-Fox et al. 2014).

1.4 The Nama Karoo

The vegetation of the Nama Karoo is comprised of dwarf shrubs and grasses, and trees are rare (Benhin 2006; Mucina & Rutherford 2006; Muller et al. 2016). The region experiences temperature extremes on both ends of the scale, with minimum temperatures as low as -5°C and maximum temperatures reaching 43°C (mean annual temperature = 14.7°C) (Weatherbase 2017). Air temperatures have been shown to be rising in the Nama Karoo region. Between 1943 and 1958, nine out of 16 years reached temperatures between -12°C and -10°C. In comparison, only three years between 1959 and 2013 reached temperatures this low (Muller et al. 2016). Ecosystem health and performance is economically relevant in this region, as large regions are utilised as rangeland for sheep and goats (Milton & Dean 2010; Palmer & Ainslie 2006). Moderately leached soils are dominant in this part of the Nama Karoo (Cowling et al. 1986).

The Nama Karoo region of South Africa is predicted to warm significantly over the next few decades, but models disagree about the sign of the change in rainfall (Conway 2008; de Wit & Stankiewicz 2006). Both increases and decreases in rainfall have been predicted for this region, resulting in a warmer but hydrologically uncertain future for this warm, semi-arid

region. It is possible that increased rainfall together with warming would lead to an increase in the amount of grass cover in the region, shifting the vegetation structure towards a grassland. Such a shift would be enhanced if the growth of local C₃ shrub species were to be adversely affected by warming. Indeed, increases in grass cover have been noted in many areas of the Nama Karoo biome (Masubelele et al. 2014).

Despite the size of the Nama Karoo (23% of the southern African subregion (Muller et al. 2016; Todd 2006)), very little ecophysiological research has been done on the vegetation of the Nama Karoo and there are even areas of this biome that remain biologically unsampled, making the impact of warming on the Karoo vegetation difficult to predict with any certainty.

1.5 Aims and objectives

Due to the gap in our knowledge of the growth and physiological behaviour of Nama Karoo vegetation and general lack of climate change studies in warm arid regions, we are not in a position to make reliable predictions regarding the future of these extensive ecologically and economically important ecosystems. This study aimed in general to improve the understanding of how such systems may be affected by future climate warming, while at the same time trialling a new and inexpensive method for applying warming in the field.

The study therefore examined in some detail the performance of a modified version of an open-top chamber system first utilised by Godfree et al (2011) in an Australian shrubland ecosystem, with the objective of assessing its efficacy and identifying any possible improvements for future use. Ecophysiologically, the study aimed to quantify the effect of simulated climate warming on the growth, water relation and gas exchange behaviour of *Eriocephalus ericoides* (L.f. Druce), a common C₃ shrub throughout the Nama Karoo, and the production of mixed stands of C₄ grasses.

2. The performance of the OTC warming system

2.1 Abstract

Anthropogenic climate change is increasingly recognised as a threat to plant species ecological performance and even survival under some future climate change scenarios. Manipulative experiments that simulate aspects of future scenarios, such as warming, are crucial to understanding the future effects in ecological settings, but are challenging and expensive to install and implement. Various systems for simulating warming under field conditions have been used in the past three decades, balancing issues of unintended effects (experimental artefacts) with cost and replication. The most commonly used method has been the open-topped chamber (OTC) system, but is criticised commonly for excessive daytime warming and insufficient night-time warming. I designed and implemented a warming experiment at a Nama Karoo site in Middelburg (Eastern Cape), South Africa, using a modified version of an OTC first described by Godfree et al in 2011 in a temperate grassland in New South Wales, Australia. The OTC included water-filled PVC pipes as a thermal buffer around the inner edge of the chambers. This design raised temperatures moderately during both day and night-time, yielding mean daytime increases of 0.89°C (max = 1.37°C) and mean night-time increases of 1.03°C (max = 1.56°C). The addition of detachable top panels during winter months is likely to have contributed to maintaining warming during these cold months, and to reducing frost frequency and severity in warmed plots. The diurnal warming pattern was more even during winter than summer months. There did not seem to be a significant rain shadow effect due to the chambers, except for one small rainfall event in July, which did not wet the soil in the warmed plots and only caused slight wetting in one of the control plots. Effects on wind were not quantified, but are likely to be the most significant artefactual effect of the presence of chambers. A small number of chambers were disarticulated in a single event, probably by a concentrated mini-whirlwind (dust-devil), but apart from this, the chambers did not require any repair or maintenance during the year of their deployment at this windy site. The OTC system described and implemented provided an inexpensive, robust and low-technology way of increasing night-time and daytime temperatures for climate warming studies at this site, and could be relatively easily implemented in similar low-stature plant communities.

2.2 Introduction

Predictions of future warming point to a rise in global surface temperature of at least 2°C (under stringent emission control); with a maximum above 4.8°C over centuries, depending on the emission pathway followed over the next few decades (described as Representative Concentration Pathways) (IPCC 2014; IPCC 2007) and as a result, climate change continues to be a major threat to the survival of many species (Broennimann, et al. 2006; Cahill et al. 2012; Conway 2008; Thomas et al. 2004; Urban 2015). Temperatures appear to be rising in the Nama Karoo region. Between 1943 and 1958, nine out of 16 years reached temperatures between -12°C and -10°C. In comparison, only three years between 1959 and 2013 reached temperatures this low (Muller et al. 2016). There is other evidence that the climate of South Africa is changing. Between 1960 and 2003, average yearly temperatures have increased by 0.13°C per decade (Benhin 2006). Analysis of long-term data from the national parks of South Africa has revealed that all of the parks for which sufficient data was available have warmed over the last 50 to 100 years, with only two exceptions (one showing no change and one showing a decrease in temperature) (van Wilgen et al. 2016).

The continued risk posed by climate change creates a need for experimental studies to assess the effect of climate change on natural systems (Mooney et al. 1991). Ecosystem warming experiments can help by determining the effect of future temperature increases on vegetation around the world.

Many methods have been developed to warm up ecosystems for climate change experiments. These methods can be divided into active and passive systems (Marion et al. 1997). Active systems are those that require an outside energy source for continued warming, such as infrared heaters and heating cables (Aronson & McNulty 2009; Beier et al. 2004). While effective, these systems are difficult to implement in study sites without access to external power. The necessity for power sources such as batteries or solar panels mean that active systems can also quickly become expensive.

Passive warming systems are usually easier to implement and maintain than active systems and can be more cost-effective. Passive warming includes night-time covering of soil and vegetation to preserve infrared radiation (Aronson & McNulty 2009). This method is inexpensive, but requires maintenance or automation to remove the covering during precipitation events and is not practical in ecosystems with high canopies.

Open-top Chambers (OTCs) are commonly used for warming experiments and have long been used in the International Tundra Experiment (Rustad et al., 2001). These systems started being used for heating after researchers struggled to minimize air temperature increases when they used similar open-topped systems for CO₂ enrichment studies (Aronson & McNulty 2009). OTCs differ in shape from simple square or round chambers up to larger hexagonal chambers. Open-top chambers have largely been used in high Northern latitudes but have also been used in temperate ecosystems (Godfree et al. 2011) and even in the Succulent Karoo in South Africa (Maphangwa et al. 2012).

The OTC system used in this experiment was based on a system designed by Godfree, Robertson, Bolger, Carnegie, and Young in 2011 and used in a warming and CO₂ enrichment study in central NSW, Australia. The authors aimed to construct an OTC system which would create stable night-time and daytime warming and decrease the incidence of extreme temperature fluctuations that are sometimes seen with the use of OTCs during the summer months. In order to achieve these aims, Godfree et al. designed an OTC system that included water-filled PVC pipes to act as a thermal mass within the chambers. The inside perimeter of the chambers were lined with 150mm PVC pipes with the sky-facing area painted black. These pipes were expected to act as a thermal mass by absorbing solar energy during the day and releasing it as heat energy during the night. Their design also featured detachable top panels, to be added when temperatures start to drop in the colder months.

Godfree et al. (2011) showed that their system was effective at significantly increasing both daytime and night-time temperatures. They showed that the presence of the thermal mass provided by the PVC pipes increased minimum daily temperatures while an increase in the height of the chambers alone did not. They also found that lowering the height of the chambers during the summer months reduced the occurrence of extreme temperature fluctuations (Godfree et al. 2011).

2.2.1 Aims and objectives

The aim of this chapter was to assess the performance of the warming system and to identify possible improvements for future use. This was done by analysing the extent of warming per month, the difference in minimum and maximum temperatures and the difference in warming pattern of the warmest and coldest months. Possible rain shadow effects were also investigated.

2.3 Methods

2.3.1 Study site

The study was located on Grootfontein (31° 25'S 25° 1'E, elevation 1279m), a research farm situated outside Middelburg, Eastern Cape and operated by Grootfontein Agricultural College. The region is classified as Eastern Upper Karoo (Mucina & Rutherford 2006) and has been suggested to represent an ecotone between the Nama Karoo to the west and the semi-arid grasslands to the east (du Toit & O'Connor 2014), with vegetation mainly comprised of dwarf C3 shrubs and annual and perennial C4 grasses. Middelburg has a mean annual temperature of 14.9°C with a mean low of 6°C and mean high of 22°C (Weatherbase 2017). Mean annual precipitation is 356mm with the most rain falling from January to March. Frost is common in winter and occurs up until October (du Toit & O'Connor 2014).

2.3.2 Experimental setup

Warming was applied by the use of Open-top Chambers (OTCs). These passive ecosystem warming systems have been widely used by scientists for temperature and atmospheric manipulation (de Frenne et al. 2010; Van Oijen et al. 1999). The OTCs used in this study were modified from a system designed by Godfree et al. (2011) which were designed to decrease extreme temperature fluctuations and to produce significant levels of night-time warming. The chambers used by Godfree et al. (2011) were hexagonal in shape and included a CO₂ injection system. The chambers installed at Middelburg were pentagonal in shape with an internal area of 4.91m². Each plot contained one target shrub (*Eriocephalus ericoides* (L.f.) Druce), situated in the middle of the plot. The inside perimeter of the chambers were lined with two rows of water-filled 110mm PVC pipes. The pipes were painted black on the side facing the sky, in order to absorb maximum energy during the day. This design was showed by Godfree et al (2011) to be effective in preventing extreme temperature increases during the summer months and producing night-time warming via the release of this energy during the night.

Six heated plots were set up with six control plots interspersed throughout.

2.3.3 Meteorological and micrometeorological data

Ambient climate data were collected adjacent to the study plots using a Campbell Scientific CR1000 Data Logger. A stainless steel stand supported a Campbell Scientific CS215-L Temperature and Relative Humidity Probe. Four GS1 Soil Moisture Probes were deployed, two in control plots and two in warmed plots, inserted into the soil close to the edge of the

target shrub canopy. Measurements were taken every 10 minutes and an hourly and daily data table was recorded by the data logger. All treatment and control plots contained an iButton (DS-1923L-F5#), situated 15cm above the soil surface and measuring temperature every 15 minutes, data from which the thermal performance of the OTCs was assessed.

2.3.4 Statistical analysis

All statistical analysis was done with R (R Core Team 2012), using RStudio version 3.4.2 and the packages agricolae, ez, lme4 and segmented.

Repeated measures ANOVA's were performed on the iButton data. Tests were run per month on the following parameters: mean monthly temperature, mean day temperature, mean night temperature, mean maximum temperature and mean minimum temperature. Monthly extreme values were also considered.

2.4 Results

Table 2.1. Results of repeated measures ANOVA's to determine the performance of the open-topped chambers. Temperatures are given in Celsius; C = control plots, W = warmed plots, diff = difference between control and warmed plots, positive if warmed plot temperatures are higher. Significantly different results ($p < 0.05$) are printed in bold.

Month	Mean monthly temp			Mean day temp			Mean night temp			Mean min temp			Mean max temp			Coldest min temp			Hottest max temp			
	C	W	Diff	C	W	Diff	C	W	Diff	C	W	Diff	C	W	Diff	C	W	Diff	C	W	Diff	
February	22.75	23.60	0.85	28.23	29.60	1.37	17.32	17.67	0.35	5.13	5.98	0.85	46.13	50.85	4.72	4.54	5.53	0.99	49.01	55.07	6.06	
	$F_{(1, 27)} = 116.36$			$F_{(1, 27)} = 79.73$			$F_{(1, 27)} = 285.40$			$F_{(1, 27)} = 128.86$			$F_{(1, 27)} = 34.72$									
March	21.41	22.04	0.63	29.50	29.88	0.38	13.31	14.20	0.89	1.45	2.17	0.72	50.20	47.77	-2.43	0.53	2.02	1.49	55.50	51.52	-3.98	
	$F_{(1, 30)} = 92.66$			$F_{(1, 30)} = 58.75$			$F_{(1, 30)} = 123.27$			$F_{(1, 30)} = 157.03$			$F_{(1, 30)} = 5.04$									
April	16.67	17.83	1.16	24.08	25.08	1.00	9.26	10.58	1.32	-3.49	-1.04	2.45	42.48	46.47	3.99	-4.49	-2.47	2.02	43.58	48.60	5.02	
	$F_{(1, 29)} = 407.15$			$F_{(1, 29)} = 202.54$			$F_{(1, 29)} = 199.56$			$F_{(1, 29)} = 321.82$			$F_{(1, 29)} = 44.99$									
May	12.73	13.70	0.97	17.55	18.32	0.77	7.90	9.08	1.18	-4.49	-2.35	2.14	34.83	36.09	1.26	-4.99	-2.97	2.2	36.54	38.08	1.54	
	$F_{(1, 30)} = 159.35$			$F_{(1, 30)} = 265.62$			$F_{(1, 30)} = 110.24$			$F_{(1, 30)} = 118.95$			$F_{(1, 30)} = 161.06$									
June	9.83	10.64	0.81	14.16	14.89	0.73	5.50	6.39	0.89	-10.10	-8.07	2.03	26.50	27.90	1.4	-10.51	-9.45	1.02	27.11	28.66	1.55	
	$F_{(1, 29)} = 202.65$			$F_{(1, 29)} = 220.75$			$F_{(1, 29)} = 117.69$			$F_{(1, 29)} = 203.31$			$F_{(1, 29)} = 87.84$									
July	8.27	9.56	1.29	13.70	14.73	1.03	2.86	4.42	1.56	-10.44	-8.37	2.07	25.33	27.40	2.07	-11.04	-8.99	2.05	25.61	28.66	3.05	
	$F_{(1, 31)} = 124.17$			$F_{(1, 31)} = 201.74$			$F_{(1, 31)} = 105.48$			$F_{(1, 31)} = 139.98$			$F_{(1, 31)} = 125.35$									

Air temperatures in warmed plots were always significantly higher than in control plots throughout most of the six month study period. Air temperature increases across all measures (except for daily maximum temperatures) due to the chambers appear to be larger from April, possibly due to the addition of the top panels (not tested due to insufficient data). Daily maximum temperatures were the most erratically affected by the OTCs, with the largest increase in February but an apparently anomalous reversal in March when mean maximum temperatures were higher in control plots than in heated plots. This result can be seen in weeks six to eight in Figure 3.1. Mean night-time warming due to the OTCs tended to be higher than daytime warming.

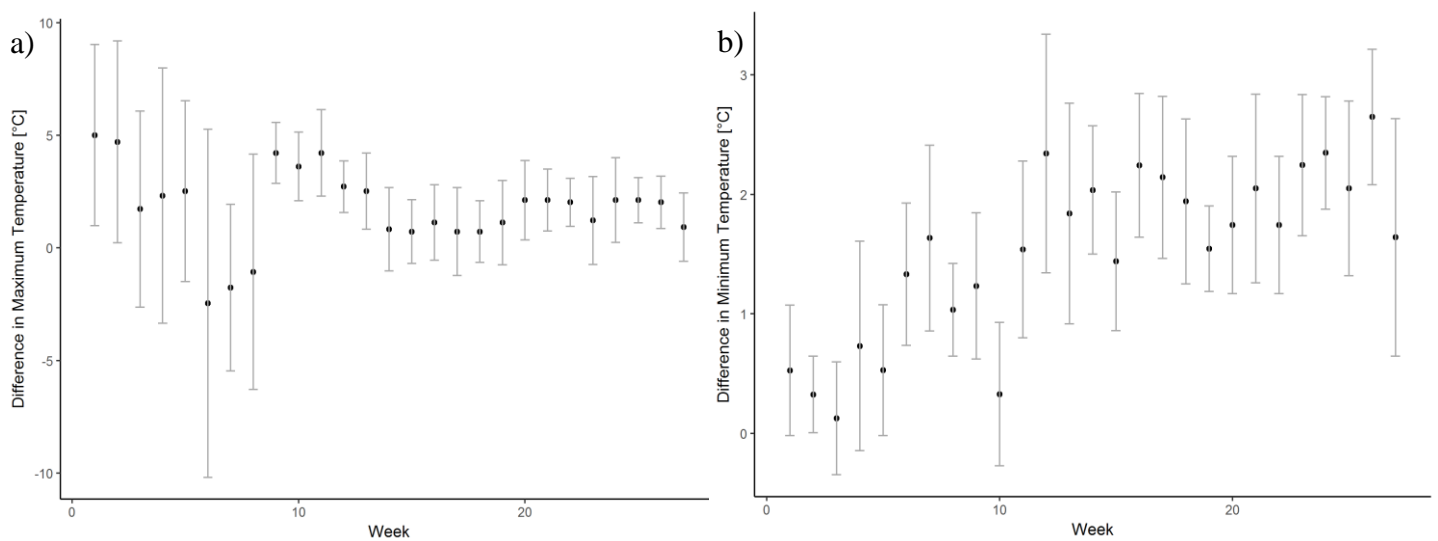


Figure 2.1. Differences in a) Maximum and b) minimum temperatures of control and heated plots averaged per week over a five month study period. Bars denote standard deviations.

The difference between control and heated plot maximum temperatures was more variable during the summer months (February and March, weeks 1-10, Figure 2.1) than during the autumn and winter months (April to July, weeks 11-27, Figure 2.1). Weeks six to eight represent an anomalous period during March when average maximum temperatures of control plots were higher than that of heated plots. The difference in mean minimum temperatures of heated and control plots was lower in the summer months than during the winter.

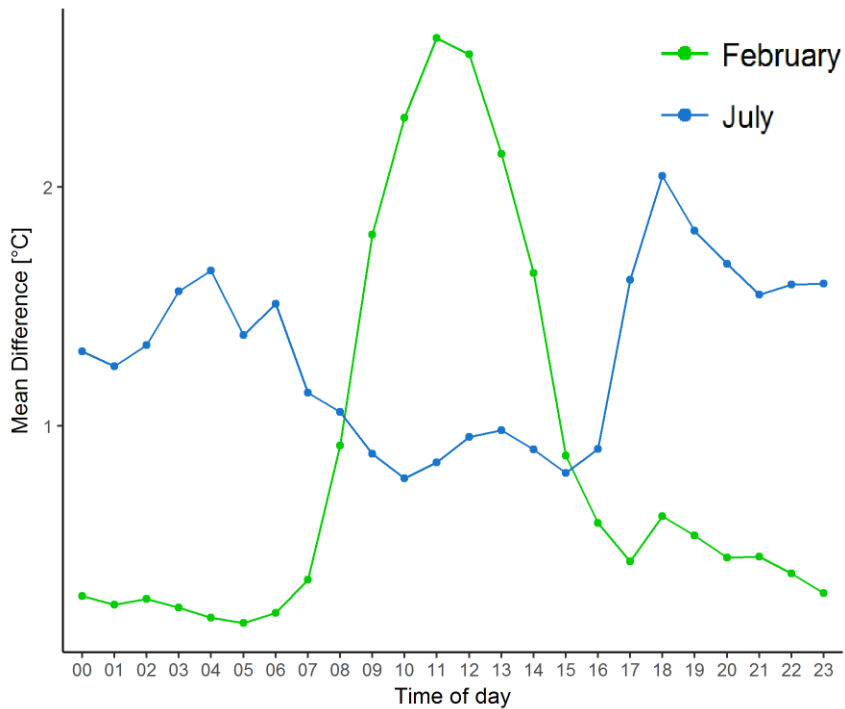


Figure 2.2. Mean hourly difference between control and heated plots during February (green) and July (blue).

Figure 2.2 represents the difference in the diurnal pattern of warming during February and July. Warming during the night and early morning was low during February and the majority of warming occurred during the day, peaking at noon. Warming during July was much less variable. Night-time and early morning warming was much higher than during February. Unlike during February, warming during the day was slightly lower than during the night and early morning.

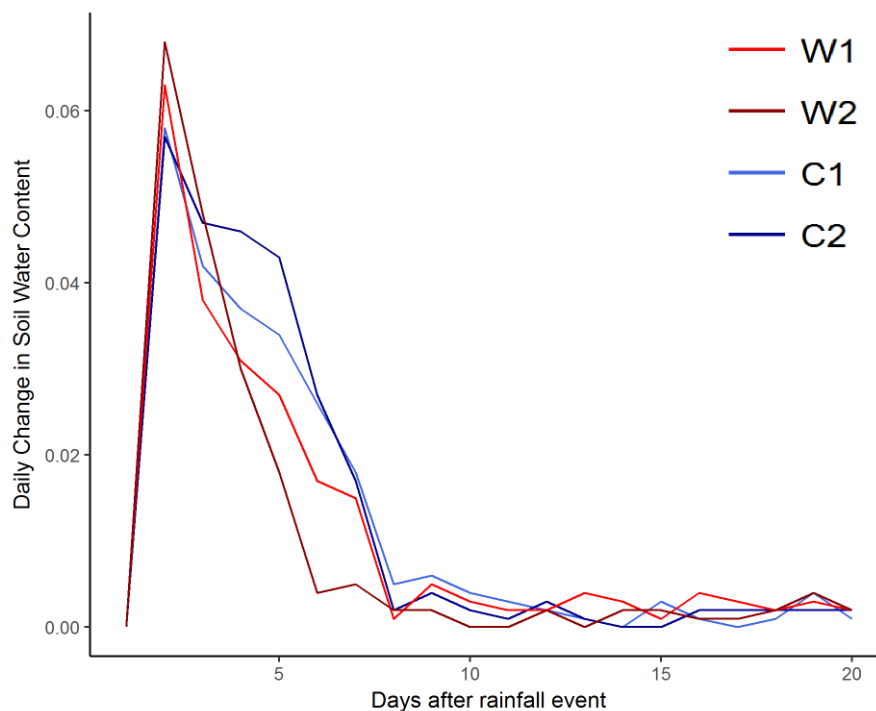


Figure 2.3. Daily loss of volumetric soil water content after a rainfall event in two control and two warmed plots. This dataset represents a 30.9mm event over three days in early March 2017.

The chambers appear to have had a slight effect on the daily loss of soil moisture content via evapotranspiration during the summer (Figure 2.3). Warmed plots experienced a slightly greater increase in soil moisture content directly after the rainfall event but soil moisture content decreased faster in the two warmed plots than in the two control plots (Figure 2.3).

The OTCs did not appear to have a significant effect on the amount of rainfall reaching the soil within the chambers, as judged by the immediate response of soil moisture content after isolated rainfall events (Figure 2.4). After a heavy 20mm rainfall event in late March, control and warming plots experienced the same amplitude of soil moisture increase (Figure 2.4a), but also note the mixed control/warming response during mid-April, assumed to be a smaller rainfall event which fell at the study site but did not register at the rainfall collection site. A small 6.5mm rainfall event only increased soil moisture in the two control plots (one significantly more than the other), suggesting that light rainfall events may not wet the surface soil of warmed plots.

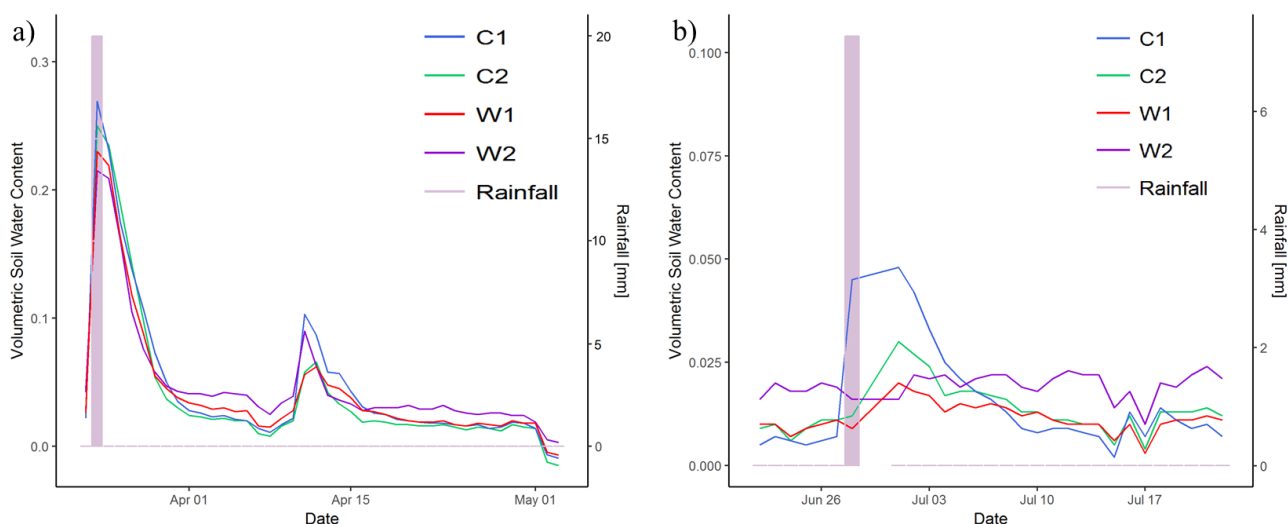


Figure 2.4. Volumetric soil moisture content during a) late March to early May and b) mid-June to end July of two heated (W1 and W2) and two control (C1 and C2) plots after isolated rainfall events.

2.5 Discussion

As expected, the open-top chambers generated significant warming, with control and heated plots differing by a global mean of 0.95°C (ie. Differencing data for all chambers from the entire six-month study period). This is similar levels of warming as those achieved by Godfree et al (2011).

2.5.1 Night-time warming

Active ecosystem heating systems might easily be used to increase minimum temperatures, as systems like infrared heaters can be set to produce stable levels of heating above ambient. However, financially and logistically, it is not always possible to use active warming systems for climate warming studies; leading to the need for passive systems capable of producing night-time warming, which remains the most significant challenge for passive systems.

Increasing night time temperatures was a core aim of the system designed by Godfree et al. (2011), as most previously used OTC systems generated minimum temperatures similar to or even lower than control temperatures (Aronson & McNulty 2009; Marion et al. 1997). This is a clear drawback, as long-term observational data show that minimum temperatures are increasing with global warming at a greater rate than maximum temperatures (Alward 1999; Aronson & McNulty 2009; Beier et al. 2004; Braganza et al. 2004; Karl et al. 1993; Kirschbaum 2004; Llorens et al. 2002; Llorens et al. 2003; Turnbull et al. 2002). Night-time

temperatures could be important for growth and respiration (Cheesman & Winter 2013). Therefore, climate change studies aiming to simulate future climate warming need to take into account the importance of increasing minimum temperatures and night-time temperatures as well as daytime temperatures.

Comparisons of differences between control and heated plots showed that night-time warming was greatest during July (1.56°C mean difference between control and heated plots). Results show that the extreme minimum temperature for July was -11.04°C for control plots and -8.99°C for heated plots. Mean minimum temperatures for July also showed significant warming caused by the addition of thermal mass. Control plots showed a mean minimum temperature of -10.44°C and heated plots showed a mean minimum temperature of -8.35°C. This is similar to the results of Godfree et al. (2011), who found that the addition of thermal mass significantly increased mean nightly temperatures and extreme minimum temperatures; and decreased the duration and severity of frost events. A decrease in frost duration and severity is likely in this study, though no frost data were collected and thus this system would be useful in studies examining the effect of frost reduction in similar settings.

February showed the lowest night-time temperature change (0.35°C mean difference between control and heated plots). Warm night-time temperatures (average night-time control temperature = 17.32°C) may preclude the release of enough energy from the thermal mass to increase the night-time temperature to a greater degree.

Mean night-time temperature increase was 1.03°C, similar to the result Godfree et al (2011) produced, which was between 0.8 and 0.9°C night-time warming. The greatest night-time increase was found in July, in mid-winter; unlike Godfree et al (2011), who found the greatest increase during spring and summer months.

2.5.2 Daytime warming

February showed the greatest increase in daytime temperature (1.37°C mean difference between control and heated plots). Extreme daytime temperatures were avoided; the mean maximum increase in temperature during February was 6.06°C. Excessive heat energy was thus absorbed by the thermal mass, and the chambers were also likely able to vent excess heat as the opening of the chamber is large without the addition of the top panels.

The second greatest increase in daytime temperature was seen in July (1.03°C mean difference between control and heated plots). Combined with the night values, July showed the greatest overall warming (1.29°C mean difference).

March showed the smallest effect on daytime temperatures (0.38°C mean difference). March is anomalous to other months in this respect, as there are three weeks in March where maximum temperatures of control plots are shown to be higher than that of heated plots. The reason for this is unclear, and careful data checking indicates that this is not an error in the data collection or analysis. Total daily radiation decreases during this time, but the lowered values continue after heated plot temperatures rise above control plots, and is unlikely to be the reason for this anomaly.

Godfree et al (2011), found daytime temperature increases of between 0.5 and 2°C throughout their study and all months in current study fall within this range, except for March.

Temperature increases of OTC systems are variable between ecosystems and designs. Warming experiments in alpine areas produced temperature differences between 1.5 to 2.3°C (Kudo & Suzuki 2003), but the chambers deployed were much smaller than the chambers used in this study and are thus expected to produce greater levels of heating. Daytime temperatures of a 3m high OTC system were between 1 and 2°C higher in heated plots (Kuster et al. 2012), similar to the temperature differences produced by this system. Grassland warming in Colorado produced mean difference of 2.6°C in chambers covering an area of 15.5m² (Nelson et al. 2004). Open-top chambers used in polar and alpine sites show a range between -0.9 and 2.1°C temperature difference (Bokhorst et al. 2013).

What is clear about the design of these OTCs is that the size and presence of a thermal buffer decreased extreme temperature fluctuations and produced greater night-time warming when compared with a previous Karoo-based study by Musil et al (2005). Musil et al (2005) deployed smaller hexagonal OTCs (0.46m in height, 1.2m in basal length and 0.72m apical length) to produce lethal levels of warming for dwarf succulent plant species. The OTCs produced maximum daily increases of 5.5°C and night-time warming of less than 0.5°C.

2.5.3 Seasonal differences in warming

The diurnal pattern of warming differed between the warmest (February) and the coldest (July) months of the study period, confirming the third hypothesis. Warming during February

was uneven; most of the warming occurred at midday, with low levels of warming during the night and early morning. The thermal mass did absorb some heat energy during the day, which was then radiated out during the night. The diurnal pattern of warming was much more stable during July. Daytime warming was slightly lower than night-time warming during July and there were no extreme increases in temperature like the midday increases in February.

2.5.4 Detachable top panels

Active warming methods can be used to achieve consistent warming over seasons, as programming can be used to ensure heated temperatures that are higher than ambient temperatures by a specified amount (Kimball & Conley 2009). Consistent temperature increases are more difficult to produce in passive systems where temperature cannot be controlled mechanistically. This is particularly relevant in ecosystems with seasonal variability of radiant energy, as this change in energy input must be managed in some way to maintain warming. The use of additional panels attached to the top of an OTC was suggested by Godfree et al (2011) as a low maintenance and inexpensive solution and for this study, additional top panels were added on the 27th of April 2017, at the end of the autumn season.

The detachable top panels featured in this OTC design theoretically allows for additional levels of warming during the winter months by increasing the height of the chamber and decreasing the size of the chamber aperture. The data collected strongly suggest that the chambers continued to successfully heat the inside environment throughout the winter months, it is difficult to contribute this to the addition of the top panels. Analysis of the air temperature for two week periods before and after the addition of the top panels showed no discernible increase in the degree of warming. This agrees with the results from Godfree et al (2011), who found that the addition of the PVC thermal buffers increased the degree of warming; the addition of the top panels did not. Nevertheless, while there was no detectable effect of the addition of the top panels in the first two weeks, it is possible that the presence of the top panels may have contributed to continued warming during the winter months, as theorised by Godfree et al (2011), especially as the level of warming was higher during the winter months than in the summer months and the diurnal warming pattern was different, with much more consistent levels of warming during the winter month (July) than the summer month (February). Due to the small number of chambers deployed in this study, it is not possible to empirically test the effect of the addition of the top panels. Further study of chambers with and without panels would elucidate the effect of the addition of the top panels.



Figure 2.5. OTCs with and without the detachable top panels.

2.5.5 Rain shadow effect

In general, the chambers did not appear to affect the amount of rain received by the heated plots, as the peak in soil moisture content is similar for all four GS1 plots after rainfall events, as seen in Figure 3.3 and Figure 3.4a. Figure 3.4b points to a possible rain shadow effect, as only one of the control plots (C1) experienced an increase in soil moisture content after a precipitation event in June. This may have been due to strong winds on the day, causing some of the rainfall to be lost against the sides of the chamber, but the effect is unclear, as one of the unchambered plots only experienced a slight increase in soil water content. The fourth hypothesis is therefore not completely resolved, as there was one incidence of possible rain shadow effect, but there is no general pattern.

2.5.6 Structural integrity

The OTC panels were attached using cable ties and were tied to the ground by ropes on two corners facing the prevailing wind direction. One of the OTCs collapsed during April and was reconstructed on the site visit at the end of the month. It is likely that the cable ties holding the chamber together were broken by the force of a whirlwind after being weakened in the sun. Some of the other chambers had broken cable ties but were still standing and only needed to be strengthened again. The addition of the top panels did not make it more likely for the chamber to suffer wind-induced stress and the extra weight helped to ground the chambers more effectively.

2.5.7 Improvements and suggestions for further study

The deployment of the OTCs in this study identified several potential improvements which could be made for future study and highlighted the need for further measurements to better quantify artefacts of the chambers themselves.

With regard to structural integrity, the OTCs could be strengthened by using mechanisms other than cable ties, which proved to be highly susceptible to UV damage. While this may be more time-consuming and expensive, is a necessity to ensure structurally sound chambers, especially on a remote site that is visited only monthly. Restraining cables could be attached to all corners of the OTCs and if necessary, the chambers could also be anchored to the ground using stronger anchoring systems.

It would be valuable to increase the number of chambers to better investigate the separate and combined effects of the PVC thermal masses and addition of the top panels in different seasons. Ideally, multiple chambers would be installed, with and without PVC pipes and with and without top panels; to determine the effect of these two factors on temperature increases. This would add to the performance data for this OTC system in a different biome and climate zone. and supplement the studies done by Godfree et al (2011) on the performance in NSW, Australia. Testing could be done to determine whether different sizes of thermal masses will result in greater effects on daytime and night-time warming.

Unlike Godfree et al (2011), I did not assess the effect of the OTCs on soil temperature, which may be a useful tool for future studies on the effect of warming in soil respiration and other soil responses to climate warming.

Humidity and wind speed sampling could be useful to assess the effect of the chambers on atmospheric conditions relevant to transpiration, as well as the effect on wind stress.

Monitoring of light levels inside the chambers would also be useful to assess the effect of the chambers on the quantity and quality of light reaching the vegetation.

2.6 Conclusions

The OTC system used in this study, a modified version of that described by Godfree et al (2011), proved to be an effective method for simulating future climate warming over the next few decades. The use of water-filled PVC pipes as thermal mass was effective in producing night-time warming and in reducing extreme temperatures during the summer months. The use of water-filled PVC pipes painted black and placed around the inside perimeter of the

chambers as a thermal mass was effective in producing significant night-time warming and reducing extreme daytime temperature fluctuations during the summer months. This approach allows investigator the option of a simple, cost-effective manipulation of temperature more representative of future climate changes. At the same time, this approach also avoids the need for a power source and complex feedback control mechanisms that are necessary in active warming systems.

The addition of the top panels did not appear to have a significant immediate effect on the degree of warming after installation but may have contributed to sustained warming throughout the winter months. There were large differences in the daily pattern of warming between winter (July) and summer (February) months, with large fluctuations during February and a more even spread of warming during July. Further study on the performance of this system in the field, especially the monitoring of ecological and ecophysiological variables, would be valuable for the continued development of this system. Experimentation with an array of chambers, with and without PVC thermal buffers and top panels, could allow investigators to set up combinations of temperature treatments in the field, such as separate daytime and night-time warming experiments.

Warming produced by the current OTC system closely resembled that of the system it was based on, as described by Godfree et al (2011) and was a marked improvement on an OTC system used in the Succulent Karoo by Musil et al (2005).

3. The effect of experimental warming under field conditions on the growth and ecophysiology of selected elements of a Nama Karoo shrub-grass community

3.1 Abstract

In warm, semi-arid ecosystems, increasing temperatures are generally expected to have detrimental effects on plant growth and survival due to exceedance of optimum temperatures. C₃ and C₄ species are expected to respond in different ways; C₃ species are expected to show more adverse effects on growth under warming than are C₄ species. Experimental studies have found variable effects of warming on plants, but little is known empirically about how warm, semi-arid ecosystems will respond to warming. We investigated this question by establishing a warming experiment in the Nama Karoo, a region comprised of mixed communities of C₄ grasses and C₃ shrubs. Six control and six heated plots were set up and the growth of a selected shrub species common throughout the Nama Karoo (*Eriocephalus ericoides* (L.f.) Druce) and grass stands was analysed during the growing season and up until the end of the apparently dormant season. Surprisingly, warmed shrubs had higher growth rates than those in control plots during the growing season (when soil moisture content as above 3.55%) but warming had no impact on grass growth at any time, regardless of water content. The C₃ shrub showed high tolerance of water stress, maintaining appreciably high rates of photosynthesis even during the dry season. This suggests that growth of one of the dominant shrubs in this region is partially limited by sub-optimal temperatures during the growing season, which is unexpected. Overall, neither moderate temperature increases nor moderate water availability decreases were sufficient to reduce the growth of this C₃ shrub, while the C₄ grass component is likely limited by reduced soil moisture availability during the growing season.

3.2 Introduction

Predictions of future warming point to a rise in global surface temperature of at least 2°C (even under stringent emission control); with a maximum above 4.8°C over centuries, depending on the emission pathway followed over the next few decades (described as Representative Concentration Pathways) (IPCC 2014; IPCC 2007) and as a result, climate change continues to be a major threat to the survival of many species (Broennimann, et al. 2006; Cahill et al. 2012; Conway 2008; Thomas et al. 2004; Urban 2015). The Nama Karoo region of South Africa is predicted to warm and to experience a decrease in precipitation (Conway 2008; de Wit & Stankiewicz 2006), resulting in a warmer and drier climate in what is already a hot, arid area with few non-perennial streams. Other models predict an increase in precipitation for central South Africa (Engelbrecht et al. 2011), which may lead to an alleviation of droughts in this arid region. Temperatures appear to be rising in the Nama Karoo region; between 1943 and 1958, nine out of 16 years reached temperatures between -12°C and -10°C. In comparison, only three years between 1959 and 2013 reached temperatures this low (Muller et al. 2016). There is other evidence that the climate of South Africa is changing. Between 1960 and 2003, average yearly temperatures have increased by 0.13°C per decade (Benhin 2006). Analysis of long-term data from the national parks of South Africa has revealed that all of the parks for which sufficient data was available have warmed over the last 50 to 100 years, with only two exceptions (one showing no change and one showing a decrease in temperature) (van Wilgen et al. 2016).

Species are expected to face mortality due to the exceedance of thermal tolerances (Cahill et al. 2012). Changes in species interactions appears to be another major cause of extinction due to climate change, especially changes in food availability (Cahill et al. 2012). The interaction of multiple climate change threats is likely to commit many species to extinction in the future (C D Thomas et al. 2004). Despite the danger of mortality, warming has been shown to increase growth in many ecosystems by lengthening the growing season and alleviating cold-temperature repression on photosynthesis and growth (Berry & Bjorkman 1980; Hughes 2000; Kirschbaum 2004; Mccarty 2001; Rötter & Van De Geijn 1999), a trend already apparent as NPP increased by 6.17% between 1982 and 1999, mainly in the Northern Hemisphere, but also in the tropics (Nemani 2003).

The Nama Karoo region is already a hot and dry region with great annual temperature variation (du Toit & O'Connor 2014; Weatherbase 2017); warming may pose a danger to

species survival in the summer, but may prove to be an advantage during the winter. The vegetation of this region is comprised mainly of C₄ grasses and C₃ dwarf shrubs (Benhin 2006; Mucina & Rutherford 2006; Muller et al. 2016). These two groups of species are thought to respond differently to climate change; grasses may gain benefit from increased temperatures while the shrubs are expected to better respond to increasing CO₂ concentrations (Collatz et al. 1998; Hoepfner & Dukes 2012). In the short term, the abundance of C₄ species is expected to increase in response to warming, but this effect will be dependent on the availability of water (Collatz et al. 1998).

Warming experiments have been established in many ecosystems around the world to determine how plants will respond to future warming and have revealed that warming temperatures are expected to affect many facets of plant form and function directly and indirectly. Northern Hemisphere warming experiments have demonstrated that warming affects plants in two ways; directly by increasing biomass during a growing season through increasing photosynthetic rates but also indirectly by lengthening the growing season and alleviating gas exchange limitation at cooler temperatures (Hughes 2000; Mccarty 2001; Reyes-Fox et al. 2014; Rötter & Van De Geijn 1999), with increasing night-time temperatures especially having been found to lengthen the growing season (Luo 2007; Turnbull et al. 2002), affecting productivity in these regions.

3.2.1 Growing season effects

Models of plant growth show that together, increased temperature and a longer growing season will lead to greater annual net primary productivity (NPP) (Ollinger et al. 2008). Warming in Northern Hemisphere ecosystems have led to earlier emergence of spring vegetation and higher spring temperatures are stimulating earlier bud burst in NH trees (Bergh et al. 2003; Nemani 2003). Similarly, models predict that later activity in autumn is expected to lengthen the growing season further (Bergh et al. 2003), with these effects potentially already occurring, as NPP increased by 6.17% between 1982 and 1999, mainly in the NH, but also in the tropics (Nemani 2003).

3.2.2 Effects on growth

Along with longer growing seasons, in some ecosystems it is predicted that plants may spend more time within the range of optimum temperature for photosynthesis and growth (Kirschbaum 2004; Rötter & Van De Geijn 1999), leading to annual growth and increased carbon sequestration (Rustad et al. 2001). However; higher temperatures in already hot, arid

environments may cause plants to experience temperatures above their thermal optimum more often, decreasing daily photosynthetic gains (Maphangwa et al. 2012; Maphangwa et al. 2013).

3.2.3 Seedling survival

Warming has led to an increase in the abundance of the only two vascular plant species in Antarctica, thought to be through an increase in seedling survival (Hughes 2000) and recruitment in the arid and semi-arid regions of the western United States is often limited by the ability of seedlings to survive harsh conditions at the soil surface (Loik et al. 2000). Most of the shrubs in this region of the Nama Karoo are thought to be long-lived (50 to over 100 years) (J du Toit, pers comm), with few small shrubs present. This indicates that extreme winter conditions may limit seedling survival in this region.

3.2.4 C₃ and C₄ species

Warming is expected to affect C₃ and C₄ species differently and may lead to changes in community composition due to these differences (Collatz et al. 1998). Old-field experiments at the University of Massachusetts found increased expansion of C₄ species in relation to C₃ species after a warm and dry summer. The same pattern has been seen in other warming experiments in grasslands (Hoepfner & Dukes 2012). C₄ plants are expected to increase in abundance under warmer temperatures, as they generally have higher optimum temperatures for photosynthesis and are better able to respond to temperature increases at current levels of atmospheric CO₂ (Berry & Bjorkman 1980; Körner 2006). Increased atmospheric CO₂ concentrations are expected to favour the growth and expansion of C₃ species instead. C₃ photosynthesis outperforms that of C₄ at lower temperatures but performance evens out between 25 and 30°C, above which C₄ photosynthesis becomes superior (Berry & Bjorkman 1980). The balance of C₃ and C₄ species within an ecosystem may thus be partially dependent on performance enhancement during different seasons.

3.2.5 Photosynthetic effects

Temperature effect on photosynthesis is a combination of changes in the underlying biochemical and biophysical components of photosynthesis (Berry & Bjorkman 1980). Studies have found contrasting results when studying the effect of temperature on photosynthesis, exhibiting decreasing as well as increasing photosynthetic rates while others found no effect of temperature (Llorens et al. 2003). C₃ species show wide ranges of optimal temperature for photosynthetic activity, suggesting that these species may be able to adapt to

changes in temperature (Sharkey & Bernacchi 2012; Yamori et al. 2014). C₃ plants are expected to be better able to respond to increased CO₂ concentrations at higher temperatures (Chartzoulakis & Psarras 2005).

Studies have shown that species exhibit different effects of warming on photosynthetic rate, stomatal conductance and transpiration rates (Bunce 2000; Leffler et al. 2016; Llorens et al. 2004; Luo 2007). Growing conditions and local adaptation will partially determine how plants will respond to temperature changes (Bunce 2000; Luo 2007); species grown in cool conditions can show decreased photosynthetic rates at higher temperatures while other species are able to photosynthesise effectively at temperatures up to 50°C (Berry & Bjorkman 1980; Kirschbaum 2004). However, extreme cases have shown optimal tree growth at temperatures up to 9°C higher than temperatures from which species originate (Way & Oren 2010). Results like these indicate that generalised predictions cannot be made across ecosystems or species.

Water availability will also influence the relationship between growth, photosynthesis and temperature. Insufficient water availability is often cited as a reason for a negative response or a lack of temperature response of growth and photosynthesis (Riedo et al. 1999; Rustad et al. 2001).

Warming may increase stomatal conductance and transpiration, depending on the hydrological strategy of the species and the water availability of the habitat. Increasing temperatures may cause plants to increase transpiration rates in order to cool down, but doing so may lead to dangerously low internal water potentials if there is not sufficient water available from the soil (Roman et al. 2015; Skelton et al. 2015). Changes in precipitation will also have an effect on the temperature dependence of gas exchange. Some models predict increases in precipitation over the Nama Karoo ecosystem (Engelbrecht et al. 2011), which may allow plants to increase stomatal conductance while maintaining necessary internal water potentials.

Interactions with other environmental factors should be kept in mind when studying the temperature response of photosynthesis (Mikkelsen et al. 2008). Increasing CO₂ was shown to improve the ability of a cool-adapted species to positively respond to temperature increases (Kirschbaum 2004). Higher levels of atmospheric CO₂ could allow plants to conserve water by decreasing their stomatal aperture, lowering their transpiration rate (Berry & Bjorkman

1980). This could be especially important in arid and semi-arid ecosystems where plants may be conditioned to closing their stomata to conserve water.

Due to the gap in our knowledge of the Nama Karoo biome and general lack of climate change studies in warm arid regions, we are not in a position to make meaningful predictions regarding the future of these ecosystems. This study aims to improve our understanding of how such systems may be affected by future climate warming, which may be increasingly pertinent in economically important regions such as the Nama Karoo.

3.2.6 Aims and objectives

The aims of the work described in this chapter were to determine the effects of warming on the growth and ecophysiology of the C₃ shrub *Eriocephalus ericoides* L.f. (Druce) and the aboveground biomass production of co-occurring stands C₄ grasses at an experimental field site at Grootfontein, Middelburg. Ecophysiological performance was tested by assessing diurnal patterns of photosynthetic rates of shrubs and seasonal differences of photosynthesis. The objectives of this chapter are to quantify the effect of experimental warming on the growth and ecophysiology in order to better understand how future anthropogenic climate warming could shift the relative performance of these two co-occurring plant functional types.

3.3 Methods

3.3.1 Study site

The study took place on Grootfontein (31° 25'S 25° 1'E, elevation 1279m), a farm situated outside Middelburg, Eastern Cape and operated by Grootfontein Agricultural College. The region is classified as Eastern Upper Karoo (Mucina & Rutherford 2006) and is thought to represent an ecotone between the Nama Karoo to the west and the semi-arid grasslands to the east (du Toit & O'Connor 2014), with vegetation mainly comprised of dwarf C₃ shrubs and annual and perennial C₄ grasses (Benhin 2006; Mucina & Rutherford 2006; Muller et al. 2016). Middelburg has a mean annual temperature of 14.9°C with a mean low of 6°C and mean high of 22°C. Mean annual precipitation is 356mm with the most rain falling between January and March (Weatherbase 2017). Frost is common in winter and occurs up until October (du Toit & O'Connor 2014).

3.3.2 Open-Top Chamber system

Warming was achieved by the use of Open-top Chambers (OTCs). These passive ecosystem warming systems have been widely used by scientists for temperature and atmospheric manipulation (de Frenne et al. 2010; Van Oijen et al. 1999). The OTCs used in this study were based on a system designed by Godfree et al. (2011) which aimed to decrease extreme temperature fluctuations and to produce significant levels of night-time warming (see Chapter 2 for details on the system designed by Godfree et al (2011)). The chambers were pentagonal in shape with an internal area of 4.91m². The inside perimeter of the chambers were lined with two rows of water-filled 110mm PVC pipes, to act as a thermal buffer, producing night-time warming and reducing the occurrence of extreme daytime temperature fluctuations during the summer months. The pipes are painted black on the side facing the sky, in order to absorb maximum energy during the day.

The experiment consisted of six heated and six control plots, each with a single *E. ericoides* individual positioned in the middle of the plot. In the few cases where other conspecific shrubs close to the size of the target shrub were present, these were removed before the start of the experiment.

3.3.3 Meteorological and micrometeorological data

Ambient climate data were collected adjacent to the study plots using a Campbell Scientific CR1000 Data Logger. A stainless steel stand supported a Campbell Scientific CS215-L Temperature and Relative Humidity Probe. Four GS1 Soil Moisture Probes were deployed, two in control plots and two in warmed plots, inserted into the soil close to the edge of the target shrub canopy. Measurements were taken every 10 minutes and an hourly and daily data table was recorded by the data logger. All treatment and control plots contained an iButton (DS-1923L-F5#), situated 15cm above the soil surface and measuring temperature every 15 minutes, data from which the thermal performance of the OTCs was assessed.

3.3.4 Growth measurements

In each plot, three shoots of the target shrub, *E. ericoides* were marked at the onset of the experiment. Using digital callipers, the width of the main stem of the marked shoots was recorded. The lengths of all the branches on the marked shoots were measured using the same callipers. Initial measurements were made before the installation of the warming chambers. These measurements were repeated at the end of every month included in the study period (Feb – June 2107).

In order to relate measured shoot lengths to biomass, a harvest of 30 terminal branches of randomly selected conspecific shrubs (adjacent to the study site) were taken to determine the relationship between length and biomass. Branch length and diameter was measured after which the branches were dried at 65° for 24 hours. Dry weights of the shoots were obtained using an electronic scale accurate to 0.001g. A linear regression was performed to determine the coefficients of a linear equation to calculate dry weight from length and diameter measurements, with length being sufficiently adequate as predictor (result $R_{(1,28)} = 0.45$, $p < 0.001$), according to the equation $W = 0.0010941L - 0.023293$, where W is the dry biomass and L is the length. Using this relationship, patterns of shrub growth could be expressed as biomass gain.

A 0.35 by 0.35m grass plot was demarcated in each plot before installation of the warming chambers. An initial harvest within each plot was done on 30th of January 2017. The grass was dried at 64°C for 24 hours and weighed on an electronic scale sensitive to 0.0001g. Repeat harvests were carried out on the same plots every month to measure the rate of grass regrowth, and harvests of green grass growth were done at the end of February and at the end of March, after which there was no new green growth on subsequent visits. Biomass gain was expressed in $\text{g}\cdot\text{plot}^{-1}$ (1.225m^2).

3.3.5 Diurnal photosynthetic measurements

Diurnal photosynthetic measurements were done using a Li-Cor 6400XT Portable Photosynthesis System. Daily patterns of photosynthesis were recorded at the end of every month of the study period commencing on 30 January 2017. During each site visit, a diurnal pattern of photosynthetic rate was recorded for two shoots of each shrub, recording five data points per shoot. Photosynthetic measures were taken every two hours, starting at 07:00 and ending at 17:00. To ensure comparable photosynthetic values, CO₂ concentration was set at 400ppm, photosynthetic flux density at $1500\text{m}^{-2}\cdot\text{s}^{-1}$ and flow rate at $300\ \mu\text{mol}\cdot\text{s}^{-1}$. Temperature was not controlled and followed the diurnal and seasonal course. The shoots were clipped at the end of the day to measure leaf area, scanning the leaves of the shoot and using ImageJ to determine the area in cm^2 . These area values were then entered into the data sheet to convert the measured rates to an estimate of projected leaf area, because of the very small size of the leaves and their tightly clustered phyllotaxy.

3.3.6 Water content measurements

Soil water content was measured during every site visit. Soil water content was sampled by taking a scoop sample of the first 7cm of soil, and placing it in a sealed plastic bag prior to weighing on a four-point electronic balance. The sample was then dried at 110°C for 24 hours after which it was weighed again. The gravimetric water content of the soil was measured as the difference between the wet weight of the soil and the dry weight of the soil, divided by the dry weight, in percentage terms. Continuous volumetric soil water measurements were also obtained from four GS1 Soil Moisture Sensors (Decagon Devices). Two of these sensors were placed in heated plots and two in control plots, being inserted into the surface soil layer near the canopy edge of the target shrubs. These sensors were logged once per hour and also recorded daily maximum values. The data from two of these sensors had to be corrected by setting zero at the lowest recorded negative value and applying this delta to all the data. The sensor in plot W2 (heated plot) was corrected by +0.041 g.m⁻³ and the sensor for plot C1 (control plot) was corrected by +0.003 g.m⁻³.

To determine the number of days for which control and warmed plots experienced soil moisture levels above the threshold necessary for growth, volumetric soil water content was converted to gravimetric soil water content using the intercept and gradient of a regression (result $R_{(1, 18)} = 0.88$, $p < 0.001$). The resulting equation is $\text{gravi} = 28.9429\text{volu} + 1.3292$, where *gravi* is gravimetric soil water content and *volu* is volumetric soil water content.

4.3.7 Statistical analysis

All statistical analysis was performed in R (R Core Team 2012) using RStudio version 3.4.2 and the packages *agricolae*, *AICmodavg*, *ez*, *lme4* and *segmented*.

The effect of warming on plant growth was tested by performing a repeated measures ANOVA, testing the effects of treatment, month and the interaction of treatment and month on the biomass gain of *E. ericoides*. The same was done for grass community growth.

To test the mechanisms of the warming effect on shrub growth, biomass gain was analysed as a function of soil moisture content. A piecewise regression was used to determine if there was a minimum required level of soil moisture for shrub growth. The effect of temperature on shrub growth was then tested by performing a regression on the subset of data when soil moisture was sufficient for shrub growth. Data from the GS1 Soil Moisture Sensors was used to determine the number of days for control and heated plots during which soil moisture was above the threshold for growth.

Monthly photosynthetic data were analysed using an ANOVA, testing the effect of treatment and time of day, as well as the interaction between the two, on the photosynthetic rate of *E. ericoides*. To investigate whether seasonal patterns differed, summer data comprised of data from February and March while winter data comprised of that from May and June. Regressions were used to test the effect of average monthly temperature on maximum and mean photosynthetic rate.

3.4 Results

3.4.1 Warming and plant growth

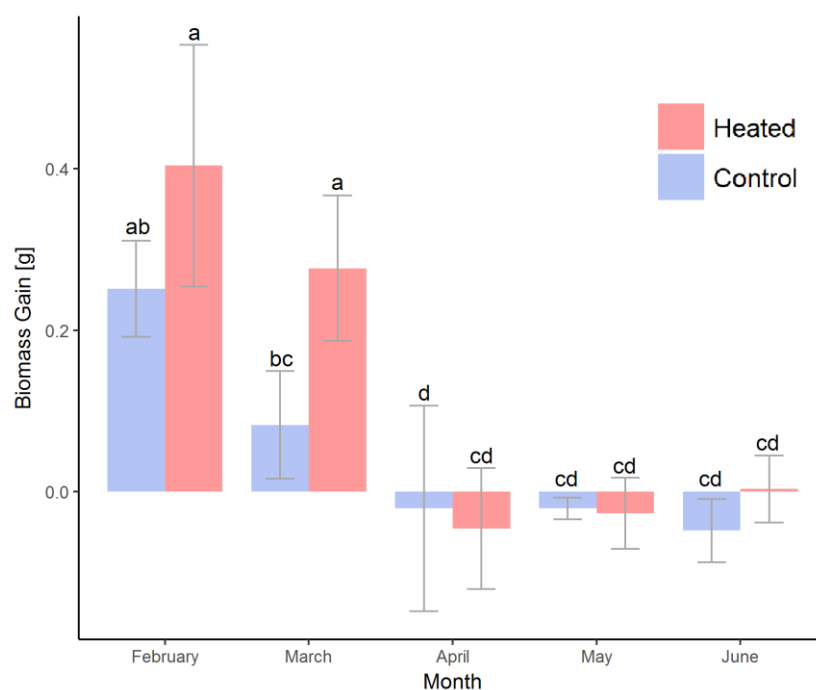


Figure 3.1. Mean biomass gain (g. shoot⁻¹) of heated (red) and control (blue) plots over a five month study period. An ANOVA revealed significant differences between treatments ($F_{(1,50)} = 14.251$, $p < 0.001$), and between months ($F_{(4,50)} = 43.046$, $p < 0.001$) A Tukey post-hoc test was performed, letters represent significantly different groups. Bars denote standard deviations.

Warmed shrubs had a significantly higher biomass gain than control plants during summer months. There was a significant effect of warming ($F_{1,50} = 14.251$, $p < 0.001$) and month ($F_{4,50} = 43.046$, $p < 0.001$) on biomass gain, but there was also a significant interaction between month and treatment ($F_{4,50} = 2.608$, $p < 0.05$), with significant biomass gains only occurring during the growing season (Figure 3.1). Warmed plots showed a trend of increased biomass during February, but this was not yet statistically significant, followed by a significant increase in biomass gain in the warmed plots during March. Thereafter, at the end of the growing season growth ceased and biomass loss can be seen in all treatments. No significant differences were detected between the treatments in the winter months (Figure 3.1).

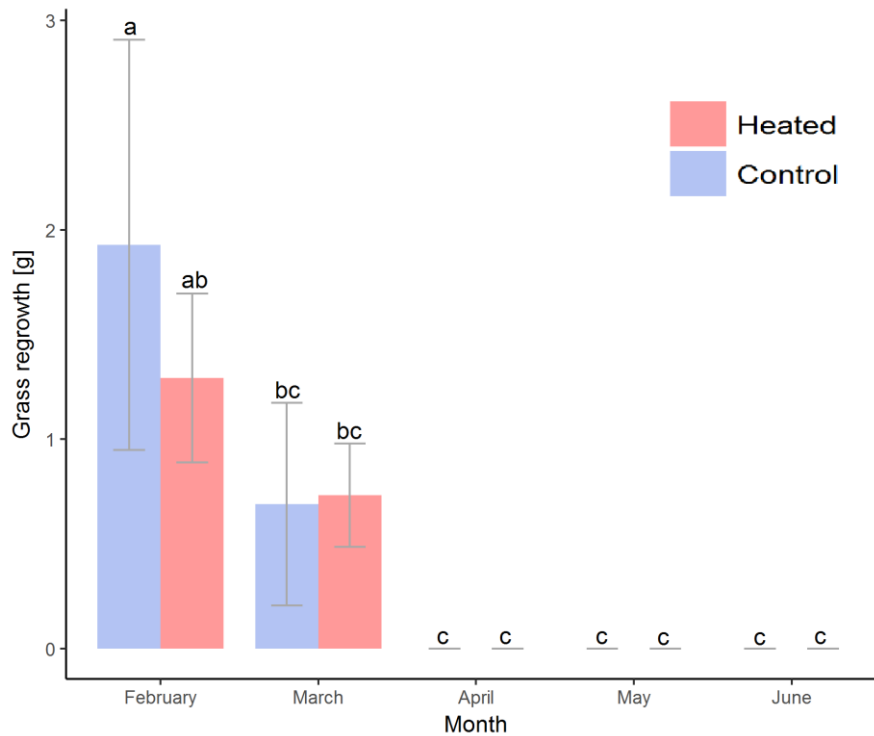


Figure 3.2. Mean grass regrowth ($\text{g}\cdot\text{plot}^{-1}$ (0.1225m^2)) of heated (red) and control (blue) plots over a five month study period. An ANOVA revealed significant differences between months ($F_{(4, 40)} = 38.684$, $p < 0.001$). There was no significant difference between treatments. A Tukey post-hoc test was performed, letters represent significantly different groups. Bars denote standard deviations.

Warming did not significantly alter patterns of grass regrowth. Instead, the time of year (month) was the only variable that had a significant effect on grass regrowth ($F_{4, 40} = 38.684$, $p < 0.001$), with grass regrowth occurring only during the growing season (February to March, Figure 3.2). Data from February suggests that warming might have suppressed grass growth somewhat, but the difference is not statistically significant.

3.4.2 The mechanisms of warming on growth

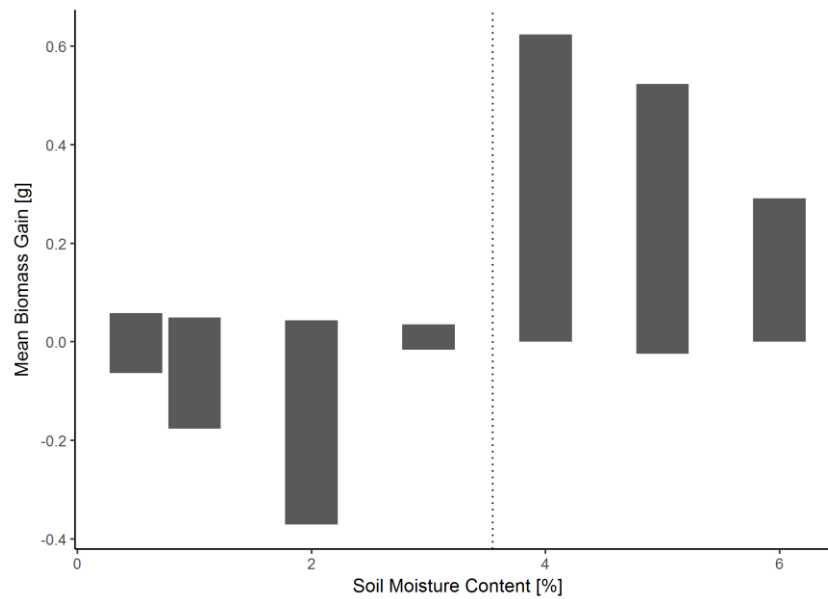


Figure 3.3. Mean biomass gain (g.shoot^{-1}) of shrubs at different levels of soil moisture content. Dashed line denotes the breakpoint (gravimetric soil moisture content = 3.55%) above which biomass gain occurs.

I investigated under what conditions warming accelerated shrub growth. It can be demonstrated that shrub biomass gain only occurred when soil moisture content was above the threshold of 3.55% (vertical line, Figure 3.3). When soil moisture is below this threshold no growth occurs, whilst significant growth occurs above this threshold. Regression analysis showed that biomass gain is positively correlated with soil moisture content above this breakpoint ($R_{(1, 58)} = 0.47$, $p < 0.001$).

Using data from the Decagon GS1 Soil Moisture Sensors, the number of days on which control plot soil moisture was above the threshold of 3.55% was shown to be 50 in C1, 43 in C2, 43 in W1 and 28 in W2. The relationship between gravimetric soil moisture content and volumetric soil moisture content was shown to not be positive at low values of gravimetric water content (Chapter 4), therefore these measures of amount of growth days may not be entirely accurate.

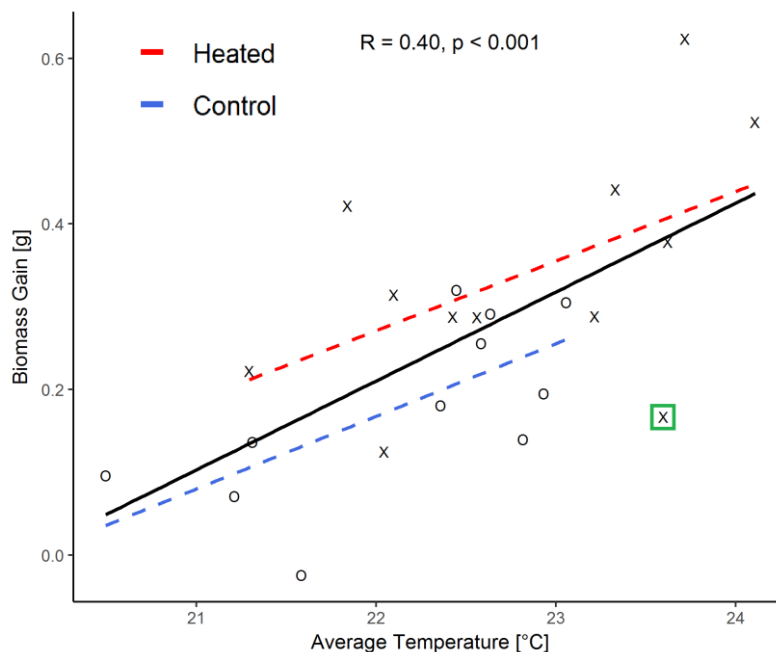


Figure 3.4. Biomass gain ($\text{g}\cdot\text{shoot}^{-1}$) of warmed (X) and control (O) plots as a function of temperature, fitted with a regression ($R_{(1,21)} = 0.40, p < 0.001$) (solid line). Dashed lines represent regression lines for heated (red) ($R_{(1,10)} = 0.19, p > 0.05$) and control (blue) ($R_{(1,10)} = 0.43, p < 0.05$) plots. These data represent the subset of data above the soil moisture threshold of 3.55% for growth. The data point marked by a green square represents an apparent outlier in the warmed plots. Removal of this data point creates a significant positive relationship ($R_{(1,9)} = 0.43, p < 0.05$) between temperature and growth in warmed plots.

I quantified how temperature affected the growth of *E. ericoides* when soil moisture was sufficient. When there is sufficient water, biomass gain increases significantly with temperature ($R_{(1,21)} = 0.40, p < 0.001$). Average monthly temperature was a significant predictor of plant growth in control plots ($R_{(1,10)} = 0.43, p < 0.05$), but not in heated plots ($R_{(1,10)} = 0.19, p > 0.05$), due to an apparent outlier in the warmed plot data set. Removal of this outlier (marked by a green square) from the warmed plot data set results in a positive relationship ($R_{(1,9)} = 0.43, p < 0.05$) between temperature and biomass gain in warmed plots. Overall, it is possible to state with some certainty that, when it is wet enough, warmed shrubs grow faster.

However, it is clear that there is an offset in the relationship between temperature and shoot biomass gain between the warmed and control shoots. If the effect of the OTC on growth

were just due to warming, one would not expect this offset, and the lines of the two regressions to overlap and lie on the common regression line for the whole data set. This offset, which is positive, implies that there is an effect on shoot growth other than through warming induced by the presence of the chamber.

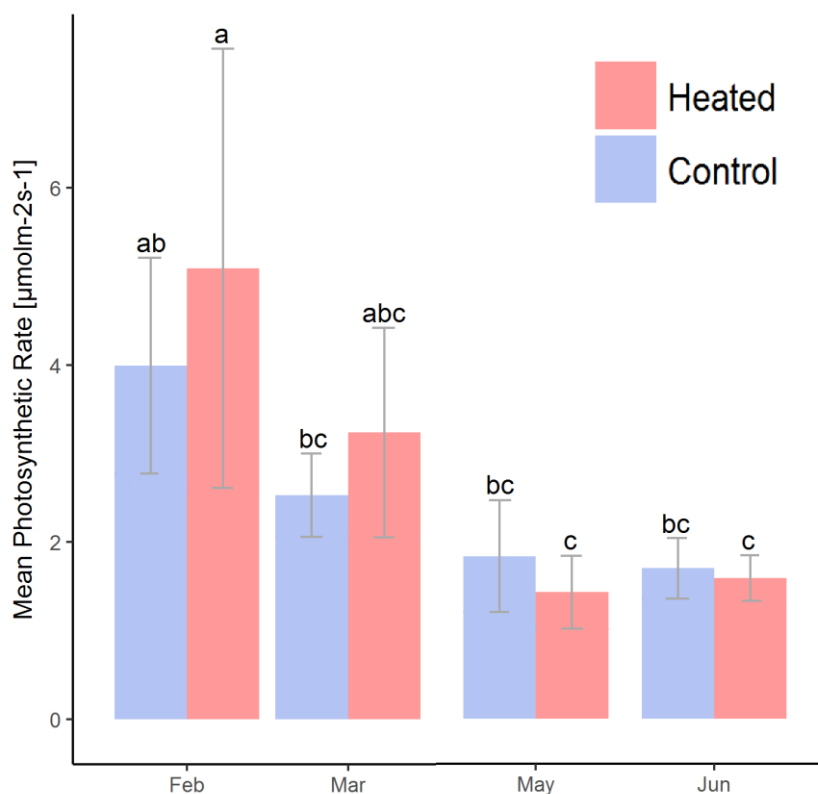


Figure 3.5. Mean monthly photosynthetic rates of control (blue) and heated (red) plots. An ANOVA revealed a significant effect of month ($F_{(11, 39)} = 14.59$, $p < 0.001$). A post-hoc Tukey test was performed, letters represent significantly different groups.

I investigated whether the increase in the growth of warmed shrubs could be explained by increased photosynthetic performance. Mean photosynthetic rate did tend to be higher in warmed shrubs during the summer months (February and March, Figure 3.5) but the difference was not statistically significant. May and June photosynthetic rates were slightly lower in warmed plots, but the difference was again not statistically significant. April is excluded from this analysis due to missing data resulting from equipment failure

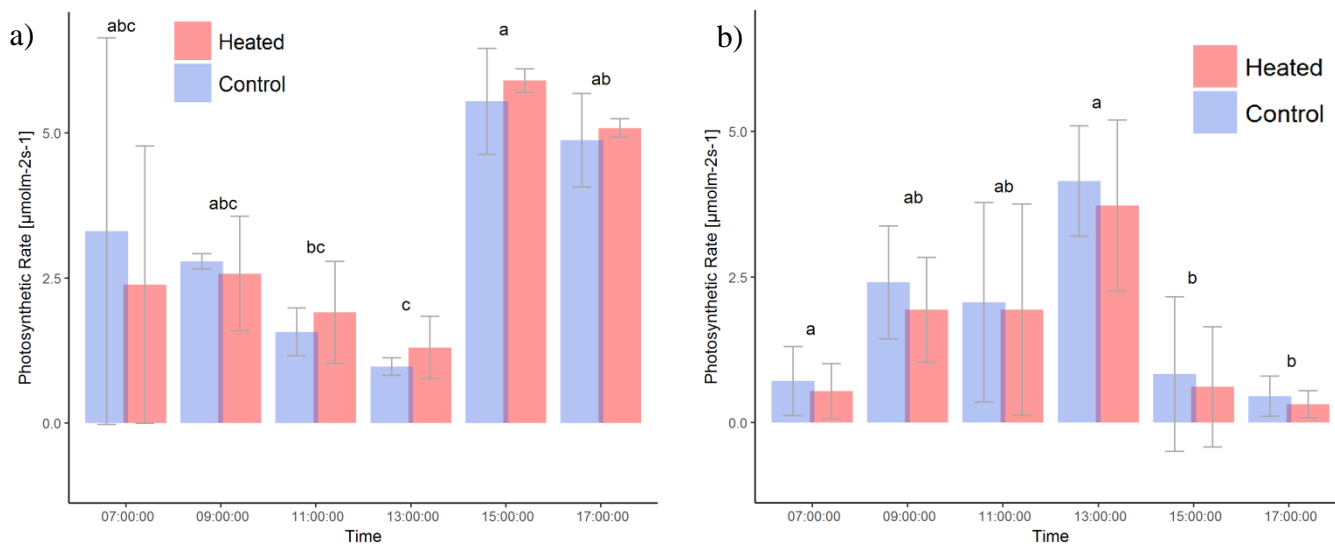


Figure 3.6. Mean a) summer and b) winter photosynthetic rates of control (blue) and heated (red) plots. An ANOVA revealed a significant effect of time on photosynthetic rate during summer ($F_{(5, 12)} = 7.59$, $p < 0.01$) and winter ($F_{(5, 12)} = 6.01$, $p < 0.01$). A post-hoc Tukey test was performed for both datasets, letters represent significantly different groups.

Whilst significantly different patterns of diurnal photosynthetic rates occurred during summer ($F_{(5, 12)} = 7.59$, $p < 0.01$) and winter ($F_{(5, 12)} = 6.01$, $p < 0.01$), there was no significant effect of warming on these diurnal patterns in either summer or winter. Patterns of photosynthetic activity differ between summer and winter, with the majority of summer activity occurring in the afternoon and the majority of winter activity occurring at midday.

Maximum photosynthetic rate increased significantly with temperature ($R_{(1, 45)} = 0.20$, $p < 0.05$), as did mean photosynthetic rate ($R_{(1, 45)} = 0.4$, $p < 0.001$).

3.5 Discussion

Warming increased shrub growth during the wet growing season, but not in the cold, dry winter months. This effect is dependent on the availability of soil moisture, as I show that growth only occurred when soil moisture content was above 3.55%. Increases in warmed plot shrub growth during the soil moisture window led to higher overall growth in heated plots during the growing season. Investigating the mechanisms of increased growth in heated plots revealed that photosynthetic rates were higher in heated plots, but no significant difference could be detected in either summer or winter. Key findings were that, contrary to predictions, only the C₃ shrubs increased growth in response to warming, while the C₄ grasses showed no significant response to warming. A positive offset in the relationship between shrub biomass gain and air temperature suggests that a factor other than warming is accelerating shrub shoot growth inside the OTC.

3.5.1 Shrub growth

Growth of *Eriocephalus ericoides* responded positively to warming, with warmed individuals showing an average increase of 37% above control shrubs by the end of the growing season (February and March). One reason for the increase in growth could be a lengthening of the growing season. Warmed shrubs gained, on average, 51% more biomass during March than control plots did, suggesting that warmed shrubs may have continued to grow after control shrubs had decreased production. This agrees with many predictions that climate warming will continue to increase the length of the growing season (Hughes 2000; Kuster et al. 2012; Mccarty 2001; Rötter & Van De Geijn 1999), especially by lengthening the period of activity in autumn (Bergh et al. 2003). Models have shown increased productivity in Northern Hemisphere systems in response to warming-induced lengthening of the growing season (Ollinger et al. 2008), a similar response could be expected in different ecosystems.

The increase in stem elongation of warmed shrubs (here converted to biomass gain) is congruent with results from other studies. Meta-analysis reveals that warming generally leads to increased growth in many ecosystems, though some systems show decreases and the magnitude of positive effect varies (Hoeppner & Dukes 2012). Deciduous tree species showed up to eight times greater stem elongation under higher temperatures (Way & Oren 2010) and evergreen alpine shrubs increase biomass allocation under warming between 1.5 and 2.3°C (Kudo & Suzuki 2003).

This shrub species behaved differently than might be expected, as many authors have predicted that species with high growing season temperatures will show negative growth responses to warming (Cahill et al. 2012). Trees from tropical and subtropical areas have shown susceptibility to decreases in growth at higher temperatures (Way & Oren 2010). While the shrubs of the Nama Karoo may be resilient to changes in temperature, as the seasonal variability is high (du Toit & O'Connor 2014; Weatherbase 2017), it is also possible that the Grootfontein site has a temperature regime that is sub-optimal for this species. This possibility could be tested using bioclimatic niche modelling, for example. The effects of warming on plant growth varies between species (Kudo & Suzuki 2003), therefore this data cannot be used to make predictions of all Karoo shrubs but provides an example of warming response in this ecosystem.

This study shows that the effect of warming on *E. ericoides* will be greatest during the summer months when soil moisture is sufficient for growth. Increased growth rate during this time appears to be capable of overriding any modest decreasing effect of warming on soil moisture.

Whilst warming did not increase growth during the colder months I found that warmed plots experienced less biomass loss during June. This may be by chance but could also be due to reduced frost damage, as frost is a common cause of topkill in Nama Karoo shrubs (Mucina & Rutherford 2006; Muller et al. 2016). Warming is expected to lead to decreased frost severity and frequency, which may affect production in the long term. Frost is common in the Nama Karoo during the winter months, though it does show great inter-annual variability (du Toit & O'Connor 2014; Muller et al. 2016). A warming-induced decrease in frost as has been shown in the Sonoran Desert (Bowers 2007) and it was well established that a chamber of this design reduced frost severity and incidence (Godfree et al. 2011).

3.5.2 Grass growth

Grass growth showed no effect of treatment during the two growing season months analysed in this study. This is contrary to what was expected, as C₄ grass species are known to increase performance under higher temperatures. Grass growth may be constrained by the low water availability of this region, as C₄ species require adequate moisture as well as favourable temperatures (Collatz et al. 1998), prohibiting grasses from positively responding to increasing temperatures. This region may therefore experience a shift to greater shrub dominance if warming is not accompanied by increased rainfall, as has happened in the

southwestern United States where precipitation loss and warming caused a shift from an arid grassland to a desert shrubland (McCarty 2001).

3.5.3 *The mechanisms of temperature on shrub growth*

Analysing the relationship between growth and soil moisture content showed that there was a minimum threshold of soil moisture content necessary for *E. ericoides* growth in this system (3.55%). This suggests that water is the main limiting factor on growth in this ecosystem. This is congruent with a study on a tallgrass prairie, where biomass increased in warmed plots only when soil moisture was adequate (Sherry et al. 2008). Of a total of 169 days, the two control plots fitted with GS1 sensors experienced soil moisture greater than the threshold for 69 and 56 days respectively. The two warmed plots experienced soil moisture greater than the threshold for 68 and 70 days respectively. The difference between the two control plots is likely due to one of the plots not experiencing the same amplitude of soil moisture increase after three rainfall events during autumn and winter. This may simply be an artefact of sensor positioning, as winds may have caused the shrub to intercept some of the rainfall. Altogether, warming did not decrease the number of days suitable for shrub growth.

Plant growth is dependent on water availability in many ecosystems (Knapp et al. 2001; Sherry et al. 2008) and growth is expected to decline if the proportion of days outside the required water availability window increases (Pires da Silva & Kay 1996). Annual production of North American plains (C_3 and C_4 species) increases across a rainfall gradient, with precipitation accounting for 90% of the variation in productivity (Knapp et al. 2001). Response of both C_3 and C_4 species to temperature and CO_2 was dependent on water availability in an arid grassland (Morgan et al. 2011). Thus, in arid and semi-arid ecosystems, the ability of any plant species to positively respond to climate change is expected to be dependent on water availability.

When soil moisture is above the threshold necessary for growth, the effect of average monthly temperature on *E. ericoides* growth is substantial. When data from heated and control plots are analysed separately, the relationship between growth and temperature is significant for control plots, but not for heated plots. Temperature response of heated plots is more variable than that of control plots (Figure 5.4), but the case of significance is dependent on one data point representing low biomass gain at high average temperature (marked by a green square on Fig. 5.4). When this data point is removed, the relationship between growth and temperature becomes significant for heated plots as well as control plots. *E. ericoides*

growth thus increases with temperature, supporting the pattern of increased growth of heated shrubs during the growing season.

Increased growth of heated shrubs occurred only when soil moisture was above the threshold necessary for growth. Growth of *E. ericoides* therefore seems to be temperature limited as well as water limited at this site. Future warming may lead to increased annual production of this shrub, provided that water availability is sufficient for growth.

With respect to the offset in the relationship between air temperature and stem growth in warmed and control shrubs, it is possible that a decrease in wind stress caused by the chambers contributed to the increased growth in warmed chambers. Stem diameter and strength is more important than stem length to protect shrubs against mechanical damage from wind and other stresses (Henry & Thomas 2002). Decreased wind stress may have allowed the shrubs to allocate fewer resources to building stronger stems and instead spend those resources on increasing shoot length and consequently photosynthetic tissue biomass. While the decrease in heated shrub stem diameter was not statistically significant during the study period (data not shown), further exposure to decreased wind stress may lead to significant differences in shoot architecture between heated and control shrubs.

3.5.4 Photosynthetic rates

Gas exchange rates (photosynthesis and respiration) generally increase with temperature to an optimum level due to improved enzyme function (Way & Oren 2010). Warm-grown species tend to acclimate to higher temperatures by shifting the optimum of both carboxylation and electron transport, thereby enhancing photosynthesis at higher temperatures (Way & Oren 2010; Yamori et al. 2014). While there was no significant difference in photosynthetic rates between treatments in this study, heated plots did show higher mean rates of photosynthesis. A regression showed that photosynthesis is positively correlated to temperatures, suggesting that the difference in temperature between control and heated plots was not enough to result in significantly higher rates of photosynthesis. Longer term monitoring of these shrubs may reveal acclimation to higher temperatures in heated shrubs, which could lead to significantly higher photosynthetic rates and carbon sequestration.

Positive effects of warming may be offset by warming-induced decreases in water availability (Hoepfner & Dukes 2012) and has been cited many times as the reason behind a lack of warming response and negative responses (Rustad et al. 2001). This may be the case with the

lower rates of photosynthesis in heated plots during winter, though the difference was not statistically significant.

3.5.5 Seasonal patterns of photosynthesis

Seasonal difference in the response of photosynthesis was also explored. Analysis of mature pine trees show a shift in the optimal temperature of photosynthesis, with summer optimums being 7-8°C higher than winter optimums (Medlyn et al. 2002). A similar phenomenon may be occurring in this system, as maximum winter photosynthetic rates weren't significantly lower than those of summer, despite much lower mean temperatures. Stomatal conductance is thought to be an important mechanism regarding this seasonal variation, but there was no difference between summer and winter stomatal conductance rates in this study (data not shown).

The diurnal pattern of photosynthesis differed quite substantially between summer and winter. Summer photosynthesis steadily declined during the morning to its lowest point at 13:00, the hottest time of day. Photosynthetic rates then increased during the afternoon until the end of sampling at 17:00. Contrary to this, in winter photosynthetic rates increased during the morning to a peak at 13:00, after which it decreased during the afternoon. The 13:00 depression of summer photosynthesis is therefore most likely due to high temperature suppression of photosynthesis, which would then be lifted as temperatures cool in the afternoon. Lower temperatures of winter cause the peak at 13:00, as this is when temperatures are closest to the thermal optimum of photosynthesis.

Species abundance and competition may shift in an environment such as this, as the trade-off between growth potential and cold tolerance during the winter months (Way & Oren 2010) changes as temperatures continue to rise. Composition and abundance will also be affected by survival, fitness and recruitment of plant species (Way & Oren 2010), which may be affected by climate change. In arid and semi-arid regions of western United States, seedling recruitment is strongly affected by their tolerance of severe conditions at the soil surface (Loik et al. 2000). This may also be the case in the Nama Karoo and climate change could lead to conditions more beneficial for seedling survival, an effect which is almost certain not to be the same among species. The dominance pattern of the region could therefore change under future climate scenarios and some species may even become locally extinct after being outcompeted.

The lack of response of C₄ grass species in this study could indicate that the region will become less grassy under future climate change if shrubs respond positively and grasses fail to respond to higher temperatures. The fate of the grasses is also dependent on the effect of climate change on the water balance of the region. Predictions regarding rainfall are currently uncertain (Conway 2008; de Wit & Stankiewicz 2006; Engelbrecht et al. 2011), but some tentative predictions have been made. Du Toit and O'Connor predicted that changes in rainfall amount and seasonality could lead to a decrease in grass cover and an increase in shrub abundance over the next 25 years (du Toit & O'Connor 2014). The increase in shrub growth could lead to increased carbon sequestration in this region, affecting the balance between being a carbon sink or source. This is of course dependent on the effect warming will have on plant and heterotrophic respiration, and as such no predictions about this balance can be made at this point.

This study has shown that growth in this region is highly dependent on water availability and ambient temperature, in combination. It has been theorised that climate change would lead to decreases in available water, due to temperature-induced increases in evaporation from the soil and transpiration from plants (Zavaleta et al. 2003). Despite this, some studies have seen increases in soil moisture under experimental CO₂ enrichment (Morgan et al. 2001; Zavaleta et al. 2003). While one study suggested the increase resulted from earlier senescence, the other postulated that it could be due to an increase in plant water use efficiency (WUE); several studies have found that CO₂ enrichment leads to increased WUE (Battipaglia et al. 2013; Feng 1999). CO₂ enrichment acts to increase photosynthetic rates and decrease stomatal conductance and consequently transpiration, leading to greater WUE (Llorens et al. 2003). Water content of the soil may thus increase under CO₂ enrichment, benefiting both shrubs and grasses in the Nama Karoo.

3.5.6 Improvements and suggestions for further study

Light intensity has an effect on the temperature dependence of photosynthetic rate (Berry & Bjorkman 1980). Possible decreases in light intensity due to the chamber sides should be monitored by placing a PYR sensor inside the chambers midway between the shrubs and the edge of the chamber. Daily gas exchange measures can be done after rainfall events to determine how heated and control shrubs differ in the response after rainfall and whether heated plots' response declines faster than control plots.

4.5.7 Conclusions

Water and temperature together were shown to be limiting factors for the growth of *Eriocephalus ericoides*, while temperature had no effect on the growth of C₄ grasses. Diurnal patterns of photosynthesis were shown to be different during summer and winter, but no significant effect of treatment was found.

4. The effect of warming on the water balance of Nama Karoo soil and shrubs

4.1 Abstract

Rainfall and soil moisture are important determinants of plant health and production and are responsible for the determination of vegetation type. Many regions of the world are expected to get drier under climate change but the prediction of these changes is less confident than predictions of future temperatures. Soil water content and plant water potential measurements were used to determine if the effect of warming might operate through the mechanism of altering the water balance of a study site in the Nama Karoo. Daily measures of volumetric soil water content revealed significantly lower soil water content in heated plots, likely due to increased evaporation and transpiration. Monthly values of soil moisture content differed between treatments and months while monthly values of plant water potential showed no effect of treatment but did differ over time. Soil water content and plant water potential were positively related at lower values of soil water content but the relationship became saturated above soil moisture levels of 5% (no further increase in plant water potential).

4.2 Introduction

Predictions of future warming point to a rise in global surface temperature of at least 2°C (under stringent emission control); with a maximum above 4.8°C over centuries, depending on the emission pathway followed over the next few decades (described as Representative Concentration Pathways) (IPCC 2014; IPCC 2007). However, changes in precipitation are more difficult to predict than changes in temperatures (IPCC 2007; IPCC 2014; Mccarty 2001; Seidel et al. 1998) and therefore, future rainfall amount and seasonal patterns of precipitation remain an uncertainty. The direct effects of climate warming include impacts on the hydrological balance of ecosystems (Bates et al. 2008; Park et al. 2010), with general consensus that warming will result in a drier world (Wetherald & Manabe 1999; Wetherald & Manabe 2002).

As precipitation in arid and semi-arid regions tend to be variable, soil water content varies in space and time more than in regions with more predictable rainfall (Schwinning & Ehleringer 2001). Precipitation events create peaks in soil moisture that can last between hours and weeks, depending on the amount of rainfall and the evaporative demand of the atmosphere (Schwinning & Ehleringer 2001).

The effect of warming on the water balance of ecosystems is an important variable to consider when making predictions of future events, particularly in arid and semi-arid ecosystems, where water is the main determinant of ecosystem function (Mccarty 2001). In already dry ecosystems, any changes in water availability are likely to have a significant effect on vegetation and ecosystem function (Bai et al. 2008).

The effect of warming on the soil moisture status of arid and semi-arid regions is thus an important facet of future climate change. In the past it was theorised that climate change would lead to decreases in available water, due to temperature-induced increases in evaporation from the soil and transpiration from plants (Zavaleta et al. 2003). Despite this, some studies have seen increases in soil moisture under experimental CO₂ enrichment (Morgan et al. 2001; Zavaleta et al. 2003). While one study suggested the increase resulted from earlier senescence, the other postulated that it could be due to an increase in plant water use efficiency (WUE). Several studies have found that CO₂ enrichment leads to increased WUE (Battipaglia et al. 2013; Feng 1999), through increasing photosynthetic rates and decreasing stomatal conductance and consequently transpiration, leading to greater WUE (Llorens et al. 2003). Changes in plant WUE would be especially important in arid and semi-

arid regions, as plants may be able to decrease their overall moisture requirements in face of increasing rainfall variability.

The response of plant water potential to warming and decreased moisture availability can inform on plant strategies for dealing with drought. When faced with conditions of drought, plants can choose to keep their stomata open in order to maximise carbon sequestration or can close their stomata in order to minimise water loss (Skelton et al. 2015; Schultz 2003). The first describes anisohydric behaviour; where plants maintain photosynthetic capacity, risking dangerously low internal water potentials that may lead to xylem cavitation. The second describes isohydric behaviour, where plants maximise water retention at the danger of carbon starvation and decreased growth (Skelton et al. 2015; Schultz 2003). This second type is often referred to as drought avoiders.

Large areas of South Africa are expected to experience a decrease in precipitation (IPCC 2014) but an increase in precipitation is expected in the east and along the southern coast. This change could have effects further inland and if so, is likely to cause a biome shift from Nama Karoo to grassland as grasses start to dominate due to increased precipitation (Driver et al. 2012). However, there is still the possibility that the Nama Karoo region will experience the decrease in precipitation expected for the rest of South Africa.

Rainfall at Grootfontein was significantly higher between 1981 and 2010 (mean annual = 413mm) than the period from 1899 to 1980 (mean annual = 347mm), but is predicted to decrease until 2025 (du Toit & O'Connor 2014). The seasonality of rain varies in the Nama Karoo, with peak rainfall occurring at any time between early January and early March. Years when peak rainfall is in early January also tend to be wet years (du Toit & O'Connor 2016).

4.3 Methods

4.3.1 *Study site*

The study took place on Grootfontein (31° 25'S 25° 1'E, elevation 1279m), a farm situated outside Middelburg, Eastern Cape and managed by Grootfontein Agricultural College. The region is classified as Eastern Upper Karoo (Mucina & Rutherford 2006) and has been suggested to represent an ecotone between the Nama Karoo to the west and the semi-arid grasslands to the east (du Toit & O'Connor 2014), with vegetation mainly comprised of dwarf C3 shrubs and annual and perennial C4 grasses. Middelburg has a mean annual temperature of 14.9°C with a mean low of 6°C and mean high of 22°C (Weatherbase 2017). Mean annual precipitation is 356mm with the most rain falling between January and March. Frost is common in winter and occurs up until October (du Toit & O'Connor 2014).

4.3.2 *Experimental setup*

Warming was achieved by the use of Open-top Chambers (OTCs). These passive ecosystem warming systems have been widely used by scientists for temperature and atmospheric manipulation (de Frenne et al. 2010; Van Oijen et al. 1999). The OTCs used in this study were based on a system designed by Godfree et al. (2011) which aimed to decrease extreme temperature fluctuations and to produce significant levels of night-time warming. The chambers were pentagonal in shape with an internal area of 4.91m². The inside perimeter of the chambers were lined with two rows of water-filled 110mm PVC pipes. The pipes are painted black on the side facing the sky, in order to absorb maximum energy during the day. This is to prevent extreme temperature increases during the summer months and to produce night-time warming by via the release of this energy during the night.

4.3.3 *Meteorological and micrometeorological data*

Ambient climate data were collected adjacent to the study plots using a Campbell Scientific CR1000 Data Logger. A stainless steel stand supported a Campbell Scientific CS215-L Temperature and Relative Humidity Probe. Four GS1 Soil Moisture Probes were deployed, two in control plots and two in warmed plots, inserted into the soil close to the edge of the target shrub canopy. Measurements were taken every 10 minutes and an hourly and daily data table was recorded by the data logger. All treatment and control plots contained an iButton (DS-1923L-F5#), situated 15cm above the soil surface and measuring temperature every 15 minutes, data from which the thermal performance of the OTCs was assessed.

4.3.4 Soil water content

Soil water content was measured during all monthly site visits, starting 30 January 2017. A sample was taken of the first 7cm of soil, weighed on an electronic scale sensitive to 0.0001g. The sample was dried at 110°C for 24 hours after which it was weighed again. The water content of the soil was measured as the difference between the wet weight of the soil and the dry weight of the soil, converted to percent. Volumetric soil water measurements were also obtained from four GS1 Soil Moisture Sensors (Decagon Devices). Two of these sensors were placed in heated plots and two in control plots.

4.3.5 Plant water potential

Plant water potential was measured using a WP4 Dewpoint Potentiometer (Decagon Devices). The WP4 uses the relative humidity of the air above a sample at temperature equilibrium, which is a direct measure of water potential. The WP4 measures water potential to a resolution of 0.1MPa (Decagon Devices 2017). One shoot was obtained from every shrub at midday and was transported to the lab for analysis. The WP4 was calibrated before measurement to ensure accuracy and all samples were analysed the afternoon of collection.

4.3.6 Rainfall data

Rainfall data were provided by Justin du Toit. Data are collected from a weather station 1.1 kilometres from the study site. Data collection was only done on working days, so any rain on a public holiday or over a weekend was recorded on the next working day.

Three precipitation events were selected to investigate the difference between soil moisture dry down of control and heated plots after a precipitation event. The three events selected were after the 3rd of March, the 24th of March and the 13th of May. These three times are taken to represent summer, autumn and winter precipitation events. No events were chosen before March, as the periods between precipitation events were too short to distinguish a difference between treatments.

4.3.7 Statistical analysis

All statistical analysis was performed in R (R Core Team 2012) using RStudio version 3.4.2 and the packages agricolae, AICmodavg, ez, lme4 and segmented.

Difference in volumetric soil water content was tested by performing a repeated measures ANOVA on the data from the Decagon GS1 soil moisture sensors. These data were combined with the rainfall data to explore the relationship between rainfall and soil moisture content.

To examine the difference in soil moisture content (by weight, collected monthly) and plant water potential, ANOVA's were used to test for differences between treatments and time. We investigated the relationship between monthly soil moisture content and plant water potential by plotting the two variables and fitting a polynomial curve to the data.

4.4 Results

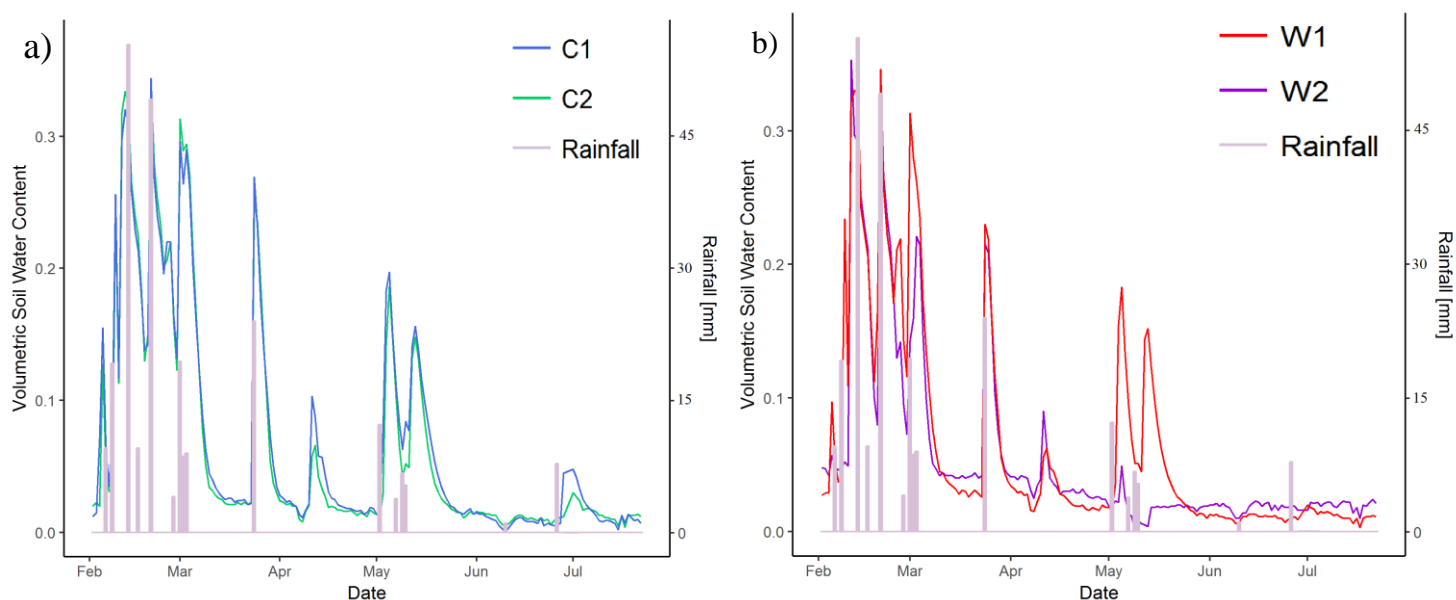


Figure 4.1. Mean volumetric soil water content for a) control and b) warmed plots combined with rainfall (coloured mauve) data. A repeated measures ANOVA revealed a significant effect of treatment ($F_{(168)} = 130.69$, $p < 0.001$). The primary y axis represents volumetric soil water content from the GS1 sensors. The secondary y axis represents rainfall amount from the Grootfontein weather station. The x axis represents time, in daily intervals.

Volumetric soil water content of plots increased sharply after precipitation events, in both warmed and control plots. A repeated measures ANOVA revealed a significant effect of warming treatment on soil moisture ($F_{(168)} = 130.69$, $p < 0.001$). There is one instance in April where soil moisture increase is not reflected by rainfall data.

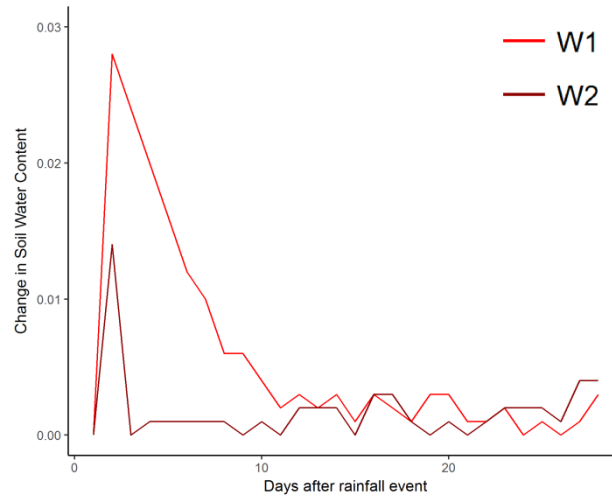
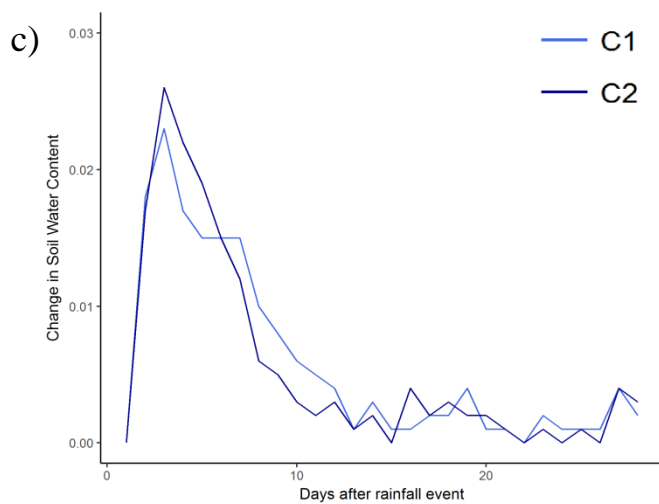
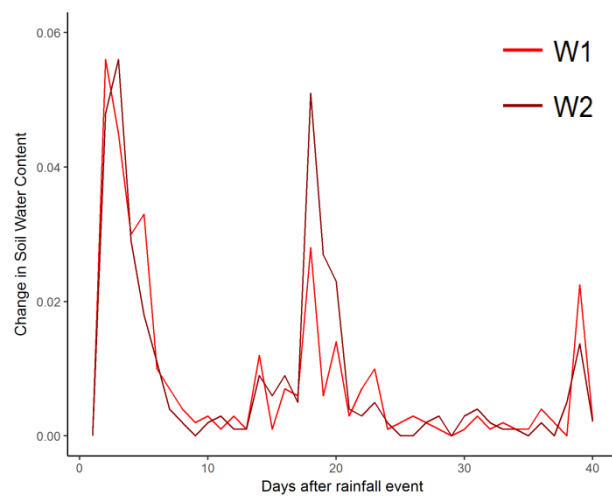
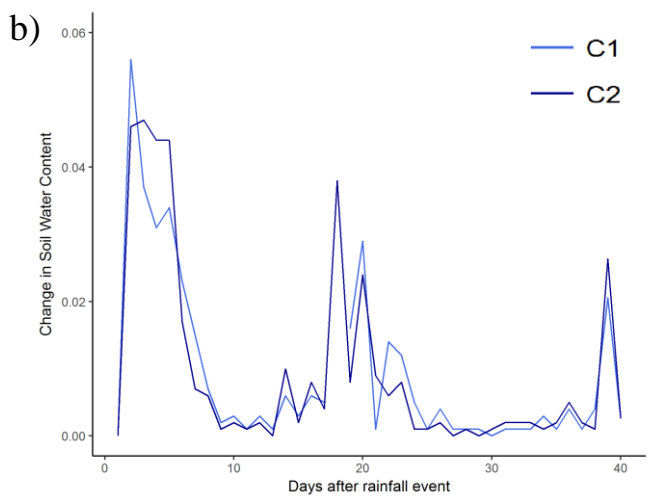
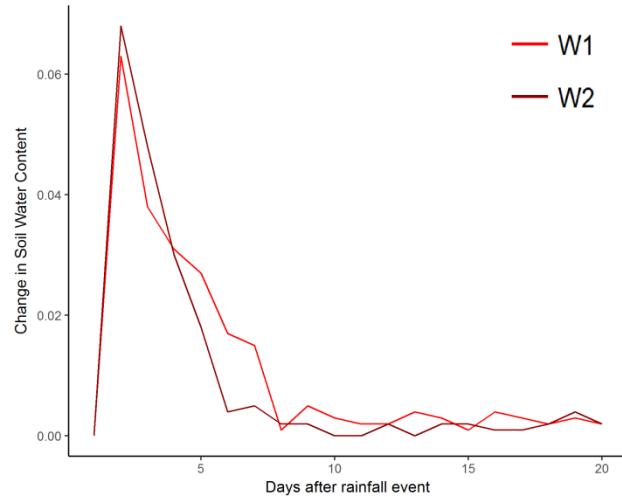
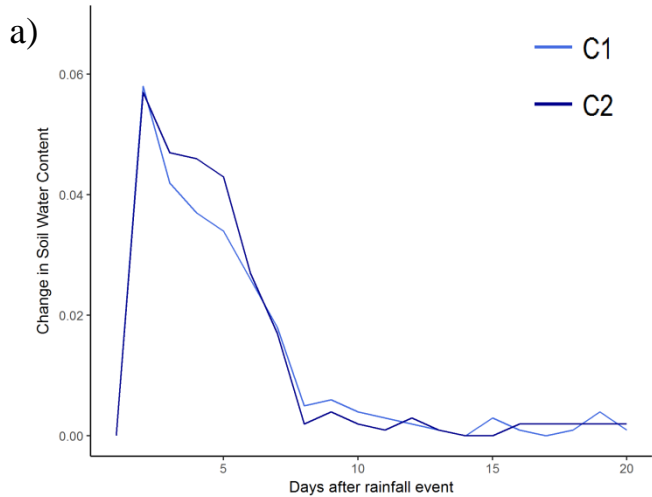


Figure 4.2. Change in soil moisture of control (left) and heated (right) plots after three rainfall events; a) After 3 March (30.9mm over three days), b) after 24 March (20mm over one day) and c) after 12 May (13.3mm over four days).

To further investigate this pattern, we compared the daily soil moisture loss after rainfall events in heated and control plots. Soil moisture seems to decrease faster after rainfall during the summer than during autumn and winter when looking at the shape of Figure 4.2a during the first ten days after rain. The only real variability came from plot W2, which decreased faster after rainfall in summer, didn't experience the same peak of moisture increase after rainfall in winter and increased more than other plots after a suspected rainfall event in autumn. Heated and control plots experienced an increase in soil moisture content during April, possibly due to a rainfall event which was not recorded at the nearby weather station.

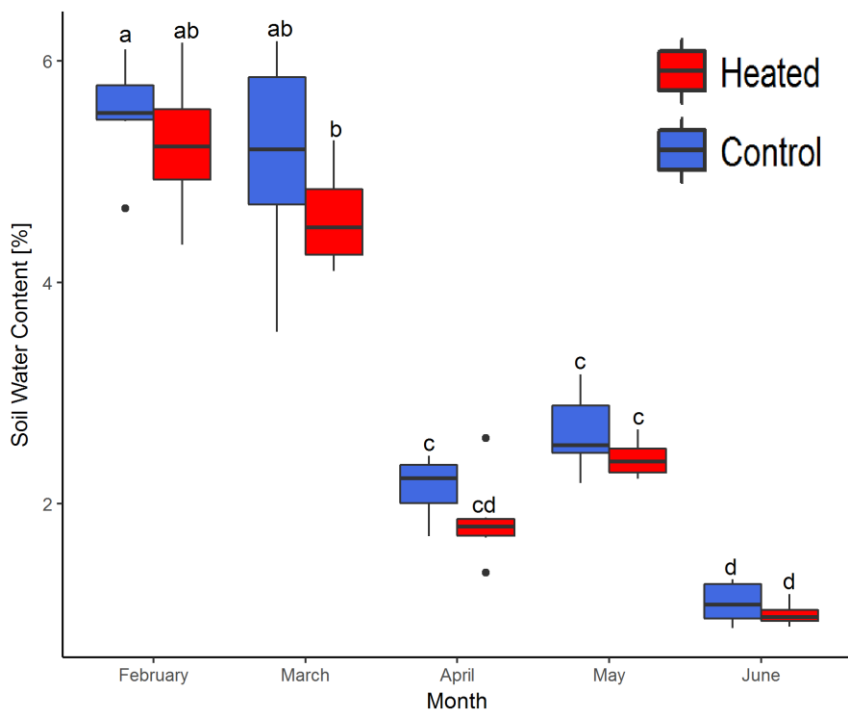


Figure 4.3. Mean monthly soil water content of control (blue) and heated (red) plots measured from physical soil samples. An ANOVA revealed a significant effect of treatment ($F_{(1, 50)} = 5.69$, $p < 0.05$), and month ($F_{(1, 1)} = 649.83$, $p < 0.001$). A Tukey post-hoc test was performed, letters represent significantly different groups.

When investigating the water content of samples taken manually in “scoop” samples from all the chambers and control plots, at one month intervals I found significant difference between

treatments (heated plots had lower water content), and between months. February and March had the highest water content and June the lowest.

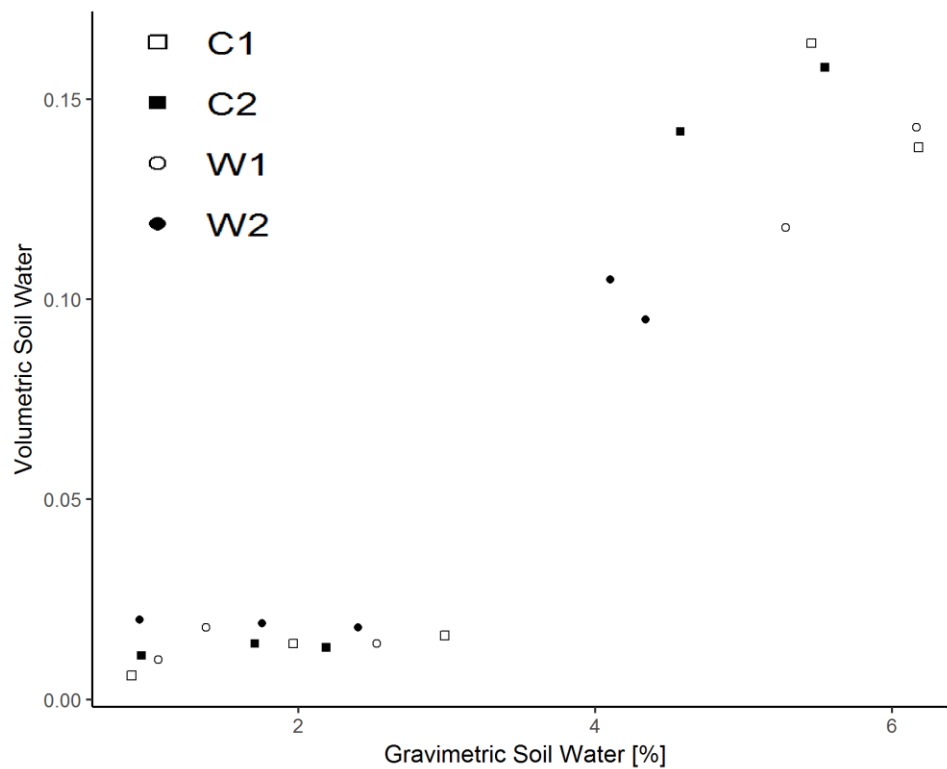


Figure 4.4. Measures of gravimetric soil water content and volumetric soil water content was positively related for all four plots. Regressions are as follows: C1: $R_{(1,3)} = 0.82$, $p < 0.05$, C2: $R_{(1,3)} = 0.2$, $p < 0.01$, W1: $R_{(1,3)} = 0.932$, $p < 0.01$, W2: $R_{(1,3)} = 0.80$, $p < 0.05$, combined: $R_{(1,18)} = 0.87$, $p < 0.001$.

The relationship between gravimetric soil moisture content and volumetric soil water content for the four plots with GS1 sensors revealed a strong positive relationship in all four plots. The relationship appears to hold for most values of gravimetric soil water content but at lower levels (below about 3% gravimetric water content) values of volumetric soil water content fail to respond as sensitively to varying water content.

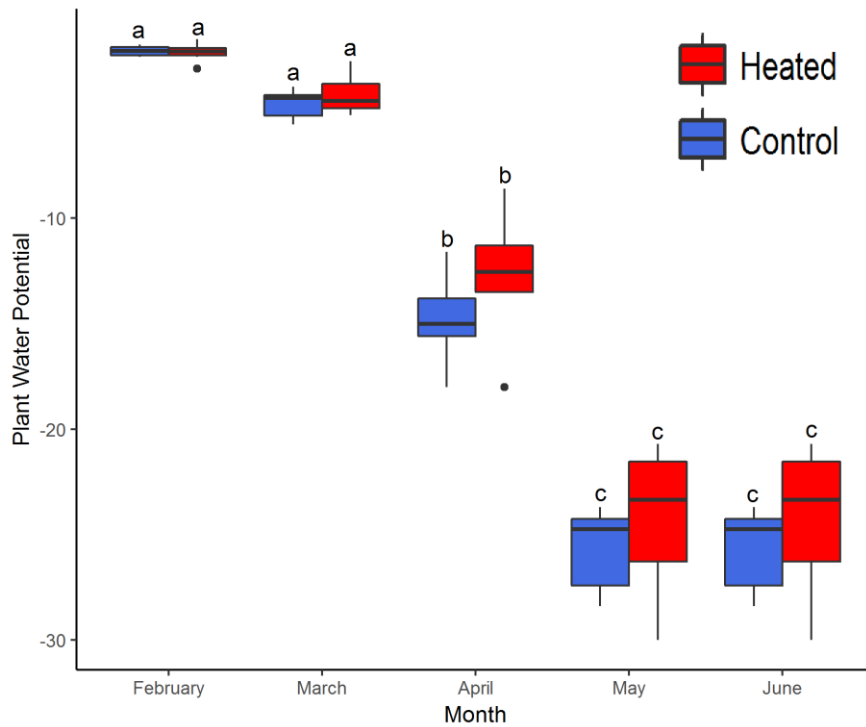


Figure 4.5. Mean monthly water potential (MPa) of *E. ericoides* in control (blue) and heated (red) plots. An ANOVA revealed a significant effect of month ($F_{(1,1)} = 1027.17$, $p < 0.001$). A Tukey post-hoc test was performed, letters represent significantly different groups.

When comparing the pattern of midday shrub water potential between treatments and across months, water potential of *E. ericoides* did not differ between treatments, but a significant effect of month was found. The water potential of the shrubs fell decidedly after the end of the growing season, and attained values of almost -26MPa during the dry winter months.

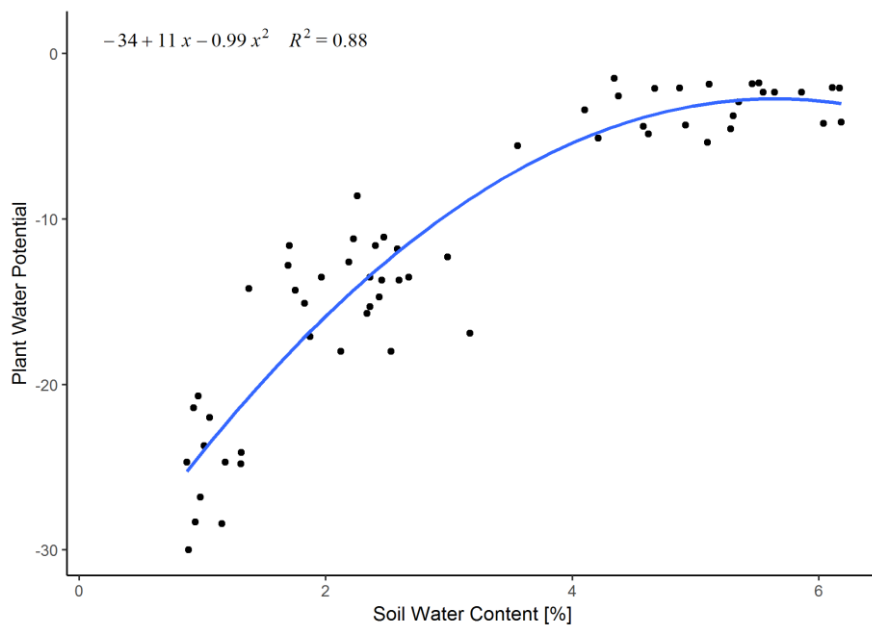


Figure 4.6. The relationship between soil water content and water potential of *E. ericoides*. Plant water potential is positively related to soil water content across a second order polynomial ($R_{(3,56)} = 0.88$).

Plant water potential was positively related to soil water content ($R_{(3,56)} = 0.88$), following a curvilinear approximated by the polynomial fit with equation $p = 11c - 0.99c^2 - 34$, where p is plant water potential and c is gravimetric soil moisture content (%).

4.5 Discussion

Soil moisture content of warmed plots was significantly lower than in control plots, when analysing the data from the Decagon GS1 sensors. Gravimetric soil water content, measured monthly, was much higher during the growing season than in the dry season, and water content was significantly higher in control plots. Warmed plots experienced the same amplitude of soil moisture gain after precipitation events, indicating little rain-shadow effect due to the OTCs, and water content was continuously lower in warmed plots after the end of the rainfall season (end of March). Warmed plots also experienced an acceleration of soil drying for the first few days after precipitation events, dipping faster below the threshold necessary for growth before control plots. Shrub water potential only differed between months; and there was no effect of warming treatment. The relationship between soil water content (from gravimetric samples) and plant water potential follows a curvilinear relationship, initially increasing then saturating at higher values of soil water content.

Warmed plots had lower values of monthly soil water content. This pattern of increased aridity with warming is likely a result of increased evapotranspiration in heated plots, as has previously been theorised (e.g. Zavaleta et al. 2003). Higher temperatures in warmed plots caused water to evaporate faster than in control plots and increased growth and transpiration could also be a factor in this relationship. As discussed in the previous chapter, warmed shrubs gained $\pm 36.72\%$ more biomass than control plots in February and $\pm 51.32\%$ more in March. This increased growth may result in higher water demand in warmed shrubs, contributing to faster utilisation of soil water. It is therefore likely that increased evaporation and transpiration contributed to the decrease in warmed plot soil moisture.

The lack of difference in plant water potential between control and warmed plots does not necessarily mean that the shrubs are not responding to a decrease in soil moisture in heated plots. Water potential of the leaves could be maintained by high root pressurisation while stomatal conductance and leaf growth are downregulated (Passioura 2002). This would correlate with the cessation of growth when soil moisture falls below the growth threshold. *E. ericoides* appears to be largely anisohydric, as midday shoot water potential dropped significantly after the end of the growing season; tracking the drop in gravimetric soil water content, while photosynthesis and transpiration were maintained. There is some evidence of isohydric behaviour in the midday depression water potential during the growing season, and in the fact that there is no difference in the mid-day water potential of droughted and

undroughted plants (Franks et al. 2007). Purely anisohydric species would experience a decrease in water potential under warming as evaporative demand increases and thus purely anisohydric droughted plants would be expected to have lower water potentials than undroughted plants (Schultz 2003). Isohydric species are classified as drought avoiders, responding to drought by reducing stomatal conductance and transpiration. This allows isohydric plants to maintain internal water potential, but could lead to carbon starvation and decreased growth in the long term (Skelton et al. 2015). Because shrubs in the Nama Karoo are exposed to prolonged drought conditions, pure isohydry would likely be detrimental in the long term, and would also increase their vulnerability to anthropogenic warming.

4.5.1 Volumetric soil water content

Warmed plots had lower levels of volumetric soil water content than control plots after some of the rainfall events during the study period and analysis of the entire data set showed that volumetric soil water content was lower in warmed plots. Many semi-arid regions in the world are expected to become noticeably drier during the second half of the 21st century (Bates et al. 2008; Wetherald & Manabe 1999; Wetherald & Manabe 2002) with southern Africa becoming drier especially during the winter. While soil moisture content was lower in heated plots overall, no difference in the amount of days available for growth was found, as discussed in the previous chapter. Other experimental warming sites have also shown these results, arctic and alpine regions experienced a decrease in soil moisture due to experimental warming (Chapin et al. 1995; Harte et al. 1995) as have experimental plots in Tennessee (Dermody et al. 2007) and warmed prairie ecosystems in Oklahoma (Sherry et al. 2008).

The role of chambers may also be having an effect on the soil moisture level of warmed plots. As it is accepted that some precipitation will be lost as an artefact of the experimental design, we placed the soil moisture sensors are placed to be directly below the central opening of the chamber. Most of the rainfall should thus be reaching the soil in this region, except if winds are especially strong and the rain is coming in from the side. We observed one data point where no increase in the soil moisture of warmed plots after 6.5mm of rain occurred, but the effect was seen in control plots. This may be a result of chamber effect. High wind speeds may have caused rain to fall from the side, prohibiting the rain from falling within the area of the chambers.

The top openings of the chambers are 1.86m wide on average and the GS1 soil moisture probes were placed underneath this opening. Thus, we feel that the warming effect, rather

than the chamber effect, is responsible for the lower soil moisture values in warmed plots. Experimental warming studies using infrared heaters have found that warming decreases soil moisture (Harte et al. 1995; Morin et al. 2010), leading us to believe that warming was the main driver of soil moisture decrease in this study.

Another data anomaly occurred in April, where an increase in soil moisture was observed with no coinciding rainfall event. Due to the distance between the experimental site and the site where rainfall data was collected (1.1km), it is possible that a localised precipitation event at the experimental site was not picked up at the rainfall collection site.

Temperatures appear to be rising in the Nama Karoo region. Between 1943 and 1958, nine out of 16 years reached temperatures between -12°C and -10°C . In comparison, only three years between 1959 and 2013 reached temperatures this low (Muller et al. 2016). There is other evidence that the climate of South Africa is changing. Between 1960 and 2003, average yearly temperatures have increased by 0.13°C per decade (Benhin 2006). Our chambers warmed the area through mean daytime increases of 0.89°C and night-time increases of 1.03°C and did reduce volumetric soil moisture content in heated plots. This suggests that warming will reduce soil moisture content in the future and drought tolerant species may benefit. The C_4 grass species present in this region may decrease in abundance, as they are highly dependent on soil moisture (Collatz et al. 1998). Deeper rooted shrubs may gain benefit, as it has been theorised that rainfall will decrease in this region over the next 25 years (du Toit & O'Connor 2014).

4.5.2 Soil water content and plant water potential

Analysing the relationship between soil water content and plant water potential showed that plant water potential tracks soil water content following a polynomial relationship. At low levels of soil water, plant water potential will increase, but the curve levels off at higher values of soil moisture content. The relationship seems to be non-existent above 5% soil moisture.

In spite of the pattern of drying under warmer conditions, we need to balance this prediction with increasing CO_2 concentrations. High CO_2 concentration is likely to affect the relationship between plants and water balance in the future. Stomatal conductance could decrease between 30 and 40% under a doubling of atmospheric CO_2 (Gerten et al. 2004), leading to decreased water use per unit growth in plants (Nelson et al. 2004). Water use efficiency of trees have increased over the last 100 to 200 years (Feng 1999), tracking the

increase of atmospheric CO₂ concentration. Trees in arid environments are thought to have grown more rapidly due to CO₂ enrichment, sequestering more carbon than before (Feng 1999). Several experimental studies have found increases in soil moisture under increased CO₂ concentration (Dermody et al. 2007; Morgan et al. 2011; Niklaus et al. 1998; Sindhoj et al. 2000; Zavaleta et al. 2003). This may balance the effect of warming, but the effects of increases in plant growth from warming and elevated CO₂ interacting with soil moisture still need to be considered.

4.5.3 Improvements and suggestions for further study

The GS1 sensors from which volumetric soil water data is collected in this study is currently only deployed in two warmed and two control plots. Deploying more would increase the accuracy of this measure but would be expensive. Temporal accuracy can be increased by more frequent physical soil samples, especially after precipitation events. Plant water potential can also be measured more frequently. Pre-dawn samples can be done to supplement the midday measurements used in this study.

Ideally, CO₂ enrichment would be included in such a climate change study to assess its effect on the hydrology of the system, especially as some studies have found that it may lead to increases in WUE.

4.5.4 Conclusion

The warming treatment induced by the OTCs appeared to cause a slight but significant reduction in surface soil moisture especially in wetter summer months. This was detected clearly in monthly “scoop” samples of gravimetric soil water content, and was supported by observations that the rate of soil drying after rainfall events in summer months was slightly elevated according to hourly data logged by a volumetric water content sensor. Warming therefore also affected the response of soil moisture to rainfall, as heated plots seem to have experienced an acceleration of water loss after precipitation events especially during summer months. This suggests an elevation of evapotranspiration in the warming chambers, an effect that is expected with future projected global warming. It is interesting to note the short duration of the most marked aspect of this effect, and that this was limited to only a few days after rainfall events.

While the reduced soil surface water availability may have played a role in preventing a response by the C₄ grass community to warming, it had no observable effect on the C₃ shrub

E. ericoides (See Chapter 3). Midday water potential in this species was not significantly affected by the soil moisture differences, and plant gas exchange was similarly not affected.

The plant water potential and gas exchange data suggest that *E. ericoides* is largely anisohydric, and does not rely upon stomatal control to avoid drought stress. This can be seen in the fact that the species shows only a low level of diurnal stomatal control (some evidence of midday stomatal closure in summer months, see Chapter 3), but that seasonally the species' water status appears to track soil water content. Overall, this strategy of maintaining continuous low plant water potential, combined with the ability of the shrub to tap a more extensive soil volume for available water, would tend to buffer the species against the hydrological feedback from atmospheric warming. Detailed work to explore stem hydraulic water flow under warming conditions would provide valuable insights into this aspect.

It is often projected that warming will result in long-term decreases in soil moisture content in semi-arid ecosystems like the Nama Karoo, but this effect may be at least partially offset by CO₂ enrichment which may lead to increased WUE efficiency in both C₃ and C₄ plants, and thereby lower water use via transpiration. The OTC system designed by Godfree et al (2011), and modified for this study, was also used to fumigate plots with elevated CO₂, and future work could add this functionality to test for such effects.

5. Conclusion

In this thesis, I present work investigating the effect of warming on plant growth in a simulated climate warming experiment near Middelburg in the Nama Karoo (Eastern Upper Karoo, Mucina and Rutherford 2006). This is a warm, semi-arid region with vegetation comprised of both C₄ grass and C₃ shrub species. The study aimed in general to improve the understanding of how such systems may be affected by future climate warming, while at the same time trialling a new and inexpensive method for applying warming in the field. The study examined in some detail the performance of a modified version of an open-top chamber system first utilised by Godfree et al (2011) in an Australian shrubland ecosystem, with the objective of assessing its efficacy and identifying any possible improvements for future use. Ecophysiologicaly, the study quantified the effect of simulated climate warming on the growth, water relations and gas exchange behaviour of *Eriocephalus ericoides* (L.f. Druce), a common C₃ shrub throughout the Nama Karoo, and the production of mixed stands of C₄ grasses.

I implemented an experimental treatment using a modified version of an open-top chamber (OTC) system first employed by Godfree et al (2011) in central NSW Australia. This system employs water-filled PVC pipes, painted black, as a thermal buffer within the chambers. This design raised temperatures moderately during both day and night-time, yielding mean daytime increases of 0.89°C (max = 1.37°C) and mean night-time increases of 1.03°C (max = 1.56°C). The addition of detachable top panels during winter months likely contributed to maintaining warming during these cold months, and to reducing frost frequency and severity in warmed plots. The diurnal warming pattern was more even during winter than summer months. Artefactual effects due to the presence of the chambers were explored as far as possible. There did not seem to be a significant rain shadow effect due to the chambers; except for one small rainfall event in July, which did not wet the soil in the warmed plots and only caused slight wetting in one of the control plots. Effects on wind were not quantified, but are likely to be the most significant artefactual effect, and could be usefully quantified in future work using wind-breaks that do not induce warming. Overall it seems that this approach provided a credible *in-situ* treatment simulating future anthropogenic warming, especially through its ability to generate night-time warming which may otherwise require expensive and high-maintenance active warming systems.

With respect to ecophysiological and growth effects on the C₃ shrub and C₄ grass community components, results generally did not conform to expectations. This highlights the inadequacy of the current knowledge base for any credible projection of vegetation responses in the vast landscapes of the Nama Karoo to anthropogenic climate change. Warmed C₃ shrubs (*Eriocephalus ericoides*) had higher growth rates than those in control plots during the growing season (when soil moisture was more freely available), but warming had no impact on grass growth at any time, regardless of water content. This result directly contradicts expectations of a greater response by the C₄ component to warming that might be inferred from ecophysiological theory and, for example, based on observations of increasing grass dominance in the Nama Karoo region (Masubele et al. 2014). The findings also contradict expectations of adverse impacts on the C₃ shrub based, for example, on (very limited) previous work by Musil et al. (2005) in the Succulent Karoo.

The C₃ shrub showed high tolerance of water stress, maintaining appreciably high rates of photosynthesis even during the dry season. During the wet season, photosynthetic rate of the C₃ shrub was higher in the warmed plots (though not significantly so), supporting observations of significantly increased stem growth. This suggests that growth of one of the dominant shrubs in the Nama Karoo region is partially limited by sub-optimal temperatures at this site during the growing season, which is unexpected. On the other hand, warming did not significantly alter patterns of grass regrowth, and the time of year (month) was the only variable that had a significant effect on grass regrowth.

Soil water content and plant water potential measurements were used to determine if the effect of warming might operate through the mechanism of altering the water balance of the soil and shrubs. Daily measures of volumetric soil water content revealed significantly lower soil water content in warmed plots, likely due to increased evaporation and transpiration. A positive grass growth response may therefore have been constrained by the reduced availability of water in the upper soil layers; partially induced by increased evaporation rates under warming, and possibly also by higher plant transpiration.

Therefore, neither moderate temperature increases nor moderate warming-induced water availability decreases were sufficient to reduce the growth of this C₃ shrub, while the C₄ grass component was likely limited by reduced soil moisture availability during the growing season. This outcome demonstrates the value of a field-based experimental approach that is

able to identify potentially complex interactions that would likely not be identified in controlled environment settings.

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