The effects of climate change, land-use and elevated carbon dioxide on tree-grass interactions in southern African savannas

Dissertation
der Mathematisch-Naturwissenschaftlichen Fakultät
der Eberhard Karls Universität Tübingen zur Erlangung
des Grades eines Doktors der Naturwissenschaften (Dr. rer. nat.)

vorgelegt von
Wellencia Clara Nesongano
aus Otjiwarongo- Namibia

Tübingen
2018

Tag der mündlichen Qualifikation:	13 July 2018
Dekan:	Prof. Dr. Wolfgang Rosenstiel
1. Berichterstatter:	Prof. Dr. Katja Tielbörger
2. Berichterstatter:	Prof. Dr. Andreas Fangmeier

Table of contents

Dedication

I dedicate this thesis to my Father in-law, Papa Mike Nesongano and my younger sister, Uaundja Sherlien Vihanga, both who passed away in 2017, during my time in Germany. May your souls rest in eternal peace.

Acknowledgments

First and foremost, I would like to give thanks to my God for carrying me through my PhD journey. I would then like to convey my sincere gratitude to my main supervisor, Katja Tielbörger for all her support, both on a personal and academic level. Thank you for bringing the savanna to my greenhouse, and for the (stuffed) companion you gave me during one of the most difficult times of my life. However subtle your support might have been at times, I noticed and highly appreciate it. To my other supervisor, Andreas Fangmeier, thank you for your relaxed approach to things, I found it very comforting.

This PhD would not have been possible without funding, and therefore I would like to thank the BMBF for funding the umbrella project (OPTIMASS) under which my PhD was housed. In the same light, I would like to acknowledge my colleagues from project OPTIMASS for being on this journey with me. Had it not been for Dirk's constant requests for contributions to reports and newsletters, I would probably not have worked through processing and understanding my results as fast as I did. So, thank you for that.

To all my friends and colleagues at the Tübingen office, especially Anu, Ellie, Liesbeth, Mark and Viki, thank you very much for being my family away from home. I would like to give a special thanks to Jürgen for assisting me with my very long and strenuous experimental setups at Hohenheim University. To Christiane and Angela, you guys made my load a whole lot lighter by carrying most of it for me, thank you. I very much enjoyed being a part of the plant ecology group, please keep up the welcoming and supportive spirit. It was a wonderful feeling to know that I was among people who cared about my academic progress as well as my general well-being.

I would also like to express my appreciation to the people from the Fangmeier group, especially Jürgen, Zorica, Thilo and Walter. Thank you very much guys for your assistance and making me feel welcome.

A special vote of thanks goes to my family and friends in Namibia and all over the world, who were with me during the ups and downs of my PhD journey. We have made it guys, this is a result of all our collective efforts.

Finally, to my wonderful, selfless husband, Mufaro Nesongano and my son (the apple of Mommy's eye), Matjiua Nesongano, I have no words to explain the sacrifice you made for me to be so far away from you, nor to express my gratitude and appreciation for that sacrifice. I am very blessed to have you guys as my family. PS: Although Mommy is about to become a "Plant doctor", I will always be your Mommy.

Abstract

Rangelands in southern Africa continue to be degraded by the thickening of bush at the expense of grasses. This is a great concern for rangeland managers, especially because there is still no consensus about the main mechanisms that drive shrub encroachment in southern African savannas, making it difficult to make recommendations regarding management. Much work has been done on the effects of fire, grazing, water and nutrient availability on rangeland productivity but the role of climate change, namely changes in CO_2 concentrations and rainfall, and their potential interaction with manageable factors, such as grazing and fire, is not understood.

This study therefore focuses on investigating the combined role of elevated CO_2, drought and land use on the balance between shrubs and grasses in southern African savannas. The study used a climate gradient in Namibia, which spans from approximately 600 mm average annual rainfall in the North to 250 mm in the South to set up experiments at a nested scale of realism and detail. I conducted semi-controlled to more controlled experiments in Germany, in the greenhouse and climate chambers, respectively. Here, the intention was to investigate how tree-grass interactions at a seedling stage are mediated by drought and land use, and CO_2 as an additional independent variable. In Namibia, I performed a field experiment in a common garden, where tree-grass interactions were monitored under the following treatments: grazing, drought and nutrient addition. Unfortunately, the drought treatment was unsuccessful, so the experiment ended up with only grazing and nutrient addition.

The general finding for this PhD is that the grass species are not less negatively affected by drought but appear to be more affected by competition than shrubs. In addition, in the field experiment, surrounding herbaceous vegetation facilitated the emergence of one of the shrub's seedlings but did not affect the other species. Elevated CO_2 did not ameliorate the negative effects of drought on shoot biomass of any of the focal species. Overall, all my experiments have one common finding, and that is that drought was the most important factor in determining plant performance during the early life stages studied during this PhD.

I therefore conclude that according to the early life stages and focal species studied here, climate change will not be decreasing shrub encroachment but that actually enhanced encroachment is possible in response to drought and elevated CO_2. This is however not necessarily due to a larger positive response of shrubs to CO_2 as predicted, but rather to a less negative response to drought and competition.

Abstract (German version)

Das Weideland im südlichen Afrika verschlechtert sich zunehmend durch die Verdichtung von Büschen und Sträuchern auf Kosten von Gräsern. Diese sogenannte Verbuschung ist ein Grund zur Besorgnis für Weidelandmanager. Zudem gibt es aktuell noch keinen Konsens darüber, welche Mechanismen hauptsächlich für das Vordringen von Sträuchern in die südlichen Savannen Afrikas verantwortlich sind. Es ist daher schwierig, fundierten Rat zum Thema Weidelandmanagement zu erteilen.

Zur Auswirkung von Feuer, Beweidung, Wasser und Nährstoffverfügbarkeit auf die Produktivität von Weideland wurde bereits viel Forschung betrieben. Die Rolle des Klimawandels, d.h. Verschiebungen in der CO2-Konzentration und Niederschlagsmengen, als auch deren mögliche Interaktion mit kontrollierbaren Faktoren wie Beweidung und Feuer ist bisher nicht geklärt.

Diese Studie konzentriert sich daher auf die Untersuchung der Wechselwirkung zwischen CO2-Anstieg, Dürre und Landnutzung im Hinblick auf das Gleichgewicht von Sträuchern und Gräsern in den Savannen des südlichen Afrikas. Die Studie machte sich für den Aufbau der Versuche den klimatischen Gradienten in Namibia zu Nutze, der sich von den nördlichen Teilen des Landes mit einem durchschnittlichen Jahresniederschlag von ungefähr 600 mm bis zu den südlichen Regionen mit einem Jahresdurchschnitt von 250 mm Regen erstreckt. Die Versuche konnten so in einer verschachtelten Skala unter natürlichen Bedingungen und Einzelheiten stattfinden.

In Deutschland führte ich halb kontrollierte bis stärker kontrollierte Versuche im Gewächshaus bzw. Klimakammern durch. Ziel war es, zu untersuchen wie sich Trockenheit und Landnutzung, mit CO2 als einer zusätzlichen unabhängigen Variablen, auf die Interaktion von Baum- und Graskeimlingen auswirkt. In Namibia wurde ein Feldexperiment in einem sogenannten „Common garden“ (Allmende) durchgeführt. Baum-Gras-Interaktionen wurden unter folgenden Behandlungen beobachtet: Beweidung, Trockenheit und Nährstoffzufuhr. Der Versuch mit dem Parameter Trockenheit war bedauerlicherweise nicht erfolgreich, weshalb der Versuch nur mit den Parametern Beweidung und Nährstoffzufuhr durchgeführt wurde.

Die grundsätzliche Erkenntnis dieser Promotion ist, dass der Grasbestand von Trockenheit nicht weniger beeinträchtigt wird als der Buschbestand, Gräser jedoch stärker unter dem Konkurrenzkampf mit Sträuchern leiden. Im Feldexperiment unterstützte die umgebende krautige Vegetation zusätzlich das Aufkommen der Keimlinge einer Strauchart, während andere Arten nicht beeinflusst wurden. Der CO2-Anstieg wirkte sich nicht erleichternd auf die negativen Auswirkungen der Trockenheit auf die oberirdische Biomasse irgendeiner der untersuchten Arten aus. Insgesamt führen alle im Laufe dieser Promotionsarbeit durchgeführten Versuche zu einer gemeinsamen Erkenntnis: In den frühen Entwicklungsphasen hatte Trockenheit den stärksten Einfluss auf die Leistung der Pflanzen.

Ich komme daher zu der Schlussfolgerung, dass für die hier untersuchten Arten in der frühen Entwicklungsphase der Klimawandel nicht zu einer Verringerung der Verbuschung führen wird, sondern dass sogar eine zunehmende Verbuschung als Reaktion auf Trockenheit und CO2-Anstieg denkbar ist. Dies ist jedoch, entgegen der ursprünglichen Prognose, nicht unbedingt darauf zurückzuführen, dass Sträucher positiv auf eine höhere CO2-Konzentration reagieren, sondern vielmehr darauf, dass sie weniger empfindlich auf Trockenheit und Konkurrenzkampf reagieren.

Declaration of my own working contribution to the present thesis

The current thesis is based on work I did in greenhouses (University of Tübingen), climate chambers (University of Hohenhein) and in the field (Namibia), during my PhD. I co-designed the greenhouse and field experiments with my main supervisor, Katja Tielbörger, and the climate chamber experiments with both my supervisors (my main supervisor as well as co-supervisor Andreas Fangmeier), but conducted all the experiments myself. The statistical analysis for the greenhouse experiments, I performed myself, with assistance from Mark Bilton. All the statistical analysis for the other experiments I performed on my own. I also wrote all the chapters of this dissertation myself, with feedback from my supervisors.

Chapter 1

General introduction

Savannas cover approximately 20 % of the earth surface and are the most prevalent ecosystems in Southern Africa (Scholes and Archer, 1997). These ecosystems, which are characteristically composed of a unique mixture of coexisting trees, shrubs and grasses (Scholes and Archer, 1997), also support millions of African people, especially those living in rural areas (Kgosikoma and Mogotsi, 2013). Therefore, any form of degradation to these ecosystems has direct implications on the means of subsistence of the majority of people in those areas. Furthermore, rangeland degradation in African savannas has the potential to have especially devastating consequences because Africa in general is said to have a low capacity to adapt to environmental changes (IPCC, 2007). It is hence important to identify and manage both anthropogenic and natural causes of degradation in savanna ecosystems.

Probably the most common type of degradation in savannas is shrub/ bush encroachment, i.e. the expansion of shrubs at the expense of grasses. This spread of woody plant species into areas previously dominated by grasses (bush encroachment) is a serious concern for rangeland managers in southern African savannas (Archer et al., 1995a; Gil-Romera et al., 2010; Kgosikoma and Mogotsi, 2013; Puttock et al., 2014; Roques et al., 2001). Bush encroachment is associated with a degradation of rangelands, due to the reduction in the cover of (palatable) grasses, and is therefore highly undesirable in regions where most people depend directly on animal husbandry (Kgosikoma and Mogotsi, 2013). In African savanna ecosystems, this form of land degradation has led to losses of rangelands, arable land (Gil-Romera et al., 2010; Kgosikoma and Mogotsi, 2013) and hunting ground for rare animals like the Cheetah (De Klerk, 2004). Despite decades of research (De Klerk, 2004; Gil-Romera et al., 2010; Higgins et al., 2000; Hudak, 1999; Roques et al., 2001; Sala and Maestre, 2014; Scholes and Archer, 1997; D. Ward, 2005), neither the mechanisms by which shrubs take over at the expense of grasses nor ways of combatting bush encroachment are fully understood (Ault et al., 2014; Bond, 2008; Bond and Midgley, 2000a; Kgope et al., 2009; H Wayne Polley et al., 1997).

Drivers/ determinants of tree-grass interactions

There are many theories regarding the mechanisms that sustain/ are responsible for tree-grass coexistence in savanna ecosystems (De Klerk, 2004; Higgins et al., 2000; Hudak, 1999; Roques et al., 2001; Sala and Maestre, 2014; Scholes and Archer, 1997; Walter, 1971; D. Ward, 2005). An often-cited and perhaps the oldest hypothesis related to water that may explain the tree-grass coexistence in savannas is the two-layer hypothesis, originally coined by H. Walter (Walter, 1971). This hypothesis states that trees and grasses co-exist because they use water at different soil depths, with grasses utilizing shallow water and trees monopolizing water at greater depths in the soil (Sala et al., 1989; Walter, 1971). Therefore, changes in soil water conditions/ distribution is likely to affect the balance between shrubs and grasses in savanna ecosystems. Though this hypothesis has been much debated (Breshears and Barnes, 1999; Holdo, 2013; Le Roux et al., 1995; Nippert and Holdo, 2015; Rossatto et al., 2014; Sala et al., 1989; Sankaran et al., 2004; Scholes and Archer, 1997; Ward et al., 2013; Weltzin and McPherson, 1997), the mechanisms that govern competitive interactions among shrubs and grasses are most likely to determine whether shrub encroachment is favoured or not. However, while it seems to have much appeal, the two-layer hypothesis is limited to life stages where niche partitioning actually applies, i.e. when plants are fully grown. Thus, it does not apply to a seedling stage, where the two life forms use essentially the same sources of water and are more likely to compete (Scholes and Archer, 1997).

In addition, changes in fire regimes and/ or grazing intensity, are believed to be two of the major causes of bush encroachment (Archer et al., 1995; Bond et al., 2003; Sankaran et al., 2005; Scholes, 2003; Skarpe, 1992). For example, overgrazing, mostly by domestic livestock reduces fuel load, making fires too weak (less intense) to control the spread of wood (Kgosikoma and Mogotsi, 2013; Roques et al., 2001). Soil nutrients is another factor identified as a determinant/ driver of tree-grass dynamics, mostly because nutrients are generally concentrated in the top layers of the soil and grasses are reportedly better competitors for resources, such as water and nutrients, when the two life forms utilize the same resources (Higgins et al., 2000; Manea and Leishman, 2015; Scholes and Archer, 1997). In addition, most trees/ shrubs, especially leguminous ones may not require an external nutrient source, particularly in the early stages of development (Ernst, 1988; Kraaij and Ward, 2006) and therefore only grasses will be affected by soil nutrients at those stages. It has also been suggested that the removal of mega-browsers, such as rhinos and elephants from rangelands, further contributed to woody species having a competitive advantage over grasses (De Klerk, 2004; Hudak, 1999; Scholes and Archer, 1997).

Possible effect of climate change on tree-grass interactions

Climate change will affect many plant and animal species with possibly detrimental impacts. However, a modified climate will not only have direct effects but may also affect the way in which organisms interact (Klanderud, 2005; Montoya and Raffaelli, 2010). These indirect effects might even be stronger than direct effects imposed by e.g. higher temperatures or decreased rainfall (Rysavy et al., 2014; Tomiolo et al., 2015). The interaction between grasses and woody plants in savannas is an essential one because it is of great importance to human livelihood. Here, a change in the balance between these life forms can lead to shrub encroachment and subsequent loss of suitability as rangeland. Climate change increases the uncertainty related to predicting the balance between trees and grasses in savannas.

For large parts of Southern Africa, global scenarios predict increasing frequencies of drought events and overall decreasing precipitation (IPCC, 2007), i.e. the water-limited savanna ecosystem will become even drier. In addition, atmospheric carbon dioxide concentrations are expected to increase due to greenhouse gas emissions (IPCC, 2007, 2014). In African savanna ecosystems, trees and grasses are mainly characterized by two different photosynthetic pathways, which are expected to respond differently to these changes. The C_4 photosynthetic pathway of most grasses, is efficient in fixing carbon at low atmospheric CO_2 concentrations, as well as reducing water loss through transpiration, making them more energy and water efficient than woody plants (Bond, 2008; Gowik and Westhoff, 2011). C_3 plants perform below optimum at low atmospheric CO_2 concentrations, and therefore, it is predicted that elevated CO_2 levels may favour C_3 over C_4 plants (Bond and Midgley, 2012; Kgope et al., 2009; H Wayne Polley et al., 1997). Therefore, more frequent and prolonged droughts have been predicted to favour the “more water efficient” C_4 grasses over C_3 trees and shrubs (Bond, 2008; Gowik and Westhoff, 2011), i.e. droughts may initiate a process that counteracts bush encroachment. On the other hand, since C_3 plants will be fixing more carbon under elevated CO_2 levels, with decreased energy loses through photorespiration, they are expected to grow faster and take less time to recover after damage (Bond and Midgley, 2000b; H Wayne Polley et al., 1997). It is therefore regrettable that there are no empirical studies comparing the performance of the two life-forms under predicted climate regimes in savannas, especially with regard to both drought and elevated CO_2 concentrations.

Importance of studying early life-stages of plants

There have been several studies aimed at determining the combined effects of some of these factors, for example: rainfall, fire (Devine et al., 2015; Higgins et al., 2000; Zimmermann et al., 2008) and herbivory (Kraaij and Ward, 2006; Roques et al., 2001); grazing and biotic interactions (Macias et al., 2014); fire and CO_2 (Bond et al., 2003; Shanahan et al., 2016); as well as land use, climate and CO_2 (Archer et al., 1995b; Manea and Leishman, 2015). The downside of most of these studies is that (1) there is no empirical evidence to support the theory, (2) they only focused on one of the two life-forms (grasses or shrubs), and/ or (3) they are not looking at the seedling stage of the plants life-cycle.

Early life stages are most likely key to understanding the balance between grasses and shrubs, because this is the stage where direct competition, and thus a shift in the balance between life forms, is more likely to occur among plants that utilize essentially the same resources (Scholes and Archer, 1997). However, almost all previous studies on tree-grass interactions focused on adult plants, i.e. on stages in the development of savannas where partitioning of root space may reduce competitive exclusion between the two life-forms. Namely, according to the resource partitioning hypothesis (Walter, 1971), grasses mostly root in shallow soil layers whereas shrubs and trees have deep taproots (Bond, 2008; Gowik and Westhoff, 2011). Therefore, when looking at the role of water availability on tree-grass interactions, it is important to capture processes at the very early life stages of grasses and trees, e.g. at a seedling stage. As it is at this stage where both life forms are expected to compete for resources most strongly (Baudena et al., 2010; Scholes and Archer, 1997) and where drought effects on tree-grass interactions should be most prominent.

There early life stages such as germination (time to germination and germination rate), and seedling survival and establishment are therefore important bottlenecks for determining population dynamics at later stages (Giménez-Benavides et al., 2007; Miller, 1987; Quintana et al., 2004; Sankaran et al., 2004), and thus particularly relevant for studying the process of tree-grass interactions. It is therefore surprising that we know very little about this key stage in the establishment of plants in the context of shrub encroachment.

Thesis objectives and outline

In an attempt to fill some of the research gaps identified above, we decided to conduct experiments in the field, as well as in the greenhouse and climate chambers. This combination of experiments was chosen because the field experiment offers a realistic view of what is naturally occurring, but with little control over conditions. On the other hand, both greenhouse and climate chamber experiments provide the opportunity to control conditions, but at the cost of realism, especially in the climate chambers. A total of seven main hypotheses were tested using the three experimental approaches, with some hypotheses being tested in more than one experiment (Table. 1).

Table. 1. The main hypotheses addressed by the study and the methods/ experiments used to test them. The tick marks indicate the method used to test the hypothesis.

Hypothesis	**Greenhouse**	**Climate chambers**	**Field**
1. Drought will lead to a stronger decrease in performance of trees compared to grass seedlings	✓	✓	**Unsuccessful**
2. Elevated CO_2 will enhance the performance of trees more than that of grasses		✓	
3. CO_2 enrichment will mitigate the adverse effects of drought, and more so for the woody species		✓	
4. Tree seedlings will suffer more from the presence of the grasses than *vice-versa*, especially under drought stress	✓	✓	✓
5. Plants locally adapted to drier and more variable climates will be less sensitive to drought than plants originating from more humid sites	✓	✓	✓
6. Nutrient addition will benefit grasses more than tree seedlings			✓
7. Seedling survival will be higher for the grasses than the tree species in general, and especially under dry conditions			✓

This thesis addresses these hypotheses in three chapters, broadly representing the three methods used to gather data, as stipulated above. **Chapter 2**, which is based on a greenhouse experiment (University of Tübingen), focuses on effects of **heterospecific interactions, change in precipitation, and origin of plants on the balance between trees and grasses at the very early life stages**. Since it was imperative to be able to accurately determine the amount of CO_2 the plants were exposed to, we used top of the art climate chambers at the University of Hohenheim, where internal conditions could be controlled. Therefore, my third chapter (**Chapter 3**), looks at **the combined effect of elevated CO_2 and drought on the establishment of two encroaching tree species and two grasses**. **Chapter 4** is based on data from a common garden experiment in the field (Namibia), that investigated **the effects of simulated grazing and nutrient addition on seedling emergence and survival of two encroaching trees and two perennial grasses in a semi-arid savanna of Namibia**. The final chapter summarises all the key findings of this thesis and highlights their possible implications in terms of bush encroachment, as well as possible management interventions.

Study species

Our focal species included two shrub/ tree species, namely *Dichrostachys cinerea* (L.) Wight & Arn. and *Senegalia mellifera* (Vahl) Seigler & Ebinger (Vahl) subsp. *detinens* (Burch) Kyal. & Boatwr. (previously known as *Acacia mellifera*). *Senegalia mellifera* (Fig. 1D) has a wide distribution throughout Namibia, while *Dichrostachys cinerea* (Fig. 1C) is commonly found in the central and northern part, i.e. the wetter parts, of the country (P. Le Roux and Müller, 2009). In the following, I refer to the woody species as shrubs. Both species are indicators of disturbance, either by grazing or poor agricultural practices, often forming dense thickets, which lowers the carrying capacity of rangelands (P. Le Roux and Müller, 2009). Hence the two species have been the focus of many bush encroachment studies (Bond and Midgley, 2012; Buitenwerf et al., 2011; Gil-Romera et al., 2010; Joubert et al., 2008; Kgosikoma and Mogotsi, 2013; Moleele et al., 2002; Kgosikoma, 2012; Ringrose et al., 2003; Rohde and Hoffman, 2012; Wiegand et al., 2006).

Two perennial grasses (*Anthephora pubescens* Nees and *Stipagrostis uniplumis var. uniplumis* (Licht. ex Roem. & Schul.) De Winter) that coexist with the above shrub species, were also used in the study. *Anthephora* (Fig. 1A) is a climax grass, which is considered one of the most palatable and valuable grasses in Namibia (Müller, 2007). The dominance of this grass in an area is an indication of a good rangeland, as this is usually one of the first grasses to disappear from disturbed, e.g. overgrazed, areas (Müller, 2007). *Stipagrostis uniplumis var. uniplumis* (Fig. 1B) is more widespread and can also be found in disturbed areas (Müller, 2007). It is a subclimax grass with palatability varying on different soils as well as seasons (Müller, 2007). Due to its wide distribution range, this grass offers good grazing in dry areas (Heath and Heath, 2010; Müller, 2007).

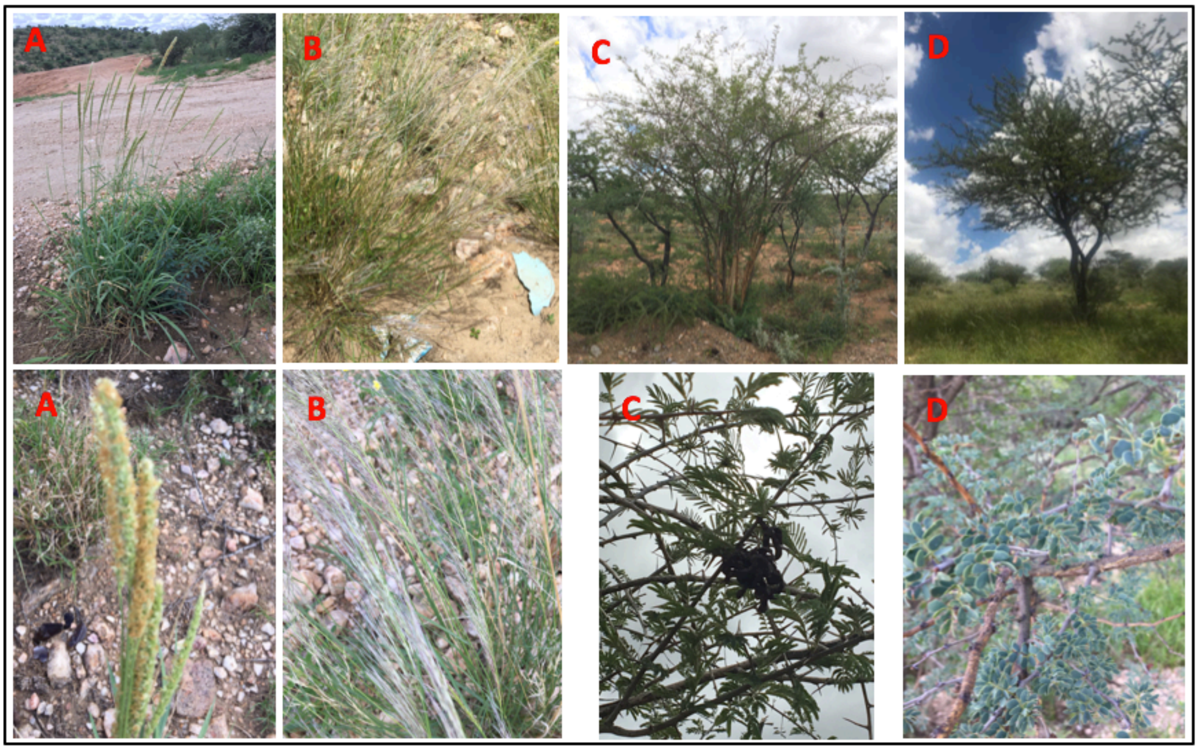


Fig. 1. Focal species for the study: *Anthephora pubescens* (A), *Stipagrostis uniplumis* (B), *Dichrostachys cinerea* (C) and *Senegalia mellifera* (D).

Study sites / seed origins

Bush encroached savanna ecosystems along a rainfall gradient in Namibia were used as sources of seeds for my study (Fig. 2). This provided the opportunity to indirectly mimic climate change in space, with respect to decreasing rainfall. The rainfall gradient represented most of the distribution range of the species and thus covered the maximum difference among sites possible. The differences in precipitation are more than two-fold between the most extreme sites, and local adaptation of plants to their areas of origin is likely. The selected study sites were: (i) Lake Otjikoto area in the north, with an average annual rainfall of about 550 mm; (ii) Waterberg and surrounding areas (average annual rainfall of about 450 mm); and Kuzikus and surrounding areas, receiving about 250 mm of rain per year (Fig. 2). In the following, I refer to these sites as Sub-humid (Lake Otjikoto area), Semi-arid (Waterberg and surrounding area) and Arid (Kuzikus and surrounding area).

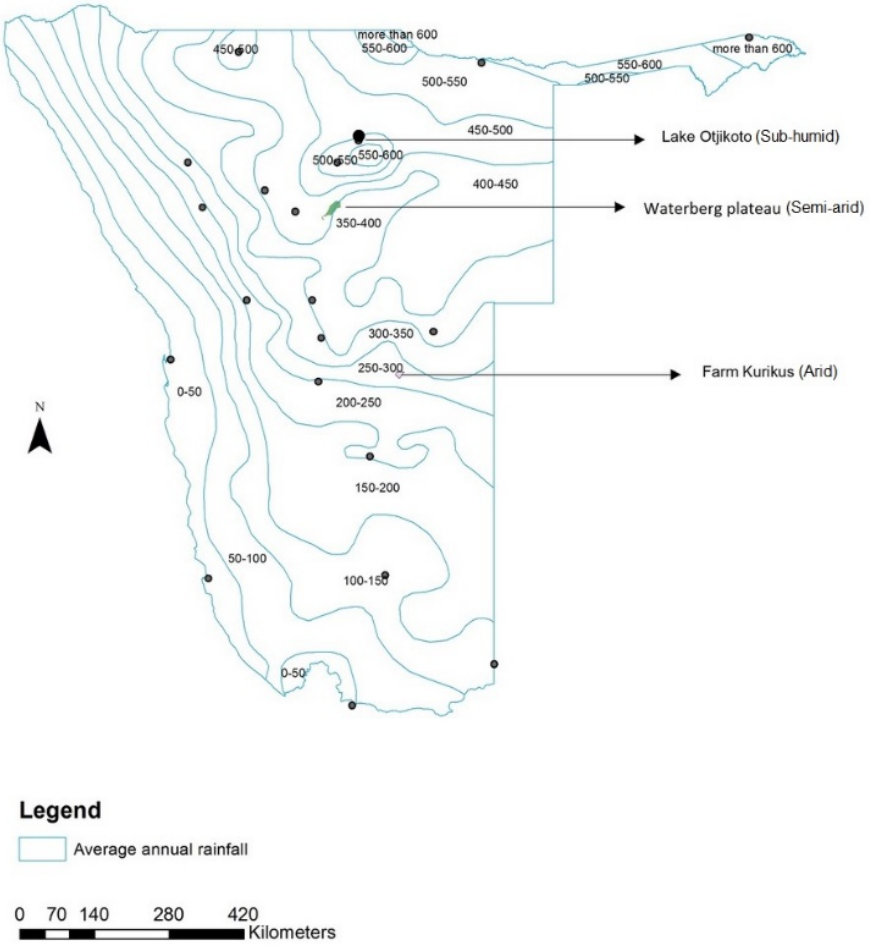


Fig. 2. Location of the study sites in Namibia including average annual rainfall isohyets. The map was constructed with data from the Ministry of Environment and Tourism (MET) (Mendelsohn, 2002).

Chapter 2

Early life-stages of two bush encroaching C_3 species and two C_4 grasses under different watering levels and heterospecific interactions - a greenhouse study

Abstract

In order to understand the mechanisms of shrub encroachment, we need to have knowledge about the interactions between the woody plants and grasses. There are a number of factors that have been put forward as determinants of shrub-grass interactions in savannas, such as herbivory, fire and competition for resources. Also, species inhabiting regions with high natural climatic variability are generally expected to be less vulnerable to climate change, because they are pre-adapted to a large range of climatic conditions. In this study, I looked at how the combination of simulated grazing (i.e. competitive release), change in precipitation, and origin of plants affect the balance between shrubs and grasses at the very early life stages. A greenhouse experiment was established for investigating germination and seedling biomass of the focal species under the following treatments: seed origin (three sites), competition (no neighbours *vs*. one heterospecific neighbour), and water (three levels). I expected that (i) grasses will be less affected by drought than shrubs, (ii) plants that stem from drier areas will cope better with drought, (iii) shrub seedling biomass will be suppressed by grasses, and (iv) this suppression will be less severe under dry conditions. The results show no evidence that grass germination and biomass was less affected by drought than trees, and the grasses seemed to suffer more from shrub competition. In addition, plants originating from drier areas did not reveal any adaptation to drought. The results of this study were therefore generally contrary to the hypotheses, which makes predicting the direction of bush encroachment under future climate even more difficult.

Introduction

Key to understanding the mechanisms of shrub encroachment is knowledge about the interactions between the woody plants and grasses. Namely, encroachment of shrubs happens at the expense of grasses, i.e. a change in competitive interactions among these two life forms. As stated in the general introduction, these shrub-grass interactions have been reported to be affected by determinants such as rainfall and herbivory (Higgins et al., 2000; Hudak, 1999;

Kgosikoma and Mogotsi, 2013; Roques et al., 2001; Scholes and Archer, 1997) because both life forms differ in their response to these two factors. For example, overgrazing by domestic livestock has been put forward as an important factor promoting shrub encroachment because these animals mostly feed on grasses (Ward 2005) and thus grazing could lead to competitive release in the coexisting woody plants. This process may be further aggravated by the simultaneous removal of mega-browsers from rangelands which mainly feed on shrubs and trees, i.e. both the increase in grazers that feed on grasses and the decrease in browsers provide woody species with an advantage over grasses (Hudak, 1999; Scholes and Archer, 1997).

Shrub-grass interactions may further be modified by climatic conditions, especially rainfall, because these two life forms differ in their water use efficiency. Namely, grasses are, as C_4 plants, more water efficient and could thus have an advantage over trees when there is a reduction in precipitation, which is the prediction for large areas in South African savannas (Bond, 2008; Gowik and Westhoff, 2011; IPCC, 2007). Therefore, bush encroachment is expected to be more rapid in wetter regions where trees are less water limited and are thus able to compete with grasses (Roques et al., 2001). In addition, according to the Stress Gradient Hypothesis (SGH), it is expected that plants originating from wetter and thus less stressful areas will be less affected by competition than those from arid environments (Bertness and Callaway, 1994). Unfortunately, while hypotheses about climate change effects on shrub-grass interactions are abundant, experimental data supporting them are rare. Especially a combination of climate manipulation and experimental change in competitive interactions is missing, thus depriving us of the ability to understand how shrub-grass interactions are modified by climate change.

One of the few existing experimental studies focusing on interactions among shrubs and herbaceous plants in drylands suggests that interactions between climate and simulated grazing could be important (Rysavy et al., 2014). Here, drought and competition effects on shrub seedling dynamics were studied along a rainfall gradient from a mesic Mediterranean to a semi-arid region in Israel. Against the initial hypothesis, annual neighbours had a stronger negative effect on shrub seedling survival under drought. This study further indicated that rainfall at the site of the experiment also affected the findings in that seedlings originating from the dry end of the rainfall gradient exhibited much higher survival. This indicates that studies conducted at single sites may not be sufficiently general to understand the phenomenon of shrub encroachment and that ecotypic variation in drought resistance should be taken into account.

There are a few observational studies on changes in woody vegetation densities along climatic gradients (Ringrose et al., 2003; Rohde and Hoffman, 2012; Wiegand et al., 2005), but they focused on whole communities with differing species composition among sites. Thus, species identity was confounded with life form, and climate-vegetation relationships were studied only in a correlative manner. Furthermore, even when the same species occurs at several sites along a gradient, local adaptation is likely (Jump and Peñuelas, 2005; McKay et al., 2001; Petrů et al., 2006; Volis, 2007) and such within-species variation in savanna ecosystems remains unexplored. This is regrettable because local adaptation is likely to interact with the ability of plants to cope with climate change. Namely, the adaptive capacity of species depends on whether or not they are narrowly adapted to the current climate (M. Davis and Shaw, 2001; Hoffmann and Sgrò, 2011).

Furthermore, it has been suggested that species inhabiting regions with high natural climatic variability would be less vulnerable to climate change, because they are pre-adapted to a large range of climatic conditions (Leger and Rice, 2007; Rysavy et al., 2014; Tielbörger et al., 2014; Volis et al., 2002). Unfortunately, there is virtually no systematic study in savannas that has covered similar species across several climatic regions to investigate whether or not the climate at the origin of the plant would determine its vulnerability to climate change and its interactions with other plant species. Such information is important because it could affect the setting of priority areas for conservation measures.

In this study, I aim at filling some of the above research gaps. Namely, I look at how the combination of simulated grazing (i.e. competitive release), change in precipitation, and origin of plants affect the balance between shrubs and grasses at the very early life stages. To study these questions, we selected an ideal study system in Namibia. The system includes a set of bush encroached savannas along a rainfall gradient, which are inhabited by similar shrub and grass species.

The following hypotheses were tested:

1. Shrubs will be more negatively affected by drought than grasses
2. Plants originating from drier sites will be better in tolerating drought, but weaker competitors
3. Grasses will be less affected by competition than shrubs
4. Competition will be less severe under drought conditions

Materials and methods

Seed collection and preparation

Grass seeds used for the study were collected at the three study sites between April and July 2015, and shrub seeds in November and early December of the same year. Unfortunately, this study was conducted during a drought period in Namibia (UNICEF, 2017), and therefore I could not obtain seeds of *A. pubescens* and *S. mellifera* at the sub-humid area that year. In addition, I depended on collecting seeds of the target species whenever we found them, i.e. sampling of plant individuals was not entirely at random. Grass seeds were collected by cutting as much of the inflorescence of the plant as possible (± 60%) and putting inflorescences of each individual plant in a separate bag. For the tree species, ripe pods were mostly collected from the trees and in a few cases picked from the ground under the individual trees. When it was not clear to which plant the seeds belonged, or very few (< 100) seeds were collected from one plant, seeds of neighbouring individuals were pooled and labelled as a bulk collection.

The reason for collecting seeds from individuals separately was to infer local adaptation among individuals from the same site. Unfortunately, due to low seed numbers per individual, it was mostly not possible to have one individual cover an entire replicate across all treatments. Thus, in the end all the seeds were treated as bulk collections and no individual response to treatments were considered in the analyses.

In Germany, the seeds were first stratified at 30 °C during the day and 20 °C, and 13-hour day length for about a month. This was done because a pilot study showed that this was necessary to break winter dormancy for some of the plants (Bruder, 2015). The conditions used for seed stratification were per the average day and night temperatures and day length for Windhoek during October 2015, which were the latest available data at the time. Since the seeds had already gone through Namibian winter before being shipped to Germany in September (grasses) and December (shrubs) 2015, there was no need for cold stratification.

Experimental design

The following treatments were applied: seed origin (three sites), species (four species), competition (no neighbours *vs*. one heterospecific neighbour), and water (three levels). All treatment combinations were replicated 20 times, apart for cases where lack of seeds limited sample sizes (Table 1).

The neighbour treatment was applied as follows: seeds of each species from each origin were either sown in a monoculture (single species per pot, thus no interaction, Fig. 1A) or in mixed culture (one shrub and one grass pair per pot, Fig. 1B). Due to a limited number of seeds, not all the neighbour combinations were possible (Table 1).

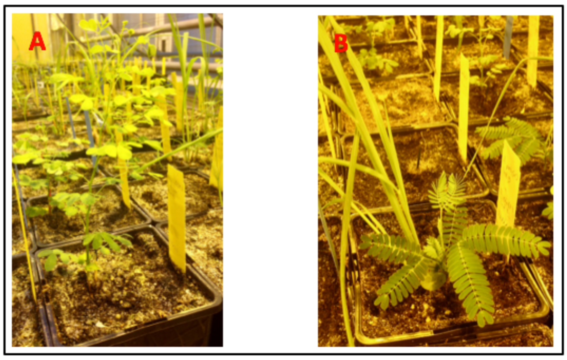


Fig. 1. An example of the neighbour treatment; *S. mellifera* growing alone (A), and *A. pubescens* and *D. cinerea* growing in the same pot (B).

Table. 1. Number of replicates per treatment for each species combination and three study sites.

Species	Arid	Semi-arid	Sub-humid
A. pubescens	20	20	0
S. uniplumis	3	20	20
D. cinerea	20	20	20
S. mellifera	20	8	0
A. pubescens & *D. cinerea*	20	20	0
A. pubescens & *S. mellifera*	20	0	0
S. uniplumis & *D. cinerea*	0	20	20
S. uniplumis & *S. mellifera*	4	8	0

Pots were arranged into 30 blocks, each representing a separate watering level to facilitate application of irrigation (Fig. 2). Thus, there were 10 blocks per watering level. The blocks were divided between two adjacent greenhouses and were randomized halfway through the experiment. The blocks also ensured that all the treatments were equally represented in the two greenhouses. In each block, the different species combinations were randomly assigned to pots, with each of the other treatments being replicated twice within a block.

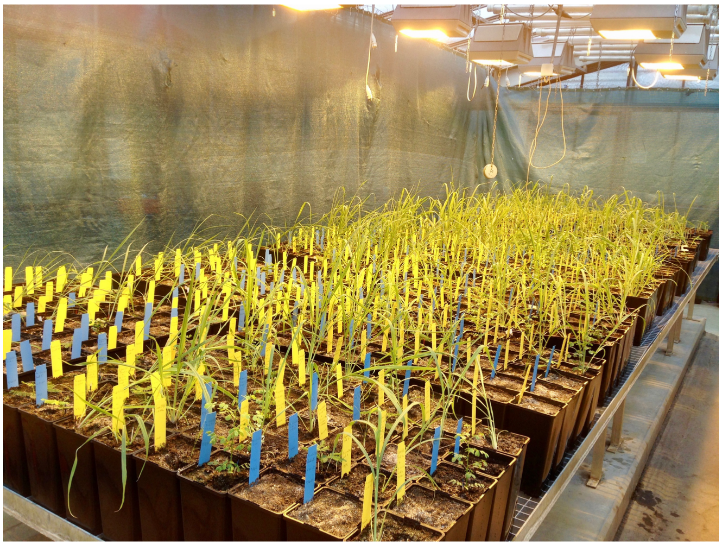


Fig. 2. An example of the experimental set-up in one of the two greenhouses.

Sowing

The seeds were sown in rose pots (11.35 cm x 11.35 cm x 20 cm (height)), to provide belowground depth for root growth. Twenty seeds per pot for each grass species and 10 seeds for the trees were sown. Seeds were sown in a one-to-one mixture of sand and "Pikiererde" soil, to allow water infiltration, while reducing the rate at which the soil will dry (especially in the high-water treatment). Seeds were sown into each pot by scattering them around the pot, and then covering them with a thin layer of soil. This was done because *S. mellifera* (Kraaij and Ward, 2006) and *S. uniplumis* (Zimmerman *et al.*, 2008) need to be slightly buried to improve germination.

Watering

The watering levels were determined after a pilot study and set at 500 ml of water for the high-water treatment, while the two lower water treatments both received 250 ml, the medium treatment after 5 days and the low water treatment after every 10 days. The initial watering levels were higher, to initiate germination. After that initial watering, the watering levels were adjusted several times throughout the study, to ensure that the high-water treatment pots always remained saturated while the other two could dry, with the low water treatment drying up the most. To that end, the high-water treatment always received enough water to ensure that water percolated out of the holes at the bottom. Due to the soil not drying as expected, the watering amounts for the two drier treatments were reduced to 200 ml and then 100 ml.

Monitoring efficiency of treatments

A TRIME-FM portable moisture measurement device was used to measure soil moisture from one pot selected randomly in each block, 17 times during the experiment, mostly before and after watering events.

Dependent variables

Germination was recorded for 20 days, and the time to germination noted. Germination fractions were calculated as the fraction between seedlings germinating and the number of seeds sown. After 20 days, the seedlings were thinned to one randomly selected plant in no-neighbour treatments and two plants in neighbour treatments, respectively. After four weeks, the remaining seedlings were harvested; their shoots dried to a constant mass at 70° C for 48 hours, and then weighed to determine individual seedling dry biomass for each species per pot.

Data analysis

All the data were analysed with SPSS Statistics for Windows (IBM, Version 24.0., 2016). The different watering levels were compared using soil moisture data, with a One-way ANOVA and Tukey HSD post hoc test. Due to the highly unbalanced design with respect to origin, biomass data were analysed separately for each species.

A Normal distribution, Generalized Linear Mixed Model (GLMM), with an identity link function was used to compare the time taken by the different ecotypes of each species to germinate under the three watering levels. The "days to germination" data for each species were log-transformed and analysed, with seed origin and water as fixed factors. To compare germination fraction of the focal species under the different treatments, I used a GLMM, with a Binomial distribution and Logit link function. The fixed factors for each species model were the same as in the "days to germination" models.

To test for the different treatment effects and their interactions on species biomass, I used a Normal distribution, GLMM and an identity link function. The fixed factors for the models were a full factorial combination of origin, water and neighbour. Since there was no neighbour combination that was represented across all ecotypes (Table 1), I was unable to test the hypothesis that "plants originating from drier sites are weaker competitors". The removal of this term (origin x neighbour) from the different species models did not change any of them (AICs remained constant). *S. uniplumis* and *D. cinerea* data were log-transformed before analysis.

For all the GLMMs, watering level was nested into block as a random factor, and the Least significant difference was used for post-hoc pairwise comparisons.

Results

All three watering levels differed significantly in terms of percentage soil moisture (F= 223.54, df= 2, p< 0.001), with the high watering level having the highest and the low the lowest soil moisture (Fig. 3). This indicates that the irrigation treatment was successful.

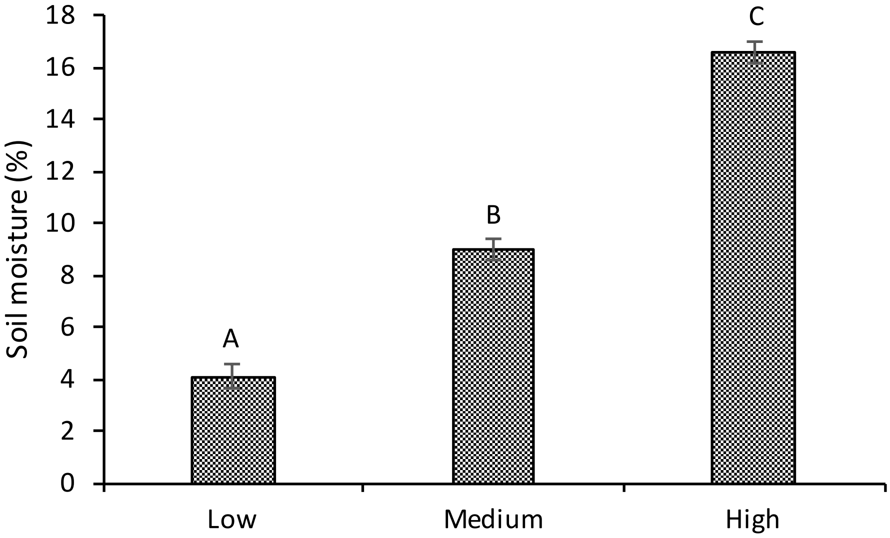


Fig. 3. Percentage soil moisture (mean ±SE) measurements from pots that received the three (low, medium, high) watering levels, respectively. Different letters indicate significant differences (Tukey HSD post hoc test).

(i) Effects of drought on shrubs vs grasses

Time to germination was generally shortest under higher watering levels, for all species (Table 2, Fig. 4A). There was a general trend of higher germination with increasing water, which was more obvious for *Anthephora* and *Dichrostachys*, although all three watering levels only differed for the latter species (Table 2, Fig. 4B). Seedling biomass also exhibited a trend of increase with increasing water (Table 2, Fig. 4C). Again, the effect was more pronounced for *A. pubescens*, although *S. mellifera* also showed a similar trend (Table 2, Fig. 4C).

Table. 2. Results for the effect of drought on the time (days) to germination (GLMMs with Normal distribution and identity link function), germination rate (GLMMs with Binomial distribution and Logit link function) and shoot biomass (GLMMs with Normal distribution and identity link function), for each of the focal species. Significant results ($p< 0.05$) are indicated in bold.

Drought effects	***A. pubescens***			***S. uniplumis***			***D. cinerea***			***S. mellifera***		
	F	**df 1**	**P**	**F**	**df 1**	**P**	**F**	**df 1**	**P**	**F**	**df 1**	**P**
Time to germ.	7.94	2	**0.001**	4.67	2	**0.014**	3.89	2	**0.028**	3.73	2	0.29
Germ. rate	6.25	2	**0.003**	0.004	2	0.996	8.059	2	**<0.001**	2.99	2	0.056
Biomass	9.05	2	**<0.001**	1.31	2	0.276	0.76	2	0.471	10.1	2	**<0.001**

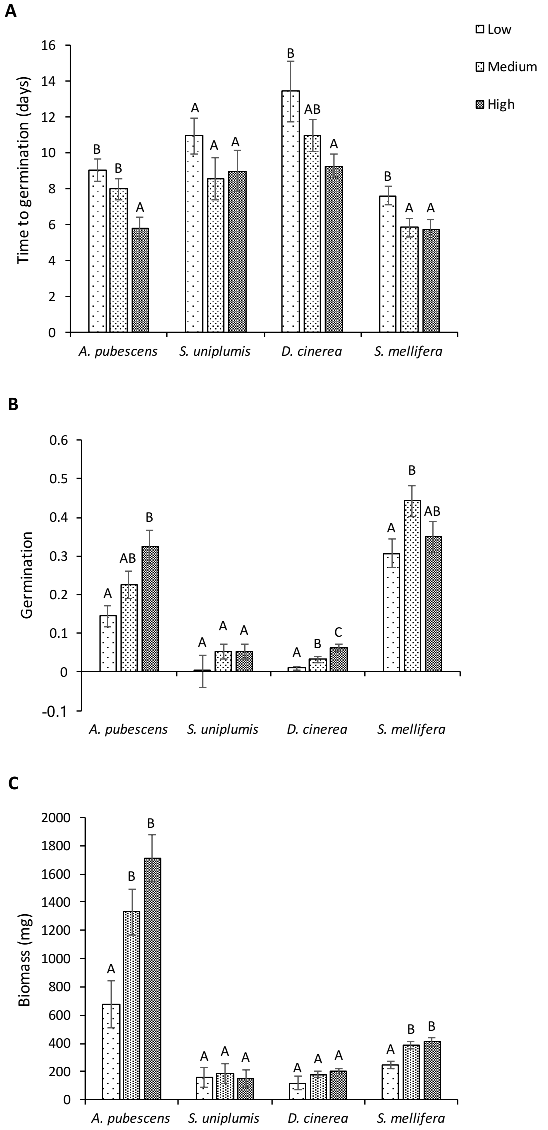


Fig. 4. Mean (±SE) time to germination (A), germination fraction (B), and shoot biomass (C), of the focal species in response to three watering levels. Different letters indicate significant differences within species.

(ii) Origin effects on drought tolerance

The germination fraction of *S. uniplumis* under the low water level was significantly higher for the two wetter ecotypes, though under the medium watering level the sub-humid ecotypes had significantly lower germination rate than the semi-arid ones (Table 3, Fig. 5). There was no significant water and origin effect on seedling biomass of all the species. However, there was a tendency suggesting a possible origin effect on drought tolerance for *S. mellifera*, as the lowest watering level for the arid ecotype had significantly lower biomass than the two higher levels, but for the semi-arid ecotypes the two lower watering levels did not differ significantly in their biomass (Table 3, Fig. 6).

Table. 3. Results of GLMMs with a Normal distribution and identity link function, testing the effect of seed origin on drought tolerance of the focal species, with seedling biomass as a response variable. Significant results ($p < 0.05$) are indicated in bold.

Origin x	***A. pubescens***			***S. uniplumis***			***D. cinerea***			***S. mellifera***		
Drought effect	**F**	**df 1**	**P**	**F**	**df 1**	**P**	**F**	**df 1**	**P**	**F**	**df 1**	**P**
Time to germ.	1.78	2	0.171	0.38	3	0.770	1.82	4	0.143	0.34	2	0.716
Germ. rate	1.34	2	0.267	3.546	4	**0.009**	1.63	4	0.169	0.09	2	0.915
Biomass	1.84	2	0.162	0.48	2	0.619	0.22	4	0.925	2.36	2	0.097

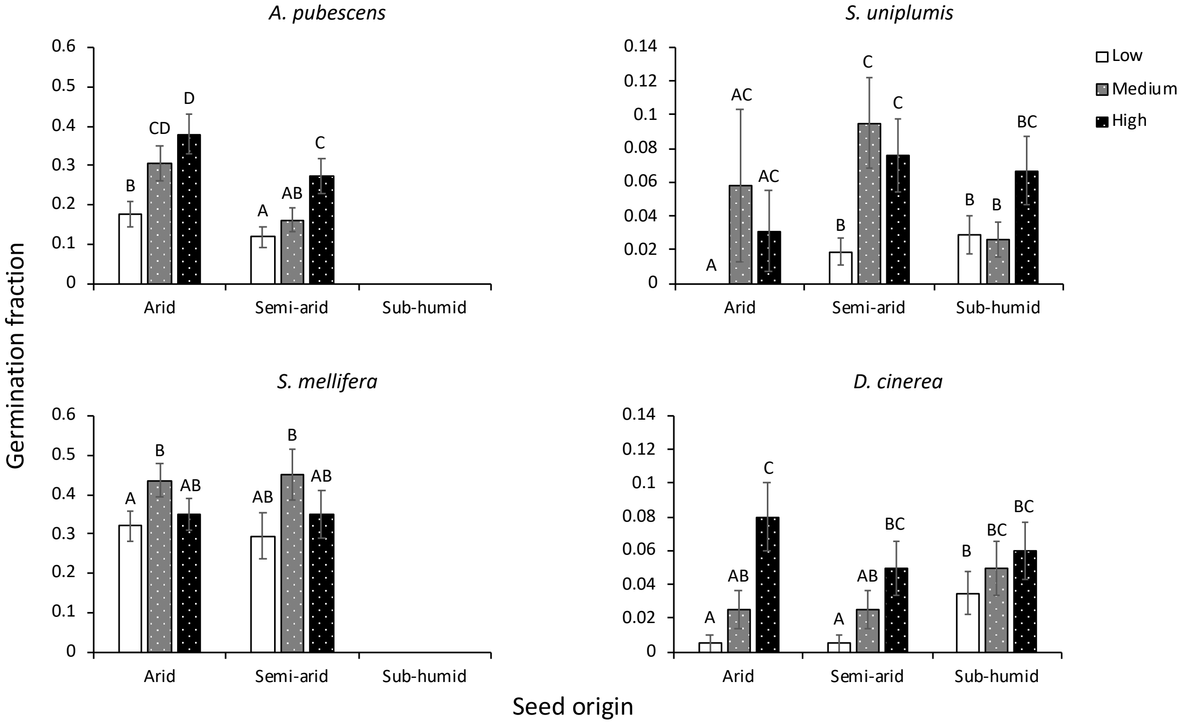


Fig. 5. Mean (+/- SE) germination fraction for the different ecotypes (seed origin) of the focal species (grasses on top and shrubs at the bottom) in response to three different watering levels. Different letters represent significant differences and are unique to each species graph.

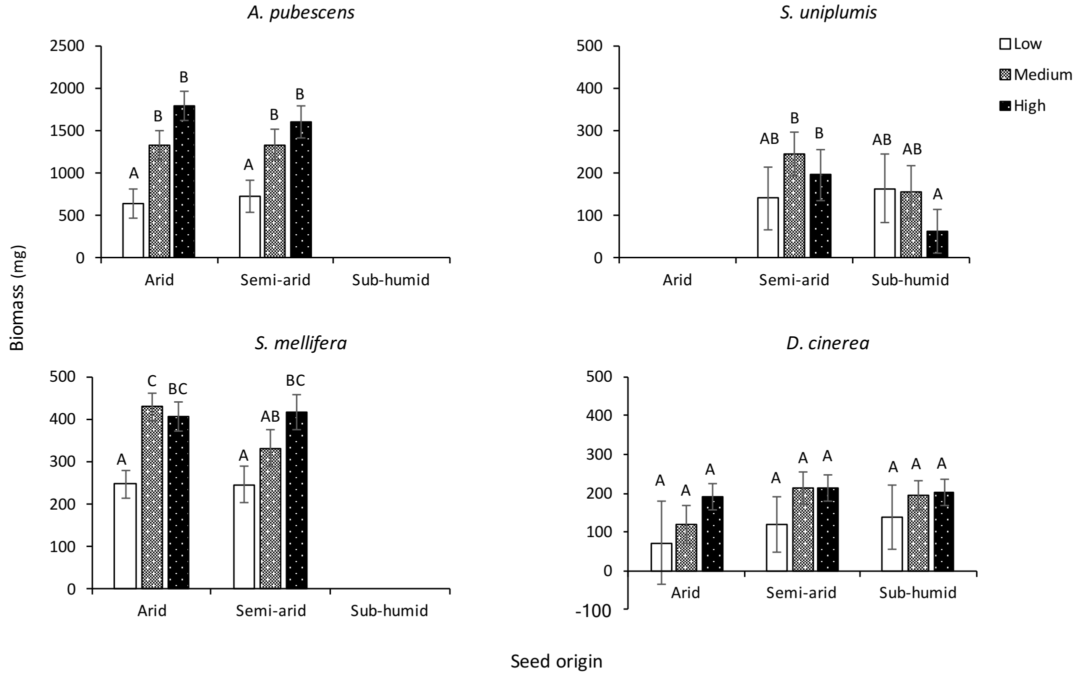


Fig. 6. Mean (+/- SE) biomass for the different ecotypes (seed origin) of the focal species (grasses on top and shrubs at the bottom) in response to three different watering levels. Different letters represent significant differences and are unique to each species graph.

(iii) Life-form and drought effects on competition

Both grasses were affected by *S. mellifera* competition, but the effect was only significant for *A. pubescens* under the two higher water levels (Table 4, Fig. 7). Although the test showed a significant effect of neighbour on *S. mellifera* (Table 4), this was due to the two grasses differing in their effects on the shrub (*A. pubescens* displaying slight overall competition and *S. uniplumis* slight facilitation), and not a difference between the control and the neighbour treatments (Fig. 7, Appendix 1). There was no significant interaction between neighbour and water for any of the species (Table 4, Fig. 7).

Table. 4. Results of GLMMs with a Normal distribution and identity link function, testing the effect of water (H_2O), seed origin (Orig.) and neighbour (Neigh.) on the focal species' seedling biomass. Significant results ($p< 0.05$) are indicated in bold.

	A. pubescens			*S. uniplumis*			*D. cinerea*			*S. mellifera*		
Effects	**F**	**df 1**	**P**	**F**	**df 1**	**P**	**F**	**df 1**	**P**	**F**	**df 1**	**P**
Orig.	3.55	1	0.061	3.34	1	0.071	0.36	2	0.697	3.38	1	0.068
Neigh.	7.86	2	< **0.001**	3.59	2	**0.031**	1.44	2	0.243	4.36	2	**0.014**
H_2O	9.05	2	< **0.001**	1.31	2	0.276	0.76	2	0.471	10.06	2	< **0.001**
Orig. x H_2O	1.84	2	0.162	0.48	2	0.619	0.22	4	0.925	2.36	2	0.097
Neigh. x H_2O	0.57	4	0.688	0.23	4	0.919	1.68	4	0.161	1.58	4	0.183

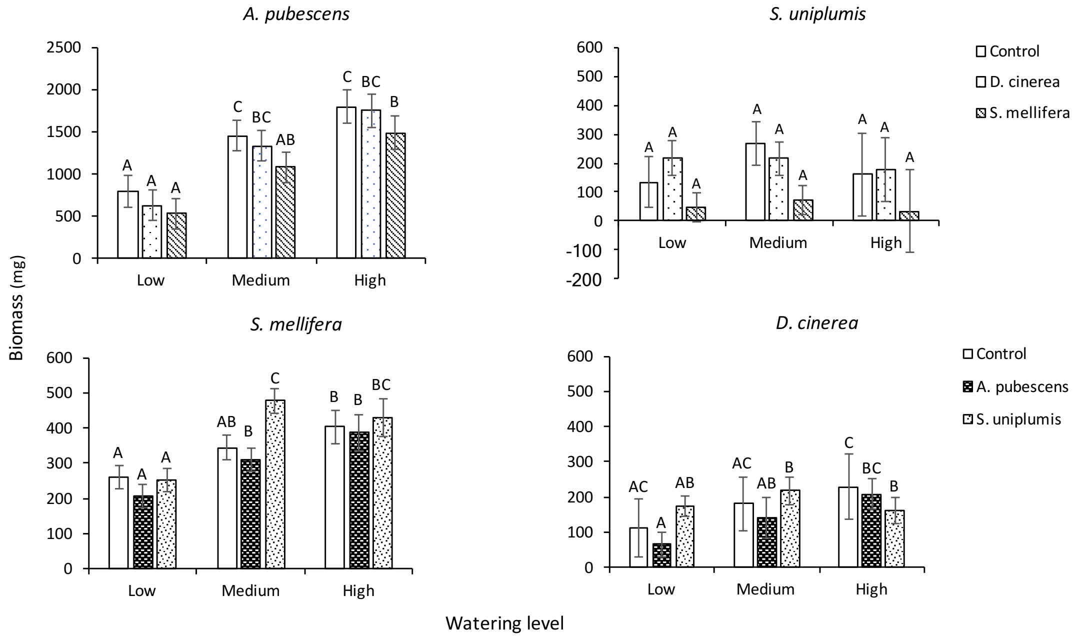


Fig. 7. Shoot biomass (mean ± SE) for the focal species, in response to three watering levels and heterospecific neighbours. The grasses are in the top row (with either *D. cinerea* or *S. mellifera* as a neighbour) and the shrubs in the bottom row (showing the effect of *A. pubescens* and *S. uniplumis* as neighbours, respectively). Different letters represent significant differences and are unique to each graph.

Discussion

Water availability and competition appeared to be the two most important factors in determining early establishment success in the focal species. These responses were also species specific, but not all responses were in the predicted direction. In the following, I discuss the findings with respect to the initial hypotheses.

(i) Shrubs will be more negatively affected by drought than grasses

The two early germinating species, namely *A. pubescens* and *S. mellifera*, had the highest germination fraction, highest biomass, and were also the strongest competitors (especially *S. mellifera*). This can be explained by the so-called priority effect, which states that species that germinate first may affect the establishment of later germinating species (Gioria and Osborne, 2014; Turkington et al., 2005; Von Gillhaussen et al., 2014; Wainwright et al., 2012) and therefore determine population dynamics at later stages (Giménez-Benavides et al., 2007; Miller, 1987; Quintana et al., 2004).

Although there was a general trend of higher germination with increasing water, only *A. pubescens* and *D. cinerea* had significantly lower germination with lowering water levels. This is not surprising because field observations during seed collection and their distribution ranges (P. Le Roux and Müller, 2009; Müller, 2007), clearly indicate that the two species commonly occur in the more wetter parts of the study area (rainfall gradient) and do not extent as far south as the other two. Suggesting that they might be more sensitive to low water conditions than the other two. Contrary to our expectations however, lower water conditions did not have a more negative effect on shrubs than grasses. In fact, the two species negatively affected by low water conditions in terms of biomass, were *A. pubescens* and *S. mellifera*, (a grass and a shrub). This goes against studies that argue that C_4 plants are more drought tolerant than C_3 plants (Hamim, 2005; Osborne and Sack, 2012; J. K. Ward et al., 1999).

On the other hand, it has also been suggested before that the drought tolerance of C_4 plants is not ubiquitous (Ibrahim, Gilbert, Ripley, & Osborne, 2008; Ripley, Frole, & Gilbert, 2010; Ripley, Gilbert, Ibrahim, & Osborne, 2007; Taylor, Ripley, Woodward, & Osborne, 2011). According to Ripley et. al. (2010), while C_3 plants response to drought is largely stomatal, C_4 plants may be limited in their assimilation of CO_2 under drought conditions, rendering them less efficient in their water usage. The difference in the performance between the two grasses, could be attributed to the fact that there was generally very low germination for the unaffected species (*S. uniplumis*), leading to fewer replicates, especially for the low water treatment. Thus, though there might have been a trend towards decreasing biomass with less water, as can been seen for the arid ecotype of *A. pubescens*, the power of the tests was probably not large enough.

Alternatively, since the species that had reduced biomass under low water were the two with the highest biomass, the drought effect on species finding might simply be a case of the fast-growing species depleting the limited water in the pots faster than the smaller ones. This could imply that recruitment during years with long breaks between rainfall events will be low for these fast-growing species, due to high seedling mortality. In which case, early germination and fast growth might not be an advantage (Wainwright et al., 2012). However, the priority effect may actually prevail for *Anthephora* during high rainfall years, leading to the possible suppression of later germinating shrub species.

(ii) Plants originating from drier sites will be better in tolerating drought

There was generally no ecotypic response to varying water availability. This is a notable contrast to previous studies which found plants from drier sites to tolerate drought better (Johnson et al., 2015; Leger and Rice, 2007; Petrů et al., 2006; Rysavy et al., 2014; Tielbörger et al., 2014; Volis et al., 2002). This uniform response to varying water availability by seedlings from the different sites along the rainfall gradient may suggest that drought, as a consequence of climate change will affect my study species similarly, irrespective of the level of aridity in their current environments. Although this will depend on which part of the gradient will experience the greatest effect of climate change, i.e. the area most affected by climate change will experience the greatest reduction in seedling biomass.

Although our study area covered a fairly extensive range within Namibia, which as a country has a mean annual rainfall range from less than 50 mm in the west to more than 600 mm in the north-east (Mendelsohn, 2002), it does not cover the entire climatic range across which savannas occur. Savannas in Africa alone are expected to fall within the limit of less than 250 mm to about 1600 mm (Lehmann et al., 2011), meaning Namibia as a whole is rather at the dry end. Furthermore, our study area may have entirely fallen within the semi-arid region of the savanna biome, which have been reported to range between 250- 500 mm mean annual rainfall (Hill, 2002; Maliva and Missimer, 2012). Therefore, since our rainfall range is not that extensive when we consider the entire savanna range, our focal species may have developed plastic mechanisms that allow them to occur under the different water conditions within the entire rainfall range within which they occur. Hence the lack of water-origin interaction, even though all three watering levels were different in terms on soil moisture.

On the other hand, this apparent lack of local adaptation has been recorded before in other systems (Dorman et al., 2009; Tomiolo et al., 2015) and could be attributed to there being other factors that are different among the study sites, such as soil water holding capacity or microbial communities (Tomiolo et al., 2015). The different ecotypes may therefore have reacted similarly due to the absence of those other factors not included in this study, since they were grown in the same substrate. This suggests that there might be a different outcome in nature when the ecotypes are grown in their native environments.

(iii) Grasses will be less affected by competition than shrubs and competition will be less severe under drought conditions

Contrary to our expectations, grasses did not suffer less from competition; in fact, both grasses suffered from *S. mellifera* competition. This is surprising because previous studies have found grasses to be better competitors when the two life forms use the same resources (Higgins et al., 2000; Manea and Leishman, 2015; Scholes and Archer, 1997), as is the case in the early life stages studied here. It also seems to contradict the idea that overgrazing by cattle may be a main reason for shrub encroachment, i.e. the removal of grasses favours shrub establishment. However, this result might also suggest that the processes happening in an established grass matrix may not be the same as in a pot with one grass seedling competing with a shrub seedling. Generally, when there are few shrub seedlings in a grass matrix, as is the case in nature, the grasses are able to exploit the resources more efficiently due to their extensive root system (Higgins et al., 2000; Manea and Leishman, 2015; Scholes and Archer, 1997). Therefore, the competitive advantage that has been recorded in grasses might be density dependent and only occur in a dense matrix. It may also only occur later, when grasses have developed more extensive roots and not in the early life stages.

The intensity of competition did not vary with the amount of water available to the plants, which was rather puzzling, because theory predicts that competition intensity should increase with increasing resource availability (Bertness and Callaway, 1994; Dohn et al., 2013; Ladd and Facelli, 2005; Roques et al., 2001). I attribute this to very low germination in the lower water treatments, reducing the power of the tests. Although there was a pattern of *A. pubescens* suffering slightly more from competition from both shrubs, under low water conditions. In addition, due to mismatched neighbour combinations across ecotypes, it was not possible to determine origin-neighbour interactions.

In conclusion, the results of this study made predicting the direction of bush encroachment under future climate even more difficult, as they were generally contrary to the hypotheses. For example, grasses were not less negatively affected by drought, but appeared to be more affected by competition than shrubs. Furthermore, plants originating from drier areas did not reveal any adaptation to drought, most probably because there might be other factors not measured in this study, which together with soil moisture might lead to differential ecotypic reactions. Future studies should therefore consider reciprocal transplant experiments, to determine how the different species respond to their native vs. foreign environmental conditions.

Chapter 3

Drought and elevated CO_2 effects on trees and grasses in southern African savannas – a climate chamber study

Abstract

Despite much research, the main causes of bush encroachment remain unclear. In addition, climate change may add further uncertainty related to predicting the balance between shrubs and grasses in savannas. For example, more frequent and prolonged droughts should favor C_4 (grasses) over C_3 (trees/shrubs) plants. Vice-versa, elevated CO_2 concentrations should favor shrubs. Unfortunately, despite myriads of conceptual and speculative studies about the effects of elevated CO_2 on shrub-grass interactions in savannas, there are virtually no experiments. Here, I determined the combined effect of elevated CO_2 and drought on the establishment of two encroaching shrub species and two grasses. Seeds were collected at three sites along a rainfall gradient in Namibia and sown in six climate-chambers with either ambient or elevated CO_2 concentrations. After germination, seedlings were randomly assigned to either a control or drought treatment and performance measures obtained from them. We expected that a) drought affects the growth of shrub seedlings more negatively than grasses, b) shrub seedlings show a stronger positive response to elevated CO_2 than grasses, and c) CO_2 enrichment mitigates the adverse effects of drought. The results suggest that drought will play a more important role in determining performance of savanna plants under predicted climate regimes than CO_2. Elevated CO_2 did not compensate for shrub fitness losses caused by drought, indicating that shrub encroachment may slow down under predicted climate change. There is however need for caution as the shrub species, particularly *S. mellifera* seemed to be less affected by drought in terms of shoot biomass, and both had longer roots under drought stress, which might suggest that they are better equipped to survive under such conditions.

Introduction

Another important factor related to climate change are elevated atmospheric CO_2 concentrations due to greenhouse gas emissions. These are particularly interesting in the context of bush encroachment because of the two photosynthetic pathways that should cause differential susceptibility to elevated CO_2. Namely, elevated CO_2 should favour C_3 plants more than C_4 plants. Therefore, a highly interesting interaction occurs between drought and CO_2 in that elevated CO_2 should counteract negative effects of drought on shrubs and trees and thus favour shrub encroachment (Bond and Midgley, 2012; Kgope et al., 2009; Polley, 1997). Unfortunately, despite myriads of speculative, conceptual and some quantitative modelling studies about the effects of elevated CO_2 on shrub-grass interactions in savannas, experimental evidence to support the theories is virtually missing. Namely, almost no experiments exist that manipulate CO_2 in shrub-grass systems of African savannas and even less that combine CO_2 effects with manipulation of water availability.

There is one study by Kgope et al. (2010) on growth responses of African savanna trees to different CO_2 concentrations. Although the study found that seedling growth was significantly enhanced by marginally elevated CO_2 concentrations for the trees (*Acacia karoo* and *Acacia nilotica*) but remained unaffected in a C_4 grass (*Themeda triandra*), this difference was not observed between 370 and 550 μmol mol^{-1}, which would represent ambient and future elevated CO_2 concentrations, respectively. Furthermore, the study was performed in open-top chambers, which have been criticized (also by Kgope et al. (2010)) for the fact that the conditions within the chambers cannot be controlled. Moreover, the plants were not germinated together, making conclusions about their interactions under drought and elevated CO_2 impossible. Therefore, there is a clear need for studying CO_2 effects on shrubs and grasses and their interactions using state-of-the art methods such as closed climate chambers or FACE systems.

This study aims to bridge these research gaps. Namely I look at the combined effects of elevated CO_2 concentration and drought on establishment of four coexisting species of southern African savannas: two encroaching C_3 shrub species and two coexisting C_4 grasses. Additionally, I looked at the effects of these changes on shrub-grass interactions.

In addition, previous work has shown that plants originating from areas with different environmental conditions (e.g. different levels of precipitation) may respond differently to changes in climatic conditions as a result of local adaptation (Franks et al., 2014; Hagenah et al., 2009; Johnson et al., 2015; Leimu and Fischer, 2008; Kgosikoma, 2012; Rysavy et al., 2014; Savolainen et al., 2007; Wang et al., 2016). Therefore, drawing general conclusions on species' reactions to predicted climate change regimes based on plants from a single locality might be misleading. For example, it has been suggested that plants that stem from more variable climates may be much less vulnerable to climatic extremes such as drought (Johnson et al., 2015; Petrů et al., 2006). Unfortunately, previous studies about shrub encroachment were usually done within a single site and ignored potential ecotypic differences among sites differing in amount and variability of precipitation (Bell and van Staden, 1993; Gil-Romera et al., 2010; Meyer et al., 2005; Roques et al., 2001; Tessema et al., 2011). The same applies to the handful of experimental studies about CO_2 effects on shrubs and grasses (Kgope et al., 2009; Manea and Leishman, 2015). Here, I compared seedlings germinated from seeds collected along a rainfall gradient to infer the degree of local adaptation to drought, as well as determine the effect of elevated CO_2 concentration.

In summary, the following hypotheses were tested:

(1) Drought will lead to a stronger decrease in performance of shrubs compared to grass seedlings
(2) Elevated CO_2 will enhance the performance of shrubs more than that of grasses
(3) CO_2 enrichment will mitigate the adverse effects of drought, and more so for the shrub species
(4) Plants locally adapted to drier and more variable climates will be less sensitive to drought than plants originating from more humid sites
(5) Elevated CO_2 will be more beneficial for ecotypes from wetter climates
(6) When grown together, the shrubs will suffer more from the presence of the grasses than *vice-versa*

Materials and methods

Experimental design

General considerations: The results of own observations in the greenhouse (Chapter 2), in the field (Chapter 4), and of a pilot study in a climate chamber (Bruder, 2015), indicated that there is a high uncertainty regarding whether the focal species produce seeds in any given year or site and whether they germinate or not. Furthermore, the high number of factors (water, CO_2, origin, competition, and species) would mean a sacrifice in sample sizes when designing the experiments. Therefore, to ensure high replication and statistical power and minimize the risk of complete failure (due to lack of seeds or insufficient germination), a two-step approach was followed. Namely, I performed two experiments with seeds collected over two consecutive years. The first experiment focused on the performance of the focal species as a function of the factors origin, CO_2, and water. It also served to optimize the experimental procedure and for deciding about a subset of species for the follow-up experiment. The second experiment was then done on one grass and one shrub species, both because these exhibited the highest germination in experiment 1 and because no seeds (*Dichrostachys*) or very few seeds (*Stipagrostis*) were available for that experiment. With half the species, we could add the remaining treatment, namely competition. The second experiment thus served mainly to investigate the effect of a heterospecific neighbour on performance. In addition, it enabled us to test whether the general patterns regarding CO_2 and water effects can be confirmed for the two species and whether there are interactions with the competition treatment.

Experiment 1

The first experiment was a full factorial design for each species separately with three factors, namely CO_2 and water, each having two levels and seed origin, each having either two levels (*S. mellifera*) or three levels. The two CO_2 levels were ambient (400 µmol mol^{-1}) and elevated (550 µmol mol^{-1}), and the water levels were regular watering and a drought treatment. We therefore had the following four treatment combinations for each species and site: ambient CO_2 with regular watering (control), ambient CO_2 with drought (drought), elevated CO_2 with regular watering (elevated CO_2), and elevated CO_2 with drought.

Seed collection and preparation

Tree seeds for the first experiment were collected from November to December 2015, while grass seeds were collected from April to May 2016. Due to a fire in the area earlier that year, it was not possible to sample *S. mellifera* seeds from the sub-humid site. Furthermore, no seeds were available from the arid site for *S. uniplumis*. Seeds were then sent to Germany for the climate chamber experiments. Based on a pilot experiment (Bruder, 2015), the seeds were stratified by first exposing them to Namibian winter temperatures and day length, and then the temperature was gradually increased and day length adjusted to reflect growing season conditions. The experiment was then conducted in six climate chambers (Vötsch BioLine, Balingen, Germany) at Hohenheim University, Germany (Fig. 1A). These are closed chambers, in which CO_2 concentrations as well as photon flux density, temperature and humidity can be controlled. Three of the climate chambers were set to ambient, while the other three were at elevated CO_2 concentrations.

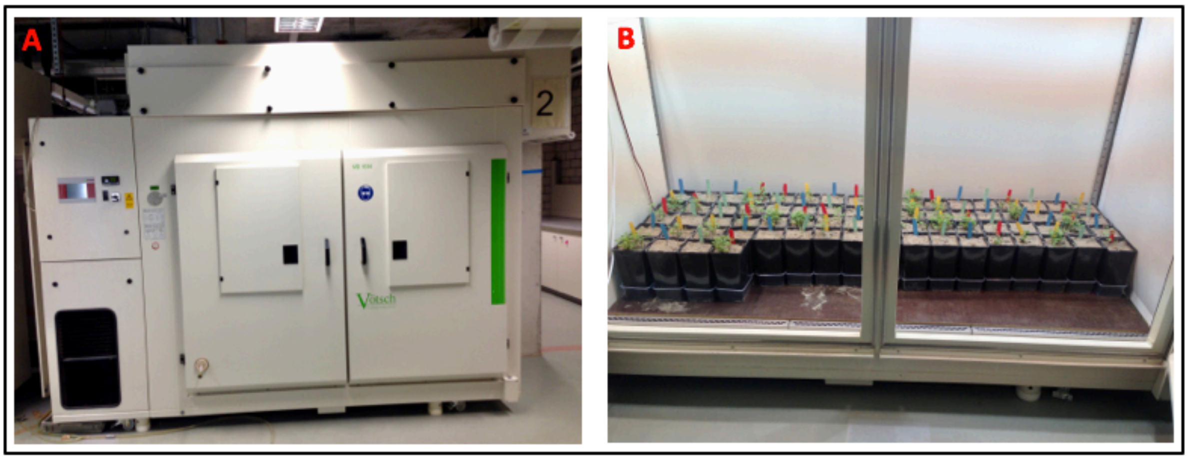


Fig. 1. An example of the state of the art climate chambers used (A) and an experimental set-up in one of the climate chambers (B).

Sowing and treatments

The seeds were sown in rose-pots (11.35 cm x 11.35 cm x 20 cm (height)), to provide belowground depth for root growth. Each species was sown separately; twenty seeds were sown per pot for each grass species and 10 seeds for each shrub species. More grass seeds were sown to increase the chances of having at least one seedling germinating per pot, as a previous greenhouse study (Bruder, 2015) showed that *S. uniplumis* has a very low germination rate. The seeds were sown in sand, to mimic natural soil conditions. Seeds were sown by covering them with a thin layer of sand (± 1 cm), because *S. mellifera* (Kraaij and Ward, 2006) and *S. uniplumis* (Zimmermann et al., 2008) need to be slightly buried to improve germination. Each pot then received four grams of Osmocote Pro Controlled Release Fertilizer (Everris), an amount determined based on the soil nutrient content in the field.

After sowing and initial watering, the pots were divided among the six climate chambers (three chambers for each CO_2 concentration) and arranged randomly within each chamber (Fig. 1 B). During the first four weeks of the experiment, all pots received the same amount of water to allow germination and seedling establishment. At the end of the fourth week, the seedlings were thinned to one per pot and randomly assigned to either a drought treatment (no subsequent watering) or control (watering every second day). Thus, the drought treatment simulated the common situation of a prolonged early drought at the seedling stage, a major bottleneck for establishment (Miller, 1987; Quintana et al., 2004). The different treatments were replicated nine times in total, i.e. three replicates per climate chamber.

To account for any possible chamber effects, pots were randomised among the chambers weekly. This was done by moving the pots to the next chamber and changing the CO_2 profiles of the chambers accordingly.

Four weeks into the drought treatment, the remaining seedlings were harvested, and their root and shoot length measured separately. Sand was shaken off the roots over a sieve to reduce the loss of fine roots, after which excess sand was gently washed off by spraying water on the roots. Both roots and shoots were first air-dried for a month, and then dried in an oven to a constant mass at 60^o C for 24 hours. After drying, the samples were weighed to determine root and shoot dry biomass for each seedling.

Experiment 2

As explained above, a second experiment was conducted that included a competition treatment. Two of the four study species, *A. pubescens* and *S. mellifera* were used for this experiment because a) they had the highest overall germination in experiment 1 and b) because only very few (*Stipagrostis*) or no seeds (*Dichrostachys*) were available for the other two species. Grass seeds for the experiment were collected at the three study areas from April to May 2016, and *S. mellifera* seeds were collected from November to December 2016. The seeds of *S. mellifera* were therefore different seeds from those in the first experiment. Due to low overall seed numbers, only eight seeds per species were sown in each pot.

The experiment had a full factorial design for each species with the addition of a competition treatment. Namely, plants were either grown alone (control) or with a heterospecific neighbour. The CO_2 concentrations were the same as in the first experiment, but the watering treatment was adjusted slightly. Seedlings in the regular watering treatment were watered every third day, while the drought treatment was watered every tenth day, to ensure all the seedlings, especially those that were two in a pot survived to the end of the experiment. At the end (four weeks after starting the drought treatment), shoot biomass was harvested and dry mass determined as described in the first experiment.

To quantify the effect of competition on performance, I calculated the relative interaction index RII (*sensu* Armas et al., 2004) as follows: Bw-Bo/Bw+Bo, where Bw= biomass with neighbour and Bo= biomass without neighbour.

To allow for the calculation of Relative Interaction Index (RII), pots were arranged into three-pot blocks: 1. *A. pubescens* alone, 2. *S. mellifera* alone and 3. both species sown together, which were always kept together during randomization. There was generally low germination of *A. pubescens*, leading to many empty pots, meaning that in some cases there was a sharing of the value of the control plants for calculation of RII between two blocks. The two sharing blocks were then kept together during randomization.

Data analysis

Our initial design for the first experiment was full factorial with four factors; seed origin, species, CO_2 and water. However, this did not end up being the case, because we only had *S. mellifera* seeds from the two drier sites. I therefore ran separate models for each species, to compare CO_2, water and origin effect. There were three dependent variables for the analyses, namely shoot biomass, and total: root biomass and length. The shoot biomass data were log-transformed and analysed using a Generalized Linear Model (GLM), normal distribution with a Logit link function. The ratios were not transformed and were analysed the same way as the shoot biomass data.

Although the second experiment had a full factorial design, there were too many factors (origin, species, CO_2, water and heterospecific interaction) to address in one model. Therefore, to avoid having a very large and confusing model, the data were analysed separately for each species as well. The data were also log-transformed and analysed using a Generalized Linear Model (GLM), with normal distribution and a Logit link function. The dependent variables for the second experiment models were shoot biomass and RIIs. A one sample t-test was used to evaluate whether the RII mean of each treatment differed significantly from zero (no neighbour effect).

All the data were analysed with SPSS Statistics for Windows (IBM, Version 24.0., 2016), and the "least significant difference" adjustment was used for all pairwise comparisons.

Results

Experiment 1

(1) Drought effects on the different ecotypes of the focal-species

(i) Shoot biomass

Drought inhibited the growth of all the focal species, but only three of the four species showed a significant drought effect according to ecotypes (Table 1, Fig. 2, Appendix 1). For two species (*S. uniplumis* and *D. cinerea*), plants from the wetter site responded less to drought (Fig. 2). For *A. pubescens*, there was a similar albeit not significant trend (Fig. 2). There was no ecotypic drought effect for *S. mellifera*, and none of the two ecotypes from this species separately exhibited a significant reduction in biomass due to drought (Table 1, Fig. 2).

Generally, all the species apart from *S. mellifera* exhibited a difference in biomass according to seed origin (Table 1). For the two grasses the wetter sites had significantly lower biomass, while for *D. cinerea* the semi-arid site had higher biomass than the arid one (Appendix 1).

Table 1. Results of GLMs with a normal distribution and logit link function, constructed to test for the effect of water (H_2O) and origin (Orig.) on shoot biomass of the four focal-species. Significant results ($p< 0.05$) are indicated in bold.

	A. pubescens			***S. uniplumis***			***D. cinerea***			***S. mellifera***		
Effects	**F**	**df**	**p-value**	**F**	**df**	**p-value**	**F**	**df**	**p-value**	**F**	**df**	**p-value**
H_2O	473.22	1	**<0.001**	33.58	1	**<0.001**	58.58	1	**<0.001**	7.415	1	**0.006**
Orig.	12.44	2	**0.002**	5.41	1	**0.020**	10.37	2	**0.006**	0.20	1	0.657
Orig. x H_2O	11.89	2	**0.003**	8.52	1	**0.004**	13.97	1	**0.001**	0.0	1	0.986

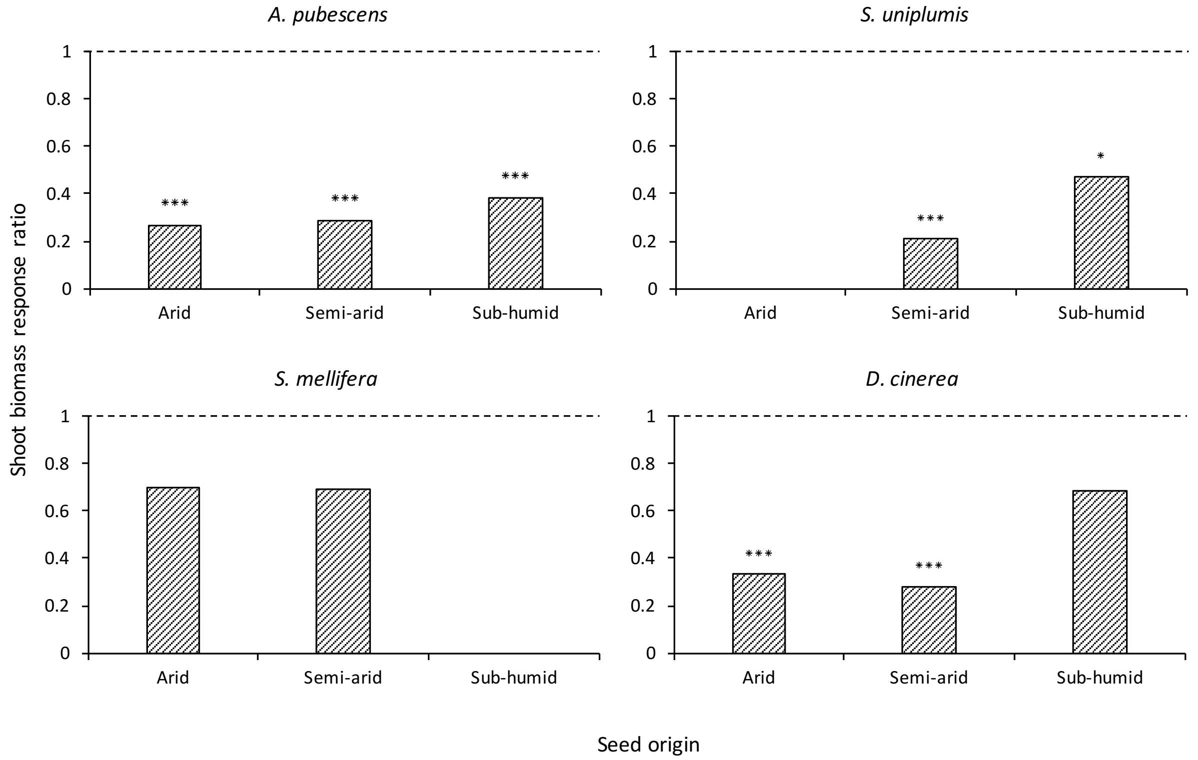


Fig. 2. The effects of drought on seedling shoot biomass of the four focal-species according to the different seed origins. The values on the y-axis (response ratio) were calculated by dividing the mean of each treatment by that of the control (treatment/ control). The control is denoted by the dashed line. Values above the control line represent a positive treatment effect and *vice-versa*. Asterisks indicate significant differences from the control and their levels of significance (*p< 0.05, **p< 0.01 and ***p< 0.001), according to the GLMs Least significant difference pairwise comparisons.

(ii) Total to root biomass allocation

Overall, biomass allocation patterns were only affected by water availability and in one case (*A. pubescens*) by origin (Table 2). All three *A. pubescens* ecotypes differed in their biomass allocation to roots, with the sub-humid ecotypes allocating the most and the semi-arid the least biomass to the roots (Table 2, Fig, 3). In general, more biomass was allocated to roots under drought stress (Fig. 3), and this pattern was particularly strong for *D. cinerea* (all sites) and *S mellifera* (arid site only) (Fig. 3).

Table 2. Results of GLMs with a Normal distribution and logit link function, constructed to test for the effect of water (H_2O) and seed origin (Orig.) on biomass allocation to roots of the four focal-species. Significant results ($p < 0.05$) are indicated in bold.

	A. pubescens			*S. uniplumis*			*D. cinerea*			*S. mellifera*		
Effects	**F**	**df**	**p-value**	**F**	**df**	**p-value**	**F**	**df**	**p-value**	**F**	**df**	**p-value**
H_2O	18.13	1	**<0.001**	5.23	1	**0.022**	69.94	1	**<0.001**	12.52	1	**<0.001**
Orig.	42.03	2	**<0.001**	0.87	1	0.351	4.47	2	0.107	1.306	1	0.253
Orig. x H_2O	1.40	2	0.497	0.34	1	0.560	0.76	2	0.685	2.24	1	0.135

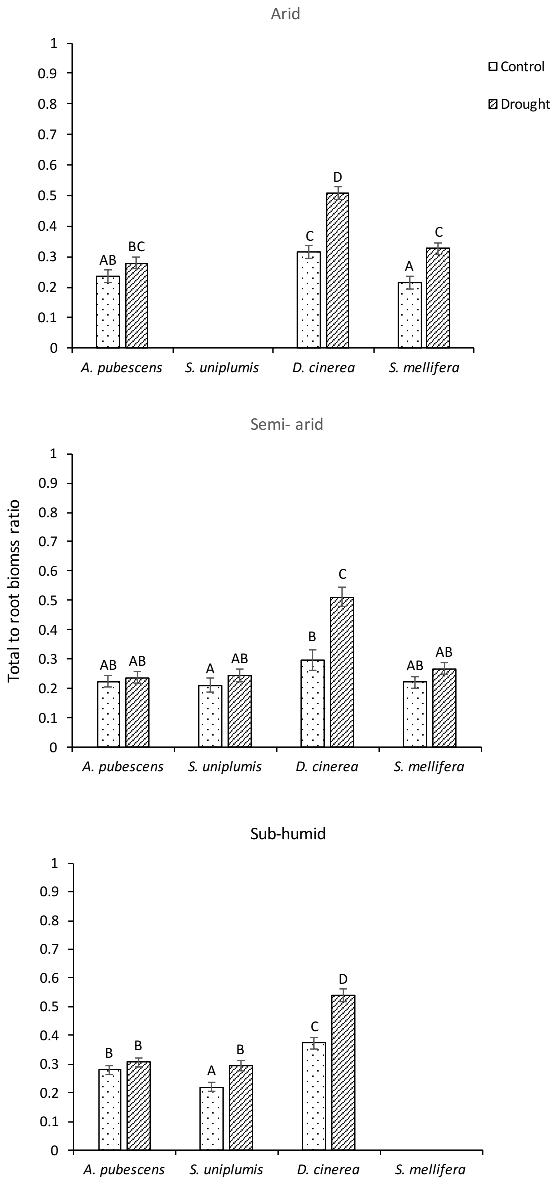


Fig. 3. The effects of drought on seedling biomass allocation to roots (mean ± SE), for the different ecotypes of our focal-species. Different letters indicate significant differences (GLMs) and are unique to each graph.

Note: I only show results for total to root biomass allocation because the general trends between the total to root biomass and length ratios were similar. See appendix 2- 4 for the total to root length ratios test outputs and figures.

(2) Effects of elevated CO_2 on the different ecotypes of the focal-species

(i) Shoot biomass

The overall (positive) effect of CO_2 on shoot biomass was very weak, and there was a slight tendency for shrubs to benefit more from CO_2 (Table 3, Fig. 4). Of all species, only *S. mellifera* exhibited a significant overall CO_2 effect (Table 3, Fig. 4). However, there were interactions with origin. Namely plants from both the sub-humid (F= 5.59, df= 1 & p= 0.018) and the arid site (F= 8.27, df= 1 & p= 0.004, Fig. 4) generally benefited from elevated CO_2, although the effect was greater and more obvious for the arid ecotypes of *A. pubescens* and *S. mellifera* (Fig. 4). *A. pubescens* from the semi-arid site was negatively affected by elevated CO_2 (Table 3, Fig. 4).

Though some of these results seem rather subtle, the same general pattern was found in the second experiment (Fig. 7, Appendix 6).

Table 3. Results of GLMs with a normal distribution and logit link function, constructed to test for the effect of elevated CO_2 and origin (Orig.) on shoot biomass of the four focal-species. Significant results ($p < 0.05$) are indicated in bold.

	A. pubescens			*S. uniplumis*			*D. cinerea*			*S. mellifera*		
Effects	**F**	**df**	**p-value**	**F**	**df**	**p-value**	**F**	**df**	**p-value**	**F**	**df**	**p-value**
CO_2	0.03	1	0.857	0.81	1	0.367	3.29	1	0.070	6.63	1	**0.010**
Orig.	12.44	2	**0.002**	5.41	1	**0.020**	10.37	2	**0.006**	0.20	1	0.657
Orig. x CO_2	10.99	2	**0.004**	0.60	1	0.440	1.37	2	0.503	0.97	1	0.325

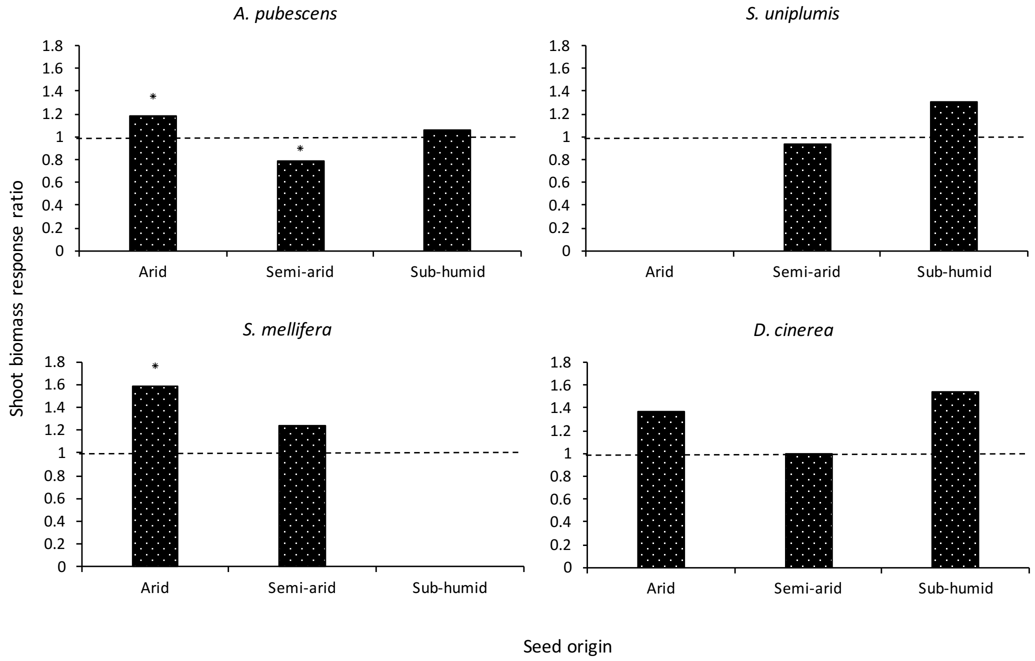


Fig. 4. The effects of elevated CO_2 on seedling shoot biomass of the four focal-species according to the different seed origins. The values on the y-axis (response ratio) were calculated by dividing the mean of each treatment by that of the control (treatment/ control). The control is denoted by the dashed line. Values above the control line represent a positive treatment effect and *vice-versa*. Asterisks indicate significant differences from the control and their levels of significance (*p< 0.05, **p< 0.01 and ***p< 0.001), according to the GLMs Least significant difference pairwise comparisons.

(ii) Total to root biomass allocation

None of the focal species revealed an elevated CO_2 effect on the allocation of biomass to roots (Table 4, Fig. 5). However, there was a difference in the allocation of biomass to the roots for *A. pubescence* based on seed origin, with the sub-humid ecotype allocating the most biomass to roots and semi-arid one the least (Table 4, Fig. 5). Since there was no overall CO_2 effect, there was also no difference in biomass allocation due to elevated CO_2 for any of the seed origins (Table 4, Fig. 5).

Table 4. Results of GLMs with a Normal distribution and logit link function, constructed to test for the effect of elevated CO_2 and seed origin (Orig.) on biomass allocation to roots of the four focal-species. Significant results (p< 0.05) are indicated in bold.

	A. pubescens			***S. uniplumis***			***D. cinerea***			***S. mellifera***		
Effects	**F**	**df**	**p-value**	**F**	**df**	**p-value**	**F**	**df**	**p-value**	**F**	**df**	**p-value**
CO_2	0.66	1	0.418	0.27	1	0.605	0.46	1	0.498	1.306	1	0.253
Orig.	42.03	2	**<0.001**	0.87	1	0.351	4.47	2	0.107	1.306	1	0.253
Orig.x CO_2	2.30	2	0.316	0.54	1	0.465	0.28	2	0.868	1.36	1	0.244

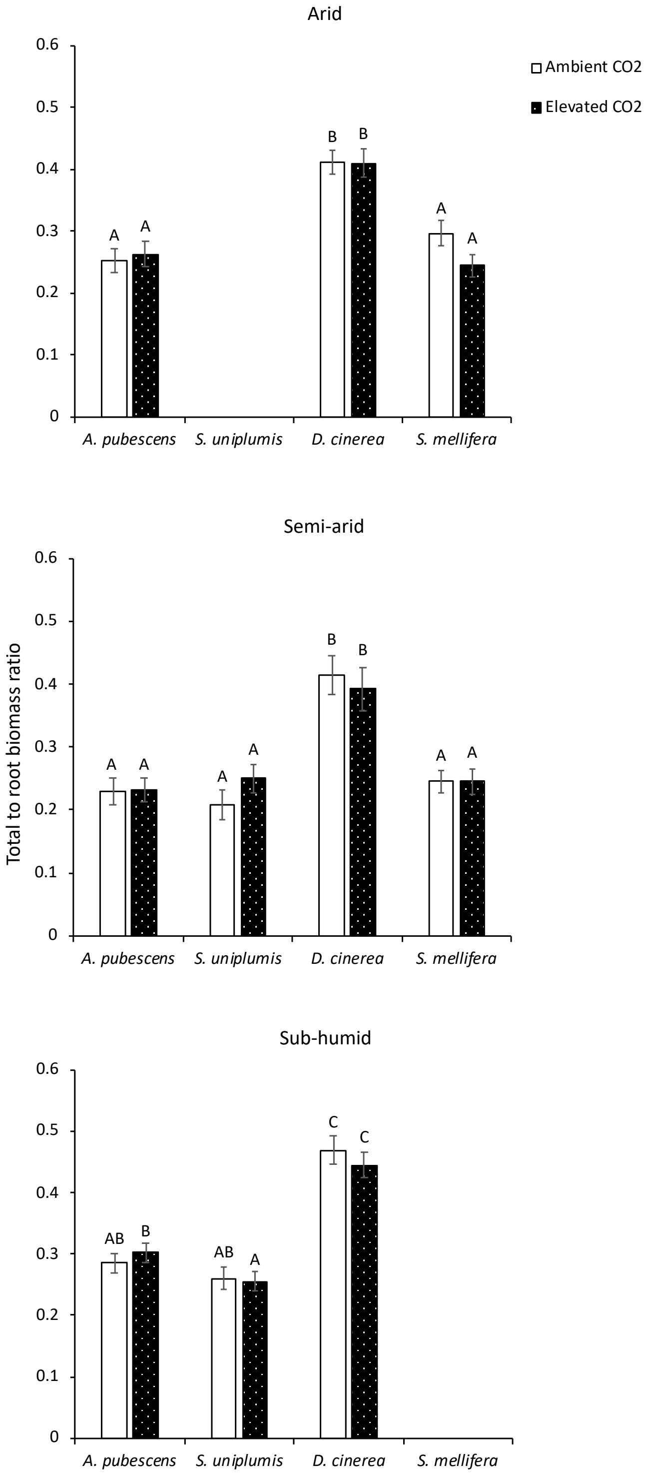


Fig. 5. The effects of elevated CO_2 on seedling biomass allocation to roots (mean ± SE), for the different ecotypes of our focal species. Different letters indicate significant differences (GLMs) and are unique to each graph.

3. The combined effect of drought and elevated CO_2 on each of the focal species

There was no CO_2 effect under drought stress for any of the focal species (Fig. 6). However, the two shrubs showed a significant interaction between CO_2 and water, both benefitting from elevated CO_2 under regular (control) watering (Table 5, Fig. 6).

Table 5. Results of GLMs with a normal distribution and logit link function, constructed to test for the combined effect of water (H_2O) and elevated CO_2 on shoot biomass of the four focal-species. Significant results ($p < 0.05$) are indicated in bold.

	A. pubescens			***S. uniplumis***			***D. cinerea***			***S. mellifera***		
Effects	**F**	**df**	**p-value**	**F**	**df**	**p-value**	**F**	**df**	**p-value**	**F**	**df**	**p-value**
CO_2 x H_2O	0.65	1	0.419	0.92	1	0.337	5.27	1	**0.022**	4.51	1	**0.034**

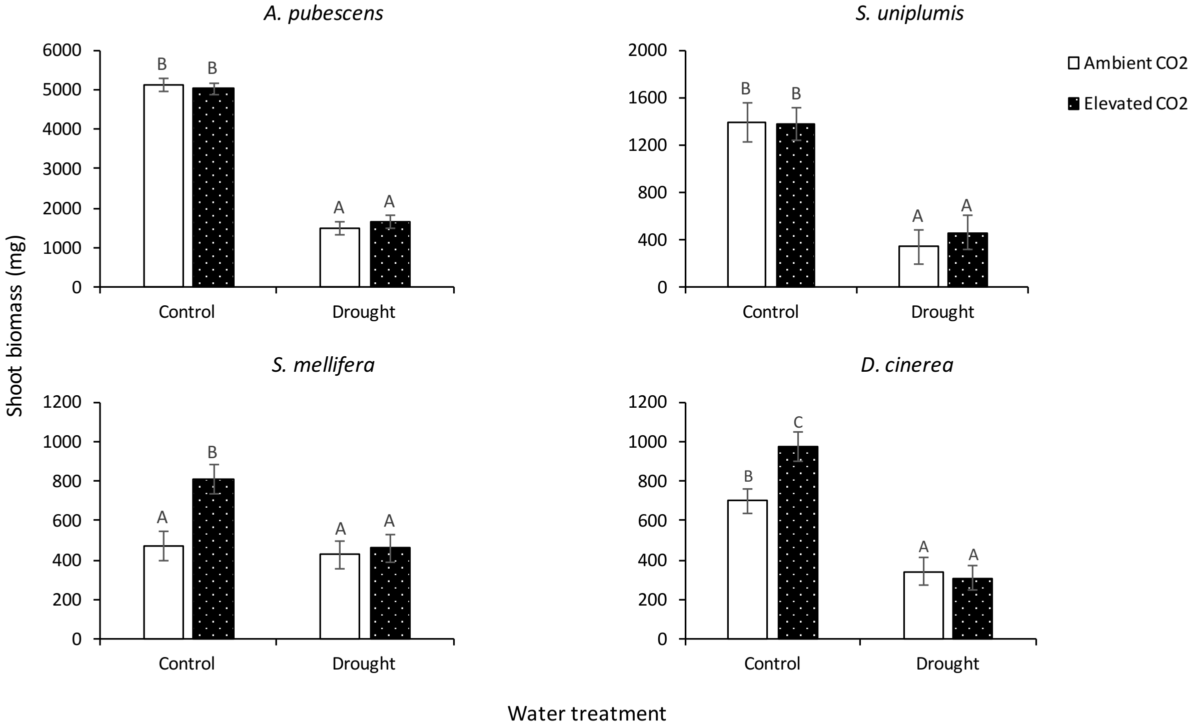


Fig. 6. Shoot biomass (mean ± SE) showing the response of the focal-species (grasses on top and shrubs at the bottom) to the interaction of two watering levels and CO_2 concentrations. Different letters indicate significant differences (GLMs) and are unique to each species graph.

Experiment 2

Drought had a similarly negative effect on biomass as in experiment 1, but this effect was not significant for the grass species (Table 6, Fig. 7A). The ecotype- and species- specific (*S. mellifera* arid) effect of CO_2 was also confirmed (Table 6, Fig. 7B).

The presence of *S. mellifera* had a significant negative effect on *A. pubescens*, but not *vice-versa* (Table 6, Figure 7C). The RIIs revealed the same results as the main test, in that the grass was generally affected by competition from the shrub and there was no significant interaction between neighbour effect and the other treatments, for any of the two species (Appendix 5 & 6).

Table 6: Results of GLMs with a normal distribution with a Logit link function constructed to test for the effect of water (H_2O), elevated CO_2 and seed origin (Orig.) and a heterospecific neighbour on biomass of the four focal-species. Significant results ($p< 0.05$) are indicated in bold.

	A. pubescens			***S. mellifera***		
Effects	**F**	**df**	**p-value**	**F**	**df**	**p-value**
CO_2	3.70	1	0.054	8.08	1	**0.004**
H_2O	2.92	1	0.087	13.26	1	**< 0.001**
Orig.	12.75	2	**0.002**	104.3	2	**< 0.001**
Neighbour	8.56	1	**0.003**	0.35	1	0.553
Orig. x CO_2	0.55	2	0.760	2.53	2	0.282
Orig. x H_2O	0.19	2	0.911	7.91	2	**0.019**
Orig. x Neighbour	0.62	2	0.734	4.18	2	0.124
CO_2 x H_2O	0.31	1	0.579	1.98	1	0.159
CO_2 x Neighbour	2.65	1	0.104	1.00	1	0.318
H_2O x Neighbour	0.08	1	0.785	1.05	1	0.307
Orig. x CO_2 x H_2O	2.26	2	0.323	6.79	2	**0.034**
Orig. x CO_2 x Neighbour	1.35	2	0.510	1.46	2	0.482
Orig. x H_2O x Neighbour	0.30	2	0.862	0.49	2	0.783
CO_2 x H_2O x Neighbour	2.28	1	0.131	0.003	1	0.954
Orig. xCO_2 x H_2O x Neighbour	2.18	2	0.336	0.96	2	0.617

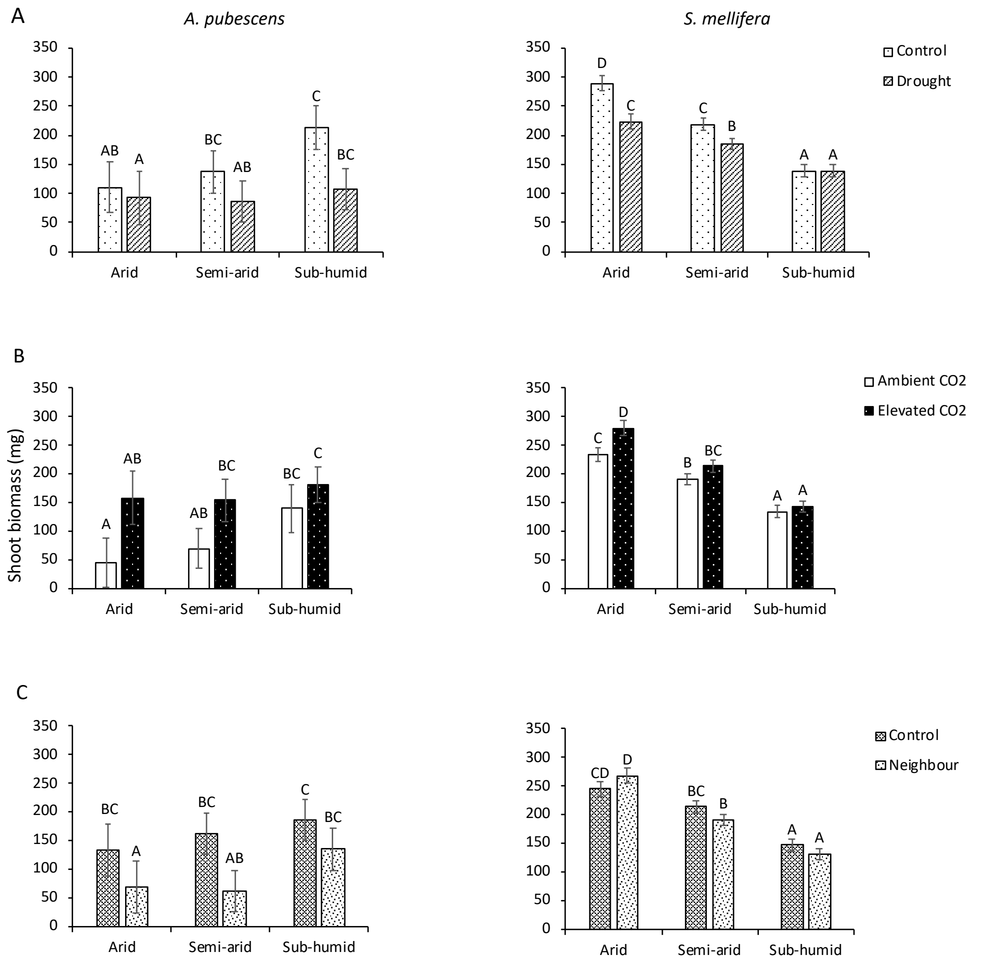


Fig. 7. The response of shoot biomass (mean ± SE) of three ecotypes (represented by origins on x-axis) of a grass (left) and shrub (right) to drought (A), elevated CO$_2$ (B) and heterospecific interaction/ neighbour (C). Different letters represent significant differences (GLMs) and are unique to each graph.

Discussion

The overall results of the climate chamber experiments suggest that drought may play a more important role in determining the performance of the focal savanna species during early establishment than elevated atmospheric carbon dioxide concentrations. In addition, responses differed between species and origins but very often not in the direction suggested up front. In the following, I discuss my findings with respect to the initial hypotheses separately.

1. *Drought effects on seedlings of the focal-species*

Drought did not have a more negative effect on shrubs than grasses. In fact, the species that was most affected by drought was a grass (*A. pubescens*) and the least affected a shrub (*S. mellifera*). Although this finding is contrary to studies that have found C$_4$ plants to be more drought tolerant than C$_3$ plants (Hamim, 2005; Osborne and Sack, 2012; J. K. Ward et al., 1999), it is similar to those found in an independent greenhouse experiment (Chapter 2), indicating that these results were robust. One explanation for these counterintuitive findings could be a suggestion by Ripley et al. (2010), who found that although C_4 plants may not be limited by excessive transpiration due to opening of stomata, they may be metabolically limited in their CO_2 assimilation under drought stress, which results in them being less water efficient even under mild drought.

However, if this explanation held true for our study species, then elevating the CO_2 concentration should have improved the performance of grasses under drought stress, but this was not the case. Therefore, another possible explanation for the finding could be that the drought treatment was too intense and was thus more pronounced for the faster growing (and therefore larger) grass (*A. pubescens*) seedlings, which may have depleted water in the pots faster than the smaller shrub (*S. mellifera*) seedlings. In fact, the second climate chamber experiment was the only one of all the experiments for this doctoral study where *A. pubescens* was not negatively affected by drought, while *S. mellifera* was. In that experiment however, seedlings of the grass species had extremely low biomass compared to the other experiments, further supporting my argument about the observed drought effect being a function of seedling size. This finding could be extrapolated to the field, to suggest that fast growing plants (i.e. the grasses) may utilize the limited water in their immediate surroundings faster, and therefore may experience drought effects way before their slow growing counterparts.

Our data may also give rise to another less speculative explanation. Namely, although all four of the study species showed a general increase in biomass allocated to roots under drought stress, this response was more pronounced in the two shrubs, particularly *D. cinerea*. Not only did the two shrubs have significantly more root biomass across ecotypes (apart from the semi-arid ecotype of *S. mellifera*), they also consistently had longer roots under drought stress. These general differences in grasses and shrubs allocation to roots, especially with regard to root length, supports the concept that shrubs predominantly get their water from greater depths, while grasses obtain their water from shallower layers of the soil (Sala et al., 1989; Walter, 1971). The shrub seedlings are therefore probably adapted to prioritise root elongation in an effort to reach ground water as fast as possible, especially under drought stress. This advantage is particularly important in an early seedling stage and may explain why in our experiment, the grasses seem to suffer more from the drought than the shrubs.

2. *The effects of elevated CO_2*

Although the response to elevated CO_2 was generally weak and sometimes ecotype specific (*A. pubescence* and *S. mellifera*), our results clearly show that shrubs benefited more from elevated CO_2 than grasses. Therefore, when considered in isolation, predicted levels of atmospheric CO_2 concentrations may lead to further bush encroachment, which agrees with the common notion that elevated CO_2 will benefit C_4 plants more than C_3 ones (Bond and Midgley, 2012; Devine et al., 2017; Hamim, 2005; Kgope et al., 2009; Polley, 1997; Polley et al., 1997). However, CO_2 might not play such a major role when considered together with drought, as discussed below.

Firstly, the general weak response to elevated CO_2 observed in our focal species could be because the difference between current ambient and near future elevated atmospheric carbon dioxide concentrations is simply not enough to cause significant increases in the growth of these species. A similar finding was also made by Kgope et al. (2009), when shoot biomass for both *Themeda triandra* (C_4 grass) and *Acacia karoo* (C_3 shrub), did not differ significantly between CO_2 concentrations of 370 and 550 μmol mol^{-1}. However, their open-top chambers did not enable any control over CO_2 concentrations. Nevertheless, the fact that we obtained similarly small responses indicates that their results could not only be affected by the manipulation approach but that indeed the predicted increase in CO_2 over the next 60 years will not have a marked effect on the establishment of shrubs and grasses in savannas. Interestingly, Kgope et al. (2009) did detect some effects between low (180-280 μmol mol^{-1}) and extremely high (370- 1000 μmol mol^{-1}) CO_2 concentrations, indicating that the subtlety in CO_2 effects detected by us could be caused by small effect sizes.

Due to the general weak response to elevated CO_2 among our focal species, it is maybe not surprising that CO_2 enrichment did not mitigate the adverse effects of drought, i.e. the plants were simply just not benefiting markedly from the higher CO_2. A possible reason is the overall small effect size for CO_2 as explained above, which was not sufficient to counteract the extreme drought effects (Perry et al., 2013). It has also been previously found in other studies that drought effects outweigh the benefits obtained from elevated CO_2 (Leakey et al., 2006; Morgan et al., 2004; Perry et al., 2013). Another possible explanation for this finding might be that the drought was so intense that the plants were forced to close their stomata to preserve the limited water or simply reached wilting point (Franks et al., 2013; Warren et al., 2011). Therefore, although elevated CO_2 is expected to ameliorate drought effects by reducing stomatal conductance (Ainsworth and Rogers, 2007; Franks et al., 2013; Polley and Derner, 2002; Xu et al., 2016), during an extreme drought the seedlings in this study would have been unable to assimilate the abundant CO_2 and therefore could not benefit from it (Duan et al., 2013; Franks et al., 2013; Warren et al., 2011). As discussed above, another indication that our drought treatment might have been too intense is the fact that all our focal species were significantly affected by drought, with the larger ones suffering the most.

Interestingly, while general performance measured in biomass did not show any mitigation effects, there was an effect on below-ground architecture in one of the species. Namely the total to root length ratio of *A. pubescens*, was larger under drought stress with elevated CO_2. This may suggest an interesting pattern, i.e. a drought mitigating effect of CO_2 belowground. Since this is the only one of our four species that demonstrated this below-ground response to elevated CO_2, we cannot draw a general conclusion on the effect of CO_2 enrichment on seedling root elongation under drought stress. Unfortunately, there are no previous studies to support our claim, not only because of the general lack of experiments with CO_2, but also because belowground responses are generally neglected (but see- Derner et al., 2003; Kgope et al., 2009). This new pattern merits more attention in future studies.

I generally suggest that the next step towards understanding the role of elevated CO_2 in tree-grass interaction in savanna ecosystems should be through “free air CO_2 enrichment” (FACE) experiments (Ainsworth and Rogers, 2007; Ceulemans and Mousseau, 1994; Derner et al., 2003; Fangmeier et al., 2016; Högy et al., 2013; Norby and Zak, 2011; Paschalis et al., 2017). It might however be difficult to retrieve all the roots of the seedlings when they are grown directly in the soil, therefore I further suggest the use of large tubes or deep pots dug into the ground, to allow for root harvesting.

3. *The outcome of heterospecific interactions*

Another intriguing finding, was that the grass suffered consistently from the presence of a shrub seedling neighbour whereas the shrubs did not respond at all to competition from the grass. This finding may on the one hand seem contradictory to findings that grasses are better competitors for shared resources than shrubs (Higgins et al., 2000; Manea and Leishman, 2015; Scholes and Archer, 1997), and the idea that the removal of grasses, through overgrazing by domestic livestock, favours shrub encroachment. However, an own parallel greenhouse study fully confirmed this pattern, indicating that this finding is robust (Chapter 2).

One explanation may be that different processes occur in an established grass matrix in nature that may not be the same as in a pot with the two life-forms competing on a one-to-one ratio. The competitive advantage that has been recorded in grasses might therefore be density dependent, and probably only observed in a dense grass matrix. Additionally, it might also be that previous studies have been assessing the effect of established grasses on shrub seedlings, again giving the competitive advantage to the older and larger plants (Manea and Leishman, 2015). Our field experiment (Chapter 4) showed no significant effect of a mature grass matrix on grass seedling emergence, but possible facilitation for *S. mellifera* emergence. Unfortunately, there were very few seedlings surviving at the end of the experiment, making it impossible to determine neighbour effect at seedling stage.

We therefore conclude, with all the caution needed to be applied when doing studies with few species, that climate change might actually enhance encroachment. This is however not necessarily due to a larger positive response of shrubs to CO_2 but rather a smaller negative response to drought. This rather unexpected effect might be due to the fact that we focused on the rarely studied bottleneck of shrub establishment, i.e. germination and early survival. Furthermore, rangeland managers should carefully monitor the recruitment of *Senegalia mellifera*, as this species benefited the most from CO_2 enrichment and suffered the least from drought. It therefore has the potential of becoming more of a problem in terms of shrub encroachment, under those conditions. To what extent the removal of grasses by livestock is a main determinant of shrub encroachment we cannot say, but at least at early life stages, *S. mellifera* (one of the problematic encroachers) seemed to be a stronger competitor than *A. pubescens* (one of the most valued/ palatable rangeland grasses).

This study provides first empirical evidence on the effect of elevated CO_2 and drought on tree-grass interactions in southern African savannas, under controlled conditions. But that means this was at a cost of realisms. Therefore, there is a need for experiments such as this (looking at early life stages) to be conducted in the field, through FACE experiments, for a more realistic view.

Chapter 4

The effect of simulated grazing and nutrient addition on early life stages of two encroaching shrub species and two perennial grasses in a southern African savanna - a field experiment

Abstract

There are a number of factors that have been identified as so-called determinants for shrub-grass interactions in savanna ecosystems, with overgrazing by domestic livestock being one of the most mentioned ones. In this study, I investigated the effects of simulated grazing and nutrient addition on seedling emergence and survival of two encroaching shrubs and two perennial grasses in a semi-arid savanna of Namibia. The original experimental design included a drought treatment in the form of rainout shelters, and as such a randomized block design with a full factorial combination of drought, grazing and nutrient treatment was used. Unfortunately, the drought treatment was unsuccessful, mainly due to constant damage of the rainout shelters. We hypothesised that simulated grazing will enhance shrub emergence more than grasses, grass shoot emergence will be enhanced by nutrient addition more than shrubs, and seedling survival will be higher for the grasses than the shrubs (especially under dry conditions). The results showed that simulated grazing negatively affected the emergence of *S. mellifera*, one of the shrub species, suggesting that the emergence of this species is facilitated by neighboring vegetation. Nutrient addition was more beneficial to the smaller seeded grass species. Overall seedling survival was significantly lower in grasses than shrubs. Due to generally lower survival, we could not compare seedling survival per treatment. As the study was conducted during a drought year in Namibia, I conclude that although not directly assed in this study, drought/ water stress seemed to continue (as found in our two previous chapters) being the most important factor affecting the early stages of establishment for our study species.

Introduction

One reason for shrub encroachment that has been repeatedly mentioned in literature, is overgrazing, particularly by domestic livestock (Bond and Midgley, 2012; Kraaij and Ward, 2006; Moleele et al., 2002; Skarpe, 1990). Some studies have found that grasses are generally better competitors for shared resources than shrubs, and as such suppress the recruitment of the former life form (Bond, 2008; Britz and Ward, 2007; Good et al., 2014; Higgins et al., 2000; Manea and Leishman, 2015; Scholes and Archer, 1997). Therefore, the mass removal of grasses through overgrazing is believed to induce competitive release for the shrubs and thus promote shrub establishment (Harrington, 1991; O'Connor et al., 2014; D. Ward, 2005). However, while this idea sounds appealing, it does not take into account that the nature and intensity of plant-plant interactions is not uniform in space. For example, the stress gradient hypothesis suggests that when environmental conditions are harsh, positive interactions among plants (facilitation) should become more dominant (Cristina Armas et al., 2011; Bertness and Callaway, 1994; Dohn et al., 2013).

Facilitation as a major interaction type among shrubs and grasses in savannas has been almost completely overlooked so far, maybe because a great deal of research has been done in systems that receive a relatively large amount of annual precipitation, i.e. semi-arid to more humid savannas (Blaser et al., 2014; Devine et al., 2015; Kgope et al., 2009; X. Le Roux et al., 1995; Roques et al., 2001; Tessema et al., 2011). However, African savannas stretch from below 250 mm to about 1600 mm mean annual rainfall (Lehmann et al., 2011), and thus the mechanisms governing shrub-grass interactions may largely differ in dry vs wet savannas. For example, in arid savannas, facilitation may be important such that some plants may provide suitable micro-habitats for others to establish and grow (Bertness and Callaway, 1994; Callaway, 1995; Vetaas, 1992). I suggest, the best way to study how shrub-grass interactions may vary under different water conditions is to apply experiments that manipulate rainfall.

In addition, shrub-grass interaction may also differ at different life stages. According to (Callaway and Walker, 1997) seedlings of species benefiting from facilitation have often been found closer to the nurse plants, while their adults were not. This is an indication that facilitation is more important during early life stages than late ones (Callaway and Walker, 1997; Kellman and Kading, 1992; Schiffers and Tielbörger, 2006). For this reason, our study focused on the effect of grazing, which could mean competitive release or the possible removal of nurse plants, on the early life stages of the two life forms.

Another factor that may affect the interactions between shrubs and grasses is soil nutrients (Cramer et al., 2010; Kraaij and Ward, 2006). The effect of soil nutrients on the two life-forms has however mostly been recorded in terms of mature plants, especially under low soil nutrient conditions, where the fast growing, shallow rooted grasses are able to access the limited nutrients in the topsoil more than the deep-rooted woody plants (Blaser et al., 2014; Cramer et al., 2010; Scholes and Archer, 1997). Unfortunately, we know nothing about the effect of nutrients on seedling emergence in savanna ecosystems.

Enhanced seedling emergence has however been recorded in temperate grassland species under higher nitrate conditions (Hilhorst and Karssen, 1989; PONS, 1989). This opens up the possibility that soil nutrient conditions may affect plant recruitment, even as early as the seedling emergence state. In general, we would expect that grasses, due to their limited supply of seed nutrients, may need to rely on soil nutrients much sooner after root radicle emergence than their woody counterparts. And thus, nutrient addition should lead to more grass shoot emergence. Shrubs, on the other hand, can survive from nutrients obtained from cotyledons for weeks before requiring an external source of nutrients (Ernst, 1988), and therefore should not be affected by soil nutrient levels in the early stages of establishment. Although, it has also been previously reported that nutrient addition may have a negative effect on shrub seedling survival and establishment (M. A. Davis et al., 1999; Kraaij and Ward, 2006).

A single previous study by Kraaij and Ward (2006) looked at the combined effects of drought, grazing and nutrients on early life stages of *Acacia mellifera* (*Senengalia mellifera)* sown in a pre-established grass matrix in South Africa. They found that frequent watering, nutrient control and grazing enhanced tree recruitment, but they did not study the effect on the grasses, i.e. the second important partner in the process of shrub encroachment.

Here, we aimed at addressing the above gaps by investigating the effects of simulated grazing and nutrient addition on seedling emergence and survival of two encroaching shrubs and two perennial grasses in a semi-arid savanna of Namibia. We hypothesised that: 1. Simulated grazing will enhance shrub emergence more than grasses, 2. Grass shoot emergence will be enhanced by nutrient addition, while the treatment is expected to have a neutral to negative effect on the shrubs, and 3. Seedling survival will be higher for the grasses than the shrubs in general, and especially under dry conditions.

Materials and methods

Experimental design

The study was conducted at Neudamm, an experimental farm belonging to the University of Namibia, located about 40 Km outside Windhoek. Neudamm receives between 350 mm to 400 mm average annual rainfall (Mendelsohn et al., 2002). Simulated grazing (clipping of grasses, Fig. 1), drought (rainout shelters intercepting 50% of the incoming precipitation) and nutrient addition (15 ml (table spoon) of crushed manure collected at a sewage water treatment in Windhoek) were applied in a full factorial design. Since the rainout shelters served as blocks, a randomised block design with the different treatments being applied in 4 x 4 m blocks (the size of the shelters), was used. Each block therefore represented one combination of the three treatments (e.g. shelter x clipped x nutrient). The focal species were sown in 20 x 20 cm plots, within each block (Fig. 2). To distinguish our seedlings from those growing from the soil seed bank, the seeds were sown in plastic rings.

A total of 20 sheltered and 20 unsheltered blocks were used, and each ecotype was replicated four times (when seed numbers allowed) and randomly distributed within a block.

Due to limited space, grass seeds from the same site where sown in individual plastic rings on opposite sides of the same 20x 20 m plots. Each plot was 50 cm from the next and 50 cm from the edge of the block (Fig. 2). The experiment relied on rainfall for germination, therefore no artificial watering was done. The soil was however moist at the time of sowing as the area received some rain during the sowing period.

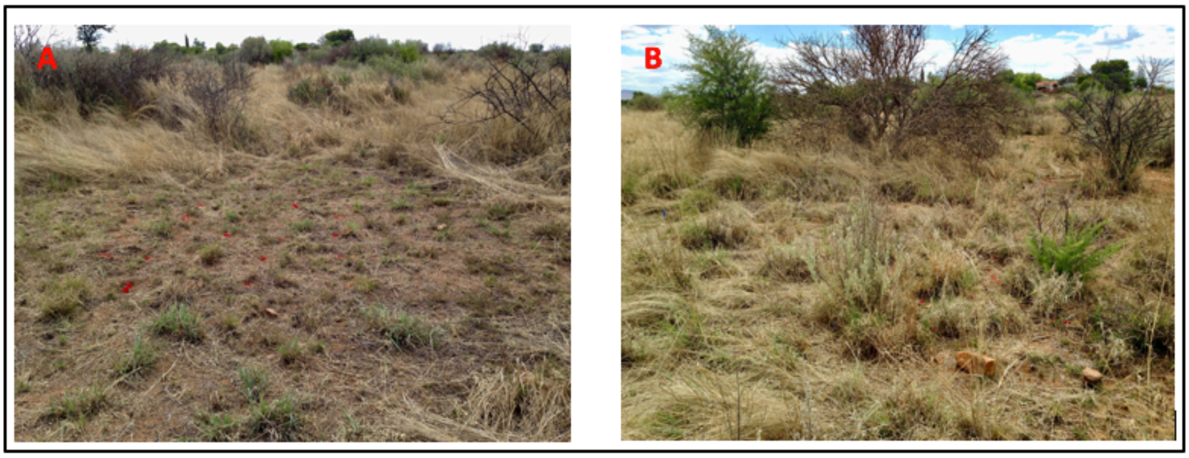


Fig. 1. Examples of the experimental setup at Neudamm showing a clipped (A) and an unclipped block (B).

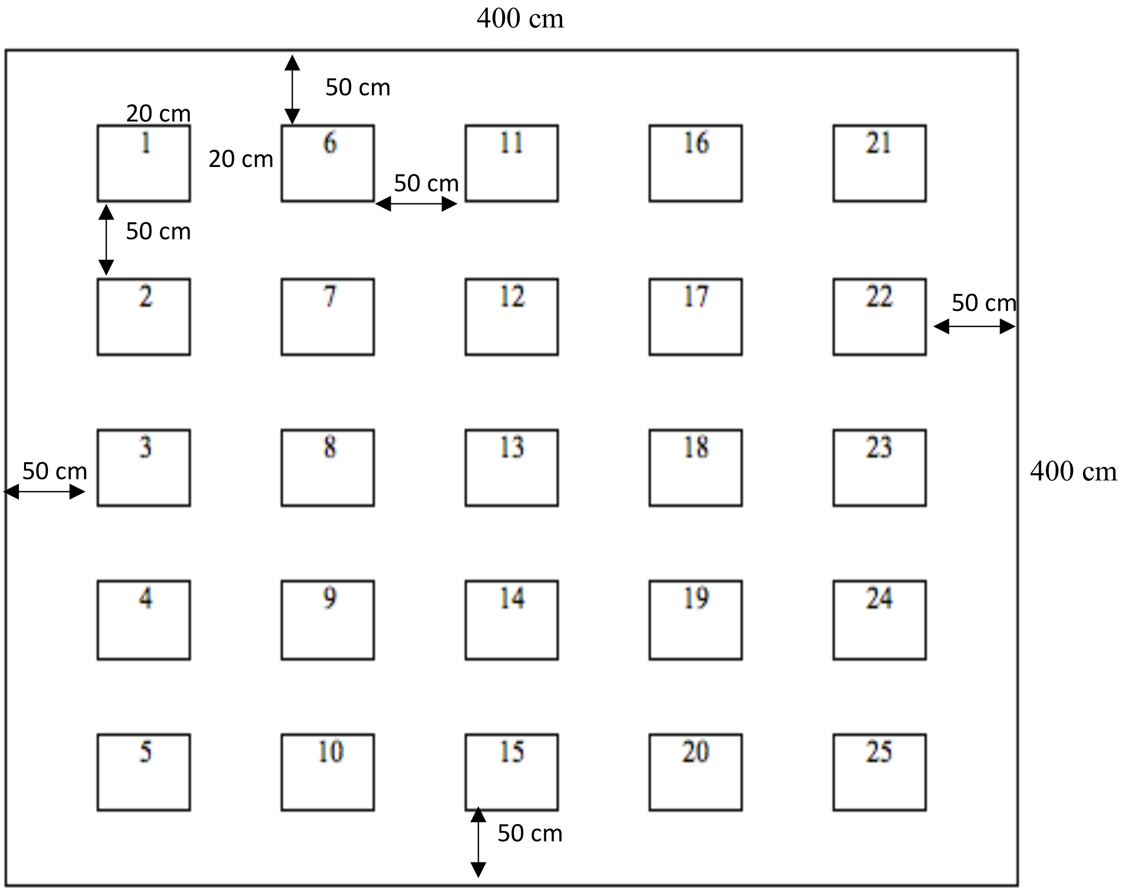


Fig. 2. An example of a single block layout.

Sowing and data collection

Sowing was done between 6-11 December 2014, by emptying a paper bag with pre-counted seeds into a plastic ring (4 cm in diameter and 2.5 cm height) positioned in the centre of the plot and covering the seeds with about a centimetre of soil. Plastic rings were used in order to protect our seeds from been blown away by the wind as well as to insure, to a certain degree, that we were counting seedlings emerging from our seeds and not from the soil seed bank. A total of eight shrub and ten grass seeds per plot were sown, to increase the chances of having at least one seedling per plot.

The first emerging seedling per plastic ring was recorded, marked with a flag and monitored for the duration of the experiment. All subsequent emerging seedlings were noted and then carefully removed. Seedling emergence was recorded once a week, or sometimes less frequent, mostly depending on rainfall, until the eighth of May 2015. Seed germination fractions was calculated as the number of emerging seedlings divided by the number of seeds sown. Seedling survival rate was determined by dividing the number of seedlings alive on the last day of data collection, by those that emerged during the period of data collection, for each species.

Changes made to the experiment

The rainout shelters were designed to intercept 50% of rainfall, but due to constant shelter damage throughout the study, there was no difference in the response variables between sheltered and unsheltered blocks. Soil moisture content also did not differ between the shelters and adjacent unsheltered areas. Since the drought treatment was unsuccessful, the sheltered blocks were used to increase the number of replicates for the clipping and nutrient treatments.

Data analysis

All the data were analysed with SPSS Statistics for Windows (IBM, Version 24.0., 2016). A Binomial distribution, Generalized Linear Mixed Model (GLMM) with a logit link function, was used to test for differences in seedling emergence and survival of the different species. I used “block” as a random factor in both models. The fixed factors for the seedling emergence model were species, origin, clipping and nutrients. Since there was no significant origin effect for any of the species, this factor was removed. The model improved (lower AIC) with the removal of origin and again with the removal of the three-way interaction of species, clipping and nutrients. Therefore, the fixed factors for the final model were species, clipping, nutrients and their two-way interactions. Due to very low survival and lack of other treatment effect, we only used species as a fixed factor for the survival model. The Least significant difference was used for all the post-hoc pairwise comparisons.

Results

The effect of clipping (simulated grazing) on seedling emergence of the focal species

Clipping generally lowered the emergence rate of our focal species (Table 1). This effect was however species specific, where three of the species, with the exception of *S. uniplumis*, portrayed a trend of lower seedling emergence in clipped blocks (Table 1, Fig. 3). Though this trend was only significant for *S. mellifera* (Fig. 3).

Table. 1. Results of GLMMs with a Binomial distribution and logit link function testing the effect of species and clipping on germination rate. Significant results ($p < 0.05$) are indicated in bold.

Effects	**F**	**df1**	**P**
Species	16.47	3	**< 0.001**
Clipping	4.78	1	**0.029**
Species x Clipping	3.74	3	**0.011**

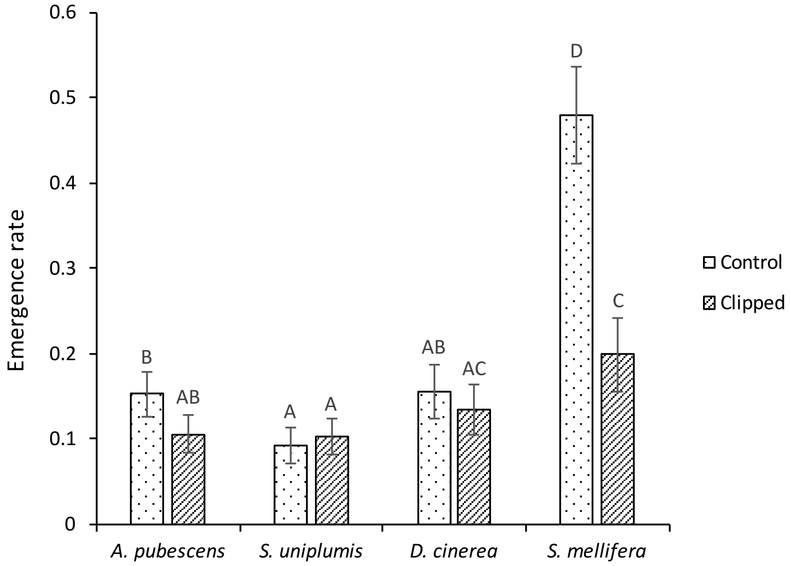


Fig. 3. Seedling emergence rate (mean ± SE), showing the response of the focal species to clipping (grazing). Different letters represent significant differences.

The effect of nutrient addition on seedling emergence of the focal species

Although nutrient addition had a significant overall enhancing effect on emergence, there was no significant interaction between nutrient addition and species (Table 1, Fig. 4). All the species clearly exhibited a similar trend of higher seedling emergence under the nutrient treatment, but this was only significant for *S. uniplumis*, i.e. there was a slight trend towards larger nutrient effects on grasses (Table 1, Fig. 4).

Table. 2. Results of GLMMs with a Binomial distribution and logit link function testing the effect of species and nutrients on germination rate. Significant results (p< 0.05) are indicated in bold.

Effects	**F**	**df1**	**P**
Species	16.47	3	**< 0.001**
Nutrients	5.50	1	**0.019**
Species x Nutrients	0.52	3	0.668

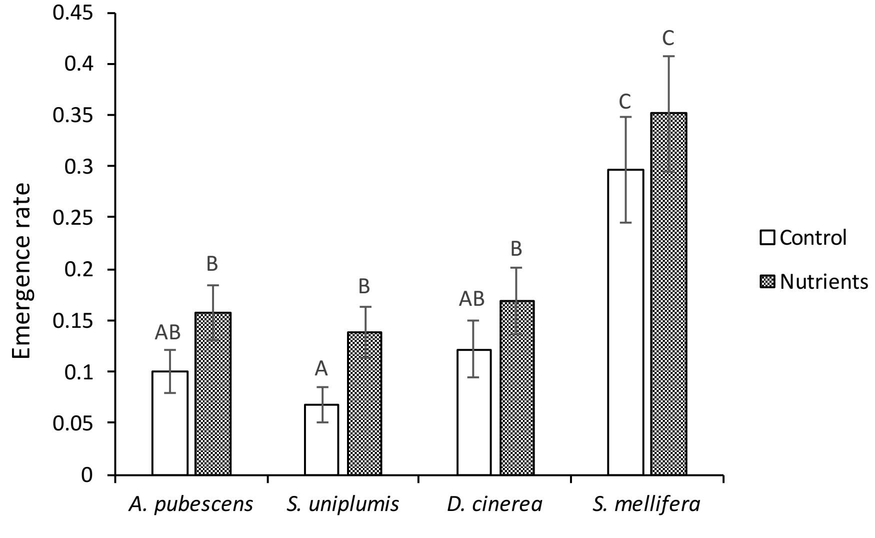


Fig. 4. Seedling emergence rate (mean ± SE) of the focal species in response to nutrient addition. Different letters represent significant differences.

Seedling survival for the four focal- species

Seedling survival was generally low, but there was a clear life-form depended difference in the survival of the different species (F= 3.77, df= 2, p< 0.025, Fig. 5). Both grasses had a significantly lower survival rate than the shrubs, and there were no within life-form differences observed (Fig. 5).

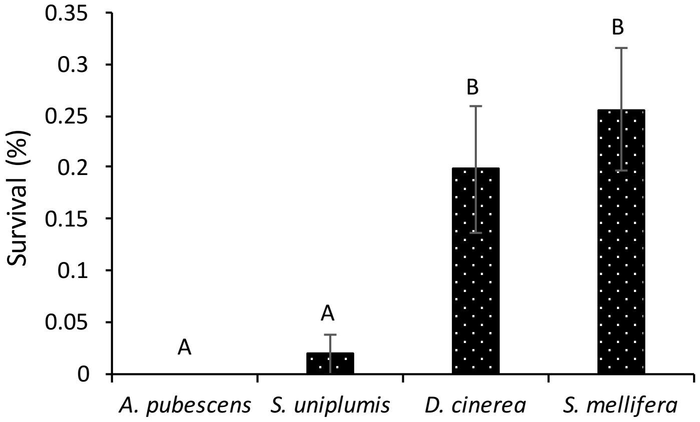


Fig. 5. Overall seedling survival (mean ± SE) for the different focal species. Different letters represent significant differences.

DISCUSSION

Our findings suggest that *S. mellifera* emergence was facilitated by the presence of neighboring vegetation and that nutrient addition may enhance emergence of *S. uniplumis*.

Contrary to our expectation, simulated grazing did not enhance shrub emergence more than grasses. We actually found clipping to significantly lower emergence of one of the shrubs, *S. mellifera*, suggesting that seedling emergence in this species is facilitated by the presence of herbaceous vegetation. This is a very interesting finding, because it is, on the one hand, in support of the stress gradient hypothesis, which expects biotic interactions to be less negative in arid environments (Bertness and Callaway, 1994; Dohn et al., 2013; Rysavy et al., 2014). On the other hand, it contrasts the common notion that shrub recruitment in savannas is greatly affected by grass competition (Ward, 2005; Skarpe, 1990; Manea and Leishman, 2015; Scholes and Archer, 1997). This finding may suggest that during the very early stages of *S. mellifera* development, facilitation by neighboring vegetation plays a more important role than competition. It has also been reported before in other studies that early life stages are more prone to being affected by positive interactions than late ones (Kellman and Kading 1992; Schiffers & Tielbörger 2006; Callaway and Walker 1997), and therefore this finding is not unique. Although, it raises the question of whether this facilitative effect will last long enough to increase shrub recruitment and therefore possible encroachment of the plant into the area.

Or whether the spread of *S. mellifera* will be controlled by subsequent competition as the plants grow larger and resources become limited (Callaway and Walker, 1997).

All four focal-species showed a trend of higher seedling emergence under higher soil nutrient conditions, which was more clearly pronounced in the grasses and only significant for *S. uniplumis*. A possible explanation for the trend of positive nutrient effect on all four species might be that the manure added to the soil surface acted not only as nutrient addition but also as a mulch, absorbing water and reducing the rate at which the soil dried. This may have created an environment of slightly higher soil moisture within the plots treated with nutrients, and in turn higher seedling emergence. However, we might not necessarily be strictly looking at the effect of nutrients addition on seedling emergence, but also early survival, because emergence was not recorded daily and therefore some seedlings might have emerged but died shortly afterwards. If this is the case, it would have been almost impossible to have spotted remnants of the dead seedlings among the other vegetation, even in the clipped blocks as the bases of the clipped plants still remained. Consequently, this result might also mean that generally more seedlings in the nutrient addition treatment survived to be counted. Therefore, it is possible that seedling size (and therefore amount of nutrient reserves) played a role in *S. uniplumis* requiring an additional supply of nutrients during this early stage of its life cycle, compared to the other relatively large seeded species (Leishman, 2001; Milberg and Lamont, 1997).

Overall seedling survival was very low, understandably so because the experiment was contacted in a drought year (UNICEF, 2017), and according to Moles and Westoby (2004), drought is responsible for 35% of seedling mortality. What was surprising however, is the fact that grass seedling survival was lower than that of shrubs. A possible explanation for this could be that the grasses, both C_4, were more sensitive to water stress caused by the generally low rainfall and long intervals between rainfall events (Qi and Redmann, 1993; B. S. Ripley et al., 2007; Westoby et al., 2002). The drought might have caused primary root death before the development of secondary roots, leading to leave death (Stichler, 2002). The higher survival in our two shrubs compared to the grasses, might be due to woody species in arid environments first allocating resources to rapid root elongation, more than shoot growth and therefore escaping the dry areas in the topsoil and reaching water at greater soil depths faster. This argument is supported by the fact that woody species in arid savannas, especially those deemed encroaching, have higher root: shoot ratios, especially under stressful situations (my climate chamber experiment (Chapter 3); Chirara and Gispen, 2002; Frost et al., 1986).

In summary, although not directly assed in this study, drought/ water stress seemed to be the most important factor affecting the early stages of establishment for all our study species. Interestingly, in early life stages, drought stress could actually cause facilitative interactions to dominate between shrubs and grasses. The effect was clearly different between the two life forms, with grass seedlings being more affected by the drought stress. This finding was confirmed by our independent greenhouse and climate chamber experiments, were especially *A. pubescens* generally suffered the most from drought. Contrary to the common perception that grazing enhances shrub seedling establishment, *Senegalia mellifera* seedling emergence was negatively affected by simulated grazing, while *D. cinerea* was not affected. Therefore, while drought may favor shrub establishment, grazing could reduce the establishment of some shrub species.

Chapter 5

General conclusion

Contrary to our prediction, drought effect was generally not stronger for shrubs, but sometimes even seem to be stronger on the grasses. In addition, the shrubs had longer roots under drought stress and therefore could probably provide their relatively shorter shoots with more water, which might explain why the drought effect on shoot biomass was slightly in their favor.

There was also no indication of adaptation to drought among plants originating from drier areas. Since the plants where grown on foreign soil in Germany, and in a common garden (Namibia) where none of the seeds sown in that experiment originated from, there is a possibility that there are other factors at the seed areas of origin and therefore not considered in our experiments, which together with soil moisture might otherwise cause plants from different origins to perform differently.

There was generally a weak or no response to elevated CO_2, which was rather species- specific, with *S. mellifera* benefiting the most. However, the benefits obtained from elevated CO_2 were not enough to counteract the adverse effects of drought, probably due to the overall small effect size for CO_2.

To what extent the removal of grasses by livestock is a main determinant of shrub encroachment we cannot say, but at least at early life stages, *S. mellifera* (one of the problematic encroachers) seemed to be a stronger competitor than *A. pubescens* (one of the most valued/ palatable rangeland grasses). Unfortunately, due to high seedling mortality in the field experiment, we could not verify that the outcome of tree-grass interactions will differ when dealing with a mature grass matrix. However, *S. mellifera* seedling emergence was facilitated by surrounding herbaceous vegetation and/ or negatively affected by grazing, while *D. cinerea* was not affected. Therefore, is seems like *S. mellifera* is generally unaffected by grass competition (seedling stage) or facilitated by surrounding herbaceous vegetation (emergence and early seedling stage).

In order to provide feedback for the main hypotheses introduced in the first chapter, each hypothesis is restated as a question in Table. 1. below.

Table. 1. Responses to the different study questions according to each experimental approach

Questions	**Greenhouse**	**Climate chambers**	**Field**
1. Are grasses better adapted to drought than shrubs?	No, a grass (*A. pubescens*) and a shrub (*S. mellifera*) were both negatively affected by drought.	No, all the focal species' shoot biomass was reduced by drought, although the shrubs seem to counteract this effect through root growth.	**Unsuccessful**
2. Does elevated CO_2 concentrations enhance the growth of shrub seedlings more than that of grasses?	**Not applicable**	Yes, especially for *S. mellifera*.	**Not applicable**
3. Do the benefits obtained from CO_2 enrichment mitigate drought effect?	**Not applicable**	No	**Not applicable**
4. Do grasses suppress the establishment of shrub seedlings?	No, grasses seem to suffer more from *S. mellifera* competition.	No, *A. pubescens* suffered more from *S. mellifera* competition.	No, grasses seem to facilitate the emergence of *S. mellifera*.
5. Is the suppression in 4 more pronounced under drought conditions?	Inconclusive: very few replicates under the low water treatment.	No, there was no significant interaction between neighbour and water for the suppressed species.	**Not applicable**
6. Do plants that stem from drier areas perform better under drought conditions?	No, the opposite was true for *S. uniplumis* germination fraction, while the other species did not exhibit a significant ecotypic response to drought.	No, the opposite was true for three of our four species, apart from *S. mellifera* that showed no ecotypic differences.	**Unsuccessful**
7. Does nutrient addition benefit grasses more than shrubs?	**Not tested**	**Not tested**	Maybe, grasses seem to benefit more but the effect was only significant for the smaller seeded *S. uniplumis*.
8. Was seedling survival higher for grasses?	**Not tested**	**Not tested**	No, it was higher for the shrubs.

The one key take-home message from this study, which is clear from all the experiments is that drought was the most important factor in determining plant performance during the early life stages studied during this PhD. I therefore conclude that according to the early life stages and focal species studied here, climate change will not be decreasing shrub encroachment but that actually enhanced encroachment is possible in response to drought and elevated CO_2. This is however not necessarily due to a larger positive response of shrubs to CO_2 as predicted, but rather a smaller negative response to drought and competition. Rangeland managers are particularly cautioned to carefully monitor the recruitment of *Senegalia mellifera*, as it benefited the most from CO_2 enrichment and suffered the least from drought and competition. It therefore has the potential of becoming more of a problem in terms of bush encroachment, under those conditions.

This study provides the first empirical evidence on the effect of elevated CO_2 and drought on shrub-grass interactions in closed climate chambers, in which the environment could be completely controlled. This was however at a cost of realisms, and as such, I recommend that the next step in researching the effect of these two factors on bush encroachment should be through FACE experiments, for a more realistic view.

References

Ainsworth, E. A., and Rogers, A. (2007). The response of photosynthesis and stomatal conductance to rising [CO2]: Mechanisms and environmental interactions. *Plant, Cell and Environment*, *30*(3), 258–270. https://doi.org/10.1111/j.1365-3040.2007.01641.x

Archer, S., Schimel, D. S., and Holland, E. A. (1995). Mechanisms of shrubland expansion: land use, climate or CO2? *Climatic Change*, *29*(1), 91–99. https://doi.org/10.1007/BF01091640

Armas, C., Ordiales, R., and Pugnaire, F. I. C. N. (2004). Measuring plant interactions: a new comarative index. *Ecology*, *85*(10), 2682–2686. https://doi.org/10.1890/03-0650

Armas, C., Rodríguez-Echeverría, S., and Pugnaire, F. I. (2011). A field test of the stress-gradient hypothesis along an aridity gradient. *Journal of Vegetation Science*, *22*(5), 818–827. https://doi.org/10.1111/j.1654-1103.2011.01301.x

Ault, T. R., Cole, J. E., Overpeck, J. T., Pederson, G. T., and Meko, D. M. (2014). Assessing the risk of persistent drought using climate model simulations and paleoclimate data. *Journal of Climate*, *27*(20), 7529–7549. https://doi.org/10.1175/JCLI-D-12-00282.1

Baudena, M., D'Andrea, F., and Provenzale, A. (2010). An idealized model for tree-grass coexistence in savannas: The role of life stage structure and fire disturbances. *Journal of Ecology*, *98*(1), 74–80. https://doi.org/10.1111/j.1365-2745.2009.01588.x

Bell, W. E., and van Staden, J. (1993). Seed structure and germination of Dichrostachys cinerea. *South African Journal of Botany*, *59*(1), 9–13. https://doi.org/http://dx.doi.org/10.1016/S0254-6299(16)30768-2

Bertness, M. D., and Callaway, R. M. (1994). Positive interactions in communities. *Trends in Ecology and Evolution*, *9*(5), 187–191. https://doi.org/10.1016/0169-5347(94)90087-6

Blaser, W. J., Shanungu, G. K., Edwards, P. J., and Olde Venterink, H. (2014). Woody encroachment reduces nutrient limitation and promotes soil carbon sequestration. *Ecology and Evolution*, *4*(8), 1423–1438. https://doi.org/10.1002/ece3.1024

Bond, W. J. (2008). What Limits Trees in C 4 Grasslands and Savannas? *Annu. Rev. Ecol. Evol. Syst*, *39*, 641–59. https://doi.org/10.1146/annurev.ecolsys.39.110707.173411

Bond, W. J., and Midgley, G. F. (2000a). A proposed CO 2 -controlled mechanism of woody plant invasion in grasslands and savannas. *Global Change Biology*, *6*, 865–869.

Bond, W. J., and Midgley, G. F. (2000b). A proposed CO 2 -controlled mechanism of woody plant invasion in grasslands and savannas. *Global Change Biology* , *6*, 865–869.

Bond, W. J., and Midgley, G. F. (2012). Carbon dioxide and the uneasy interactions of trees and savannah grasses. *Phil. Trans. R. Soc. B*, *367*, 601–612. https://doi.org/10.1098/rstb.2011.0182

Bond, W. J., Midgley, G. F., and Woodward, F. I. (2003). The importance of low atmospheric CO2 and fire in promoting the spread of grasslands and savannas. *Global Change Biology*. https://doi.org/10.1046/j.1365-2486.2003.00577.x

Breshears, D. D., and Barnes, F. J. (1999). Interrelationships between plant functional types and soil moisture heterogeneity for semiarid landscapes withing the grassland/forest conintuum: a unified concept. *Landscape Ecology*, *14*(5), 465–478. https://doi.org/10.1023/A:1008040327508

Britz, M.-L., and Ward, D. (2007). The effects of soil conditions and grazing strategy on plant species composition in a semi-arid savanna. *African Journal of Range & Forage Science*, *24*(2), 51–61. https://doi.org/10.2989/AJRFS.2007.24.2.1.155

Bruder, J. (2015). *Dormanz- und Keimungsversuche mit Pflanzensamen aus Namibia*. BSc Thesis, University of Tuebingen.

Buitenwerf, R., Bond, W. J., Stevens, N., and Trollope, W. S. W. (2011). Increased Tree Densities in South African Savannas: 50 Years of Data Suggests CO2 as a Driver. https://doi.org/10.1111/j.1365-2486.2011.02561.x

Callaway, R. M. (1995). Positive interactions among plants. *Botanical Review*, *61*(4), 306–349. https://doi.org/10.1007/bf02912621

Callaway, R. M., and Walker, L. R. (1997). Competition and facilitation: A synthetic approach to interactions in plant communities. *Ecology*, *78*(7), 1958–1965. https://doi.org/10.1890/0012-9658(1997)078[1958:CAFASA]2.0.CO;2

Ceulemans, R., and Mousseau, M. (1994). Effects of elevated atmospheric woody plants on. *New PhyloL*, *127*.

Chirara, C., and Gispen, W. H. (2002). *Tree Invasion In a Semi-arid Savanna In Zimbabwe*. *Faculteit Biologie* (Vol. PhD-van). Retrieved from http://igitur-archive.library.uu.nl/dissertations/2002-0325-100407/full.pdf

Cramer, M. D., Van Cauter, A., and Bond, W. J. (2010). Growth of N2-fixing African savanna Acacia species is constrained by below-ground competition with grass. *Journal of Ecology*, *98*(1), 156–167. https://doi.org/10.1111/j.1365-2745.2009.01594.x

Davis, M. A., Wrage, K. J., Reich, P. B., Tjoelker, M. G., Schaeffer, T., and Muermann, C. (1999). Survival, growth, and photosynthesis of tree seedllings competing with herbaceous vegetation along a water-light-nitrogen gradient. *Plant Ecology*, *145*, 341–30.

Davis, M., and Shaw, R. (2001). Range shifts and adaptive responses to Quaternary climate change. *Science*, *292*(April), 673–679. https://doi.org/10.1126/science.292.5517.673

De Klerk, J. N. (2004). Bush Encroachment in Namibia. Report on Phase 1 of the Bush Encroachment Research, Monitoring and Management Project.

Derner, J. D., Johnson, H. B., Kimball, B. a, Jr, P. J. P., Polley, H. W., Tischler, C. R., … Robert, L. (2003). Above- and below-ground responses of C 3 ± C 4 species mixtures to elevated CO 2 and soil water availability, *3*, 452–461.

Devine, A. P., McDonald, R. A., Quaife, T., and Maclean, I. M. D. (2017). Determinants of woody encroachment and cover in African savannas. *Oecologia*. https://doi.org/10.1007/s00442-017-3807-6

Devine, A. P., Stott, I., Mcdonald, R. A., and Maclean, I. M. D. (2015). Woody cover in wet and dry African savannas after six decades of experimental fires. *Journal of Ecology*. https://doi.org/10.1111/1365-2745.12367

Dohn, J., Dembélé, F., Karembé, M., Moustakas, A., Amévor, K. A., and Hanan, N. P. (2013). Tree effects on grass growth in savannas: Competition, facilitation and the stress-gradient hypothesis. *Journal of Ecology*. https://doi.org/10.1111/1365-2745.12010

Dorman, M., Sapir, Y., and Volis, S. (2009). Local adaptation in four Iris species tested in a common-garden experiment. *Biological Journal of the Linnean Society*, *98*(2), 267–277. https://doi.org/10.1111/j.1095-8312.2009.01265.x

Duan, H., Amthor, J. S., Duursma, R. A., O'Grady, A. P., Choat, B., and Tissue, D. T. (2013). Carbon dynamics of eucalypt seedlings exposed to progressive drought in elevated [CO2] and elevated temperature. *Tree Physiology*, *33*(8), 779–792. https://doi.org/10.1093/treephys/tpt061

Ernst, W. H. O. (1988). Seed and seedling ecology of Brachystegia spiciformis, a predominant tree component in miombo woodlands in South Central Africa. *Forest Ecology and Management*, *25*(3–4), 195–210. https://doi.org/10.1016/0378-1127(88)90087-4

Fangmeier, A., Torres-Toledo, V., Franzaring, J., and Damsohn, W. (2016). Design and performance of a new FACE (free air carbon dioxide enrichment) system for crop and short vegetation exposure. *Environmental and Experimental Botany*, *130*, 151–161. https://doi.org/10.1016/j.envexpbot.2016.06.002

Franks, P. J., Adams, M. A., Amthor, J. S., Barbour, M. M., Berry, J. A., Ellsworth, D. S., … von Caemmerer, S. (2013). Sensitivity of plants to changing atmospheric CO 2 concentration : from the geological past to the next century. *New Phytologist*, *197*, 1077–1094.

Franks, S. J., Weber, J. J., and Aitken, S. N. (2014). Evolutionary and plastic responses to climate change in terrestrial plant populations. *Evolutionary Applications*, *7*(1), 123–139. https://doi.org/10.1111/eva.12112

Frost, P., Medina, E., Menaut, J., Solbrig, O., Swift, M., and Walker, B. (1986). Responses of savannas to stress and disturbance. *Biology International*, (10), 1–82. https://doi.org/ISSN 02532069

Gil-Romera, G., Lamb, H. F., Turton, D., Sevilla-Callejo, M., and Umer, M. (2010). Long-term resilience, bush encroachment patterns and local knowledge in a Northeast African savanna. *Global Environmental Change*, *20*(4), 612–626. https://doi.org/10.1016/j.gloenvcha.2010.04.008

Giménez-Benavides, L., Escudero, A., and Iriondo, J. M. (2007). Local adaptation enhances seedling recruitment along an altitudinal gradient in a high mountain mediterranean plant. *Annals of Botany*, *99*(4), 723–734. https://doi.org/10.1093/aob/mcm007

Gioria, M., and Osborne, B. A. (2014). Resource competition in plant invasions: emerging patterns and research needs. *Frontiers in Plant Science*, *5*(September), 1–21. https://doi.org/10.3389/fpls.2014.00501

Good, M. K., Clarke, P. J., Price, J. N., and Reid, N. (2014). Seasonality and facilitation drive tree establishment in a semi-arid floodplain savanna. *Oecologia*, *175*(1), 261–271. https://doi.org/10.1007/s00442-014-2886-x

Gowik, U., and Westhoff, P. (2011). The path from C3 to C4 photosynthesis. *Plant Physiology*, *155*(1), 56–63. https://doi.org/10.1104/pp.110.165308

Hagenah, N., Munkert, H., Gerhardt, K., and Olff, H. (2009). Interacting effects of grass height and herbivores on the establishment of an encroaching savanna shrub. *Herbaceous Plant Ecology: Recent Advances in Plant Ecology*, 189–202. https://doi.org/10.1007/978-90-481-2798-6_15

Hamim. (2005). Photosynthesis of C3 and C4 Species in Response to Increased CO 2 Concentration and Drought Stress. *Hayati : Journal of Biosciences*, *12*(4), 131–138. https://doi.org/10.1016/S1978-3019(16)30340-0

Harrington, G. (1991). Effects of Soil Moisture on Shrub Seedling Survival in Semi-Arid Grassland. *Ecology*, *72*(3), 1138–1149.

Heath, A., and Heath, R. (2010). *Field guide to the plants of Northern Botswana including the Okavango Delta*. (D. Goyder, Ed.). Richmond, Surrey: Kew Publishing, Royal Botanic Gardens, Kew.

Higgins, S. I., Bond, W. J., Winston, S., and Trollope, W. (2000). Fire , resprouting and variability : a recipe for grass ± tree coexistence in savanna, 213–229.

Hilhorst, H. W. M., and Karssen, C. M. (1989). Nitrate reductase independent stimulation of seed germination in Sisymbrium officinale L. (hedge mustard) by light and nitrate. *Annals of Botany*, *63*(December), 131–137.

Hill, M. (2002). *Arid and semi-arid environments*. London: Hodder and Stoughton.

Hoffmann, A. A., and Sgrò, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, *470*(7335), 479–485. https://doi.org/10.1038/nature09670

Högy, P., Brunnbauer, M., Koehler, P., Schwadorf, K., Breuer, J., Franzaring, J., … Fangmeier, A. (2013). Grain quality characteristics of spring wheat (Triticum aestivum) as affected by free-air CO2 enrichment. *Environmental and Experimental Botany*, *88*, 11–18. https://doi.org/10.1016/j.envexpbot.2011.12.007

Holdo, R. M. (2013). Revisiting the Two-Layer Hypothesis: Coexistence of Alternative Functional Rooting Strategies in Savannas. *PLoS ONE*, *8*(8). https://doi.org/10.1371/journal.pone.0069625

Hudak, a. T. (1999). Rangeland Mismanage m ent in South A frica : Failu re to A pp ly Ecolo gical Kn owledge. *Human Ecology*, *27*, 55–78. https://doi.org/10.1023/A:1018705300730

Ibrahim, D. G., Gilbert, M. E., Ripley, B. S., and Osborne, C. P. (2008). Seasonal differences in photosynthesis between the C3 and C 4 subspecies of Alloteropsis semialata are offset by frost and drought. *Plant, Cell and Environment*, *31*(7), 1038–1050. https://doi.org/10.1111/j.1365-3040.2008.01815.x

IPCC. (2007). *Climate Change 2007 Synthesis Report. Intergovernmental Panel on Climate Change [Core Writing Team IPCC*. https://doi.org/10.1256/004316502320517344

IPCC. (2014). Climate Change 2014 Synthesis Report Summary Chapter for Policymakers.

Johnson, L. C., Olsen, J. T., Tetreault, H., Delacruz, A., Bryant, J., Morgan, T. J., … Maricle, B. R. (2015). Intraspecific variation of a dominant grass and local adaptation in reciprocal garden communities along a US Great Plains' precipitation gradient: Implications for grassland restoration with climate change. *Evolutionary Applications*, *8*(7), 705–723. https://doi.org/10.1111/eva.12281

Joubert, D. F., Rothauge, A., and Smit, G. N. (2008). A conceptual model of vegetation dynamics in the semiarid Highland savanna of Namibia, with particular reference to bush thickening by Acacia mellifera. *Journal of Arid Environments*. https://doi.org/10.1016/j.jaridenv.2008.07.004

Jump, A. S., and Peñuelas, J. (2005). Running to stand still: Adaptation and the response of plants to rapid climate change. *Ecology Letters*, *8*(9), 1010–1020. https://doi.org/10.1111/j.1461-0248.2005.00796.x

Kellman, M., and Kading, M. (1992). Facilitation of tree seedling establishment in a sand dune succession. *Journal of Vegetation Science*, *3*, 679–688. https://doi.org/10.2307/3235836

Kgope, B. S., Bond, W. J., and Midgley, G. F. (2009). Growth responses of African savanna trees implicate atmospheric [CO2] as a driver of past and current changes in savanna tree cover. Au... *Austral Ecology*, *35*, 451–463.

Kgosikoma, O. E., and Mogotsi, K. (2013). Understanding the causes of bush encroachment in Africa: The key to effective management of savanna grasslands. *Tropical Grasslands – Forrajes Tropicales*, *1*, 215–219. Retrieved from www.tropicalgrasslands.info

Klanderud, K. (2005). Climate change effects on species interactions in an alpine plant community. *Journal of Ecology*, *93*(1), 127–137. https://doi.org/10.1111/J.1365-2745.2004.00944.X

Kraaij, T., and Ward, D. (2006). Effects of rain, nitrogen, fire and grazing on tree recruitment and early survival in bush-encroached savanna, South Africa. *Plant Ecology*. https://doi.org/10.1007/s11258-006-9125-4

Ladd, B. M., and Facelli, J. M. (2005). Effects of competition, resource availability and invertebrates on tree seedling establishment. *Journal of Ecology*, *93*(5), 968–977. https://doi.org/10.1111/j.1365-2745.2005.01026.x

Leakey, A. D. B., Uribelarrea, M., Ainsworth, E. A., Naidu, S. L., Rogers, A., Ort, D. R., … Biology, P. (2006). Photosynthesis , Productivity , and Yield of Maize Are Not Affected by Open-Air Elevation of CO 2 Concentration in the Absence of Drought 1 [OA], *140*(February), 779–790. https://doi.org/10.1104/pp.105.073957.et

Le Roux, P., and Müller, M. (2009). *Trees and Shrubs of Namibia*. (C. A. Mannheimer & B. A. Curtis, Eds.). Windhoek: Macmillan Education Namibia.

Le Roux, X., Bariac, T., and Mariotti, a. (1995). Spatial partitioning of the soil water resoucre between grasses and shrub compnents in a west African humid savanna. *Oecologia*, *104*, 145–155. https://doi.org/10.1007/BF00328579

Leger, E. A., and Rice, K. J. (2007). Assessing the speed and predictability of local adaptation in invasive California poppies (Eschscholzia californica). *Journal of Evolutionary Biology*, *20*(3), 1090–1103. https://doi.org/10.1111/j.1420-9101.2006.01292.x

Lehmann, C. E. R., Archibald, S. A., Hoffmann, W. A., and Bond, W. J. (2011). Deciphering the distribution of the savanna biome. *New Phytologist*, *191*(1), 197–209. https://doi.org/10.1111/j.1469-8137.2011.03689.x

Leimu, R., and Fischer, M. (2008). A meta-analysis of local adaptation in plants. *PLoS ONE*, *3*(12), 1–8. https://doi.org/10.1371/journal.pone.0004010

Leishman, M. R. (2001). Does the seed size/number trade-off model determine plant community structure? An assessment of the model mechanisms and their generality. *Oikos*, *93*(2), 294–302. https://doi.org/10.1034/j.1600-0706.2001.930212.x

Macias, D., Mazía, N., and Jacobo, E. (2014). Grazing and neighborhood interactions limit woody encroachment in wet subtropical savannas. *Basic and Applied Ecology*, *15*, 661–668. https://doi.org/10.1016/j.baae.2014.09.008

Maliva, R., and Missimer, T. (2012). Arid Lands Water Evaluation and Management, (1948), 21–40. https://doi.org/10.1007/978-3-642-29104-3

Manea, A., and Leishman, M. R. (2015). Competitive interactions between established grasses and woody plant seedlings under elevated CO2 levels are mediated by soil water availability. *Oecologia*. https://doi.org/10.1007/s00442-014-3143-z

McKay, J. K., Bishop, J. G., Lin, J.-Z., Richards, J. H., Sala, A., and Mitchell-Olds, T. (2001). Local adaptation across a climatic gradient despite small effective population size in the rare sapphire rockcress. *Proceedings of the Royal Society B: Biological Sciences*, *268*(1477), 1715–1721. https://doi.org/10.1098/rspb.2001.1715

Mendelsohn, J. (2002). Atlas of Namibia.

Mendelsohn, J., Jarvis, A., Roberts, C., and Robertson, T. (2002). *Atlas of Namibia: a portrait of the land and its people*. Cape Town, South Africa: David Philips Publishers.

Meyer, K. M., Ward, D., Moustakas, A., and Wiegand, K. (2005). Big is not better: Small Acacia mellifera shrubs are more vital after fire. *African Journal of Ecology*, *43*(2), 131–136. https://doi.org/10.1111/j.1365-2028.2005.00559.x

Milberg, P., and Lamont, B. B. (1997). Seed/cotyledon size and nutrient content play a major role in early performance of species on nutrient-poor soils. *New Phytologist*, *137*(4), 665–672. https://doi.org/10.1046/j.1469-8137.1997.00870.x

Miller, T. E. (1987). Effects of emergence time on survival and growth in an early old-field plant community. *Oecologia*. https://doi.org/10.1007/BF00379278

Moleele, N. M., Ringrose, S., Matheson, W., and Vanderpost, C. (2002). More woody plants? the status of bush encroachment in Botswana's grazing areas. *Journal of Environmental Management*, *64*(1), 3–11. https://doi.org/10.1006/jema.2001.0486

Moles, A. T., and Westoby, M. (2004). What do seedlings die from and what are the implications for evolution of seed size? *OIKOS* , *106*(1), 193–199.

Montoya, J. M., and Raffaelli, D. (2010). Climate change, biotic interactions and ecosystem services. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1549), 2013–2018. https://doi.org/10.1098/rstb.2010.0114

Morgan, J. A., Pataki, D. E., Körner, C., Clark, H., Del Grosso, S. J., Grünzweig, J. M., … Shaw, M. R. (2004). Water relations in grassland and desert ecosystems exposed to elevated atmospheric CO2. *Oecologia*, *140*(1), 11–25. https://doi.org/10.1007/s00442-004-1550-2

Müller, M. A. N. (2007). *Grasses of Namibia*. (J. van Eck, Ed.). Windhoek: Ministry of Agriculture, Water and Forestry, Namibia.

Nesongano, W. C., Fangmeier, A., and Tielbörger, K. (n.d.). *Drought and elevated CO2 effects on trees and grasses in southern African savannas*. University of Teubingen.

Nippert, J. B., and Holdo, R. M. (2015). Challenging the maximum rooting depth paradigm in grasslands and savannas. *Functional Ecology*, *29*(6), 739–745. https://doi.org/10.1111/1365-2435.12390

Norby, R. J., and Zak, D. R. (2011). Ecological Lessons from Free-Air CO 2 Enrichment (FACE) Experiments. *Annu. Rev. Ecol. Evol. Syst*, *42*, 181–203. https://doi.org/10.1146/annurev-ecolsys-102209-144647

O. E. Kgosikoma. (2012). Bush encroachment in relation to rangeland management systems and environmental conditions in Kalahari ecosystem of Botswana. *African Journal of Agricultural Reseearch*, *7*(15), 2312–2319. https://doi.org/10.5897/AJAR11.2374

O'Connor, T. G., Puttick, J. R., and Hoffman, M. T. (2014). Bush encroachment in southern Africa : changes and causes. *African Journal of Range and Forage Science*, *31*(2). https://doi.org/10.2989/10220119.2014.939996

Osborne, C. P., and Sack, L. (2012). Evolution of C4 plants: a new hypothesis for an interaction of CO2 and water relations mediated by plant hydraulics. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367*(1588), 583–600. https://doi.org/10.1098/rstb.2011.0261

Paschalis, A., Katul, G. G., Fatichi, S., Palmroth, S., and Way, D. (2017). On the variability of the ecosystem response to elevated atmospheric CO2 across spatial and temporal scales at the Duke Forest FACE experiment. *Agricultural and Forest Meteorology*. https://doi.org/10.1016/j.agrformet.2016.09.003

Perry, L. G., Shafroth, P. B., Blumenthal, D. M., Morgan, J. A., and Lecain, D. R. (2013). Elevated CO2does not offset greater water stress predicted under climate change for native and exotic riparian plants. *New Phytologist*, *197*(2), 532–543. https://doi.org/10.1111/nph.12030

Petrů, M., Tielbörger, K., Belkin, R., Sternberg, M., and Jeltsch, F. (2006). Life history variation in an annual plant under two opposing environmental constraints along an aridity gradient. *Ecography*, *29*(1), 66–74. https://doi.org/10.1111/j.2005.0906-7590.04310.x

Polley, H. W. (1997). Implications of rising atmospheric carbon dioxide concentration for rangelands. *Journal of Range Management*, *50*(6), 562–577. https://doi.org/10.2307/4003450

Polley, H. W., and Derner, J. D. (2002). Publications from USDA-ARS / UNL Faculty Soil- and Plant-Water Dynamics in a C3 / C4 grassland exposed to a Subambient to superambient CO2 Gradient Soil- and plant-water dynamics in a C3 / C4 grassland exposed to a subambient to superambient CO 2 gradient. *Global Change Biology*, 1118–1129.

Polley, H. W., Mayeux, H. S., Johnson, H. B., and Tischler, C. R. (1997). Viewpoint: Atmospheric CO*, soil water, and shrub/grass ratios on rangelands. *J. Range Manage*, *50*(3), 278–284.

PONS, T. L. (1989). Breaking of seed dormancy by nitrate as a gap detection mechanism. *Annals of Botany*, *63*(1), 139–143. Retrieved from http://aob.oxfordjournals.org/content/63/1/139.short

Puttock, A., Dungait, J. A. J., Macleod, C. J. A., Bol, R., and Brazier, R. E. (2014). Organic Carbon From Dryland Soils. *Journal of Geophysical Research: Biogeosciences*, *119*, 2345–2357. https://doi.org/10.1002/2014JG002635

Qi, M. Q., and Redmann, R. E. (1993). Seed germination and seedling survival of C3 and C4 grasses under water stress. *Journal of Arid Environments*, *24*, 277–285.

Quintana, J. R., Cruz, A., Fernández-González, F., and Moreno, J. M. (2004). Time of germination and establishment success after fire of three obligate seeders in a Mediterranean shrubland of central Spain. *Journal of Biogeography*. https://doi.org/10.1111/j.1365-2699.2004.00955.x

Ringrose, S., Matheson, W., Wolski, P., Huntsman-Mapila, P., and Oppenheimer, H. (2003). Vegetation cover trends along the Botswana Kalahari transect. *Journal of Arid Environments*, *54*, 297–317. https://doi.org/10.1006/jare.2002.1092

Ripley, B., Frole, K., and Gilbert, M. (2010). Differences in drought sensitivities and photosynthetic limitations between co-occurring C3 and C4 (NADP-ME) Panicoid grasses. *Annals of Botany*, *105*(3), 493–503. https://doi.org/10.1093/aob/mcp307

Ripley, B. S., Gilbert, M. E., Ibrahim, D. G., and Osborne, C. P. (2007). Drought constraints on C4 photosynthesis: Stomatal and metabolic limitations in C3 and C4 subspecies of Alloteropsis semialata. *Journal of Experimental Botany*, *58*(6), 1351–1363. https://doi.org/10.1093/jxb/erl302

Rohde, R. F., and Hoffman, M. T. (2012). The historical ecology of Namibian rangelands: Vegetation change since 1876 in response to local and global drivers. *Science of the Total Environment*. https://doi.org/10.1016/j.scitotenv.2011.10.067

Roques, K. G., O'Connor, T. G., and Watkinson, A. R. (2001). Dynamics of shrub encroachment in an African savanna: Relative influences of fire, herbivory, rainfall and density dependence. *Journal of Applied Ecology*. https://doi.org/10.1046/j.1365-2664.2001.00567.x

Rossatto, D. R., Silva, L. C. R., Sternberg, L. S. L., and Franco, A. C. (2014). Do woody and herbaceous species compete for soil water across topographic gradients? Evidence for niche partitioning in a Neotropical savanna. *South African Journal of Botany*, *91*, 14–18. https://doi.org/10.1016/j.sajb.2013.11.011

Rysavy, A., Seifan, M., Sternberg, M., and Tielbörger, K. (2014). Shrub seedling survival under climate change - comparing natural and experimental rainfall gradients. *Journal of Arid Environments*. https://doi.org/10.1016/j.jaridenv.2014.07.004

Sala, O. E., Golluscio, R. A., Lauenroth, W. K., and Soriano, A. (1989). Resource partitioning between shrubs and grasses in the Pategonian steppe. *Oecologia*, *81*(4), 501–505.

Sala, O. E., and Maestre, F. T. (2014). Grass-woodland transitions: Determinants and consequences for ecosystem functioning and provisioning of services. *Journal of Ecology*, *102*(6), 1357–1362. https://doi.org/10.1111/1365-2745.12326

Sankaran, M., Hanan, N. P., Scholes, R. J., Ratnam, J., Augustine, D. J., Cade, B. S., … Zambatis, N. (2005). Determinants of woody cover in African savannas. *Nature*, *438*(7069), 846–849. https://doi.org/10.1038/nature04070

Sankaran, M., Ratnam, J., and Hanan, N. P. (2004). Tree-grass coexistence in savannas revisited - Insights from an examination of assumptions and mechanisms invoked in existing models. *Ecology Letters*, *7*(6), 480–490. https://doi.org/10.1111/j.1461-0248.2004.00596.x

Savolainen, O., Pyhäjärvi, T., and Knürr, T. (2007). Gene Flow and Local Adaptation in Trees. *Annual Review of Ecology, Evolution, and Systematics*, *38*(1), 595–619. https://doi.org/10.1146/annurev.ecolsys.38.091206.095646

Schiffers, K., and Tielbörger, K. (2006). Ontogenetic shifts in interactions among annual plants. *Journal of Ecology*, *94*(2), 336–341. https://doi.org/10.1111/j.1365-2745.2006.01097.x

Scholes, R. J. (2003). Convex Relationships in Ecosystems Containing Mixtures of Trees and Grass. *Environmental and Resource Economics*.

https://doi.org/10.1023/B:EARE.0000007349.67564.b3

Scholes, R. J., and Archer, S. R. (1997). Tree-Grass interactions in savannas. *Annual Review of Ecology and Systematics*, *28*, 517–544.

Shanahan, T. M., Hughen, K. A., Mckay, N. P., Overpeck, J. T., Scholz, C. A., Gosling, W. D., … Heil, C. W. (2016). CO 2 and fire influence tropical ecosystem stability in response to climate change. *Nature Publishing Group*. https://doi.org/10.1038/srep29587

Skarpe, C. (1990). Structure of the Woody Vegetation in Disturbed and Undisturbed Arid Savanna , Botswana. *Vegetation*, *87*(1), 11–18.

Skarpe, C. (1992). Dynamics of savanna ecosystems. *Journal of Vegetation Science* , *3*, 293–300.

Stichler, C. (2002). Grass growth and development. Retrieved December 20, 2017, from http://counties.agrilife.org/kerr/files/2011/09/grass-growth-and-development_3.pdf

Taylor, S. H., Ripley, B. S., Woodward, F. I., and Osborne, C. P. (2011). Drought limitation of photosynthesis differs between C3 and C4 grass species in a comparative experiment. *Plant, Cell and Environment*, *34*(1), 65–75. https://doi.org/10.1111/j.1365-3040.2010.02226.x

Tessema, Z. K., de Boer, W. F., Baars, R. M. T., and Prins, H. H. T. (2011). Changes in soil nutrients, vegetation structure and herbaceous biomass in response to grazing in a semi-arid savanna of Ethiopia. *Journal of Arid Environments*. https://doi.org/10.1016/j.jaridenv.2011.02.004

Tielbörger, K., Bilton, M. C., Metz, J., Kigel, J., Holzapfel, C., Lebrija-Trejos, E., … Sternberg, M. (2014). Middle-Eastern plant communities tolerate 9 years of drought in a multi-site climate manipulation experiment. *Nature Communications*, *5*(October), 5102. https://doi.org/10.1038/ncomms6102

Tomiolo, S., Van Der Putten, W. H., Tielborger, K., and Allison, S. D. (2015). Separating the role of biotic interactions and climate in determining adaptive response of plants to climate change. *Ecology*, *96*(5), 1298–1308. https://doi.org/10.1890/14-1445.1.sm

Turkington, R., Goldberg, D. E., Olsvig-Whittaker, L., and Dyer, A. R. (2005). Effects of density on timing of emergence and its consequences for survival and growth in two communities of annual plants. *Journal of Arid Environments*, *61*(3), 377–396. https://doi.org/10.1016/j.jaridenv.2004.09.016

UNICEF. (2017). *Namibia Humanitarian Situation Report- Report #1*. Retrieved from https://www.unicef.org/appeals/files/UNICEF_Namibia_Humanitarian_SitRep_Feb_2017.pdf

Vetaas, O. R. (1992). Micro-site effects of trees and shrubs in dry savannas. *Journal of Vegetation Science*, *3*(3), 337–344. https://doi.org/10.2307/3235758

Volis, S. (2007). Correlated patterns of variation in phenology and seed production in populations of two annual grasses along an aridity gradient. *Evolutionary Ecology*, *21*(3), 381–393. https://doi.org/10.1007/s10682-006-9108-x

Volis, S., Mendlinger, S., and Ward, D. (2002). Differentiation in populations of *Hordeum spontaneum* along a gradient of environmental productivity and predictability: life history and local adaptation. *Biological Journal of the Linnean Society*, *77*(1987), 479–490.

Von Gillhaussen, P., Rascher, U., Jablonowski, N. D., Plückers, C., Beierkuhnlein, C., and Temperton, V. M. (2014). Priority effects of time of arrival of plant functional groups override sowing interval or density effects: A grassland experiment. *PLoS ONE*, *9*(1). https://doi.org/10.1371/journal.pone.0086906

Wainwright, C. E., Wolkovich, E. M., and Cleland, E. E. (2012). Seasonal priority effects: Implications for invasion and restoration in a semi-arid system. *Journal of Applied Ecology*, *49*(1), 234–241. https://doi.org/10.1111/j.1365-2664.2011.02088.x

Walter, H. (1971). *Ecology of tropical and subtropical vegetation*. Edinburgh: Oliver and Boyd, Edinburgh.

Wang, T., Wang, Z., Xia, F., and Su, Y. (2016). Local adaptation to temperature and precipitation in naturally fragmented populations of Cephalotaxus oliveri, an endangered conifer endemic to China. *Scientific Reports*, *6*(1), 25031. https://doi.org/10.1038/srep25031

Ward, D. (2005). Do we understand the causes of bush encroachment in African savannas? *African Journal of Range & Forage Science*, *22*(2), 101–105. https://doi.org/10.2989/10220110509485867

Ward, D., Wiegand, K., and Getzin, S. (2013). Walter's two-layer hypothesis revisited: Back to the roots! *Oecologia*, *172*(3), 617–630. https://doi.org/10.1007/s00442-012-2538-y

Ward, J. K., Tissue, D. T., Thomas, R. B., and Strain, B. R. (1999). Comparative responses of model C3 and C4 plants to drought in low and elevated CO2. *Global Change Biology*, *5*, 857–867.

Warren, J. M., Norby, R. J., Wullschleger, S. D., and Oren, R. (2011). Elevated CO2 enhances leaf senescence during extreme drought in a temperate forest. *Tree Physiology*, *31*(2), 117–130. https://doi.org/10.1093/treephys/tpr002

Weltzin, J. F., and McPherson, G. R. (1997). Spatial and temporal soil moisture resource partitioning by trees and grasses in a temperature savanna, Arizona, USA. *Oecologia*, *112*(2), 156–164.

Westoby, M., Falster, D. S., Moles, A. T., Vesk, P. A., and Wright, I. J. (2002). Plant Ecological Strategies: Some Leading Dimensions of Variation Between Species. *Annual Review of Ecology and Systematics*, *33*(1), 125–159. https://doi.org/10.1146/annurev.ecolsys.33.010802.150452

Wiegand, K., Saltz, D., and Ward, D. (2006). A patch-dynamics approach to savanna dynamics and woody plant encroachment - Insights from an arid savanna. *Perspectives in Plant Ecology, Evolution and Systematics*. https://doi.org/10.1016/j.ppees.2005.10.001

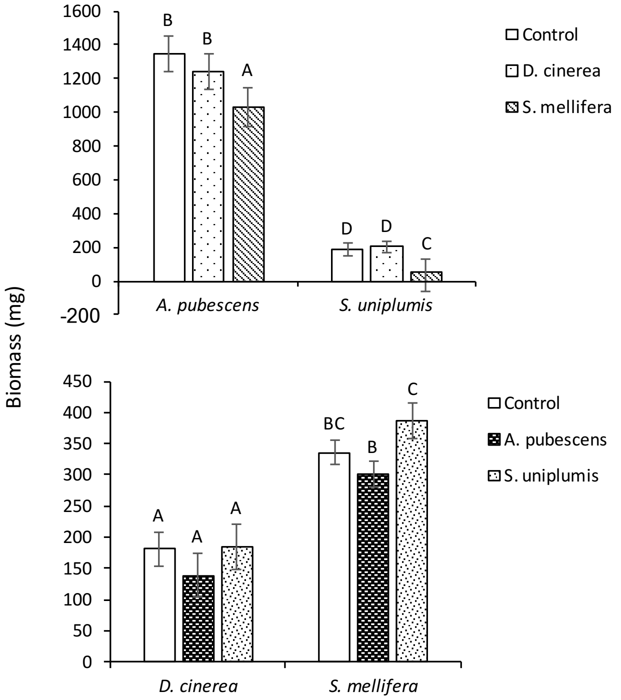
Wiegand, K., Ward, D., and Saltz, D. (2005). Multi-scale patterns and bush encroachment in an arid savanna with a shallow soil layer. *Journal of Vegetation Science*, *16*(3), 311–320. https://doi.org/10.1111/j.1654-1103.2005.tb02369.x

Xu, Z., Jiang, Y., Jia, B., and Zhou, G. (2016). Elevated-CO2 Response of Stomata and Its Dependence on Environmental Factors. *Frontiers in Plant Science*, *7*(May), 1–15. https://doi.org/10.3389/fpls.2016.00657

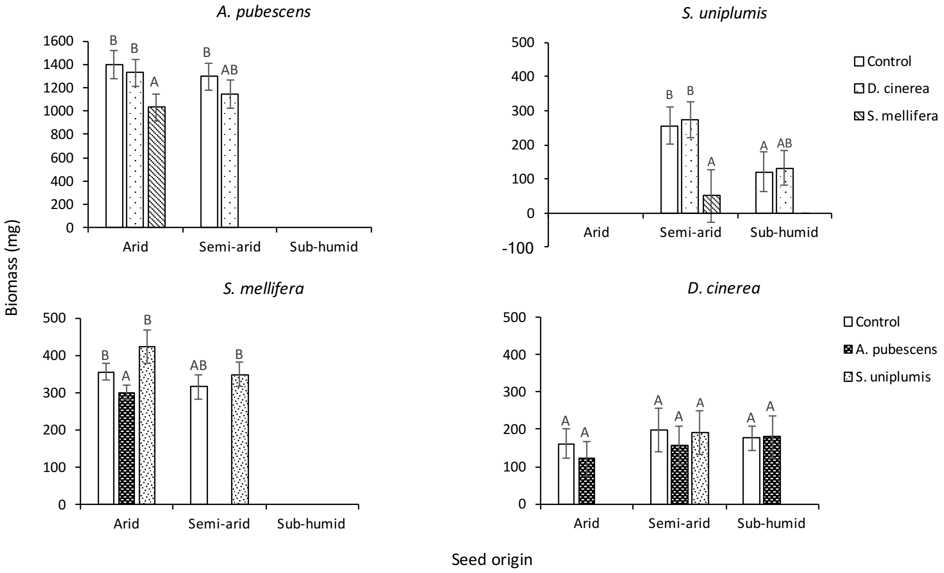
Zimmermann, J., Higgins, S. I., Grimm, V., Hoffmann, J., Münkemüller, T., and Linstädter, A. (2008). Recruitment filters in a perennial grassland: The interactive roles of fire, competitors, moisture and seed availability. *Journal of Ecology*, *96*(5), 1033–1044. https://doi.org/10.1111/j.1365-2745.2008.01409.x

Appendices

Chapter 2



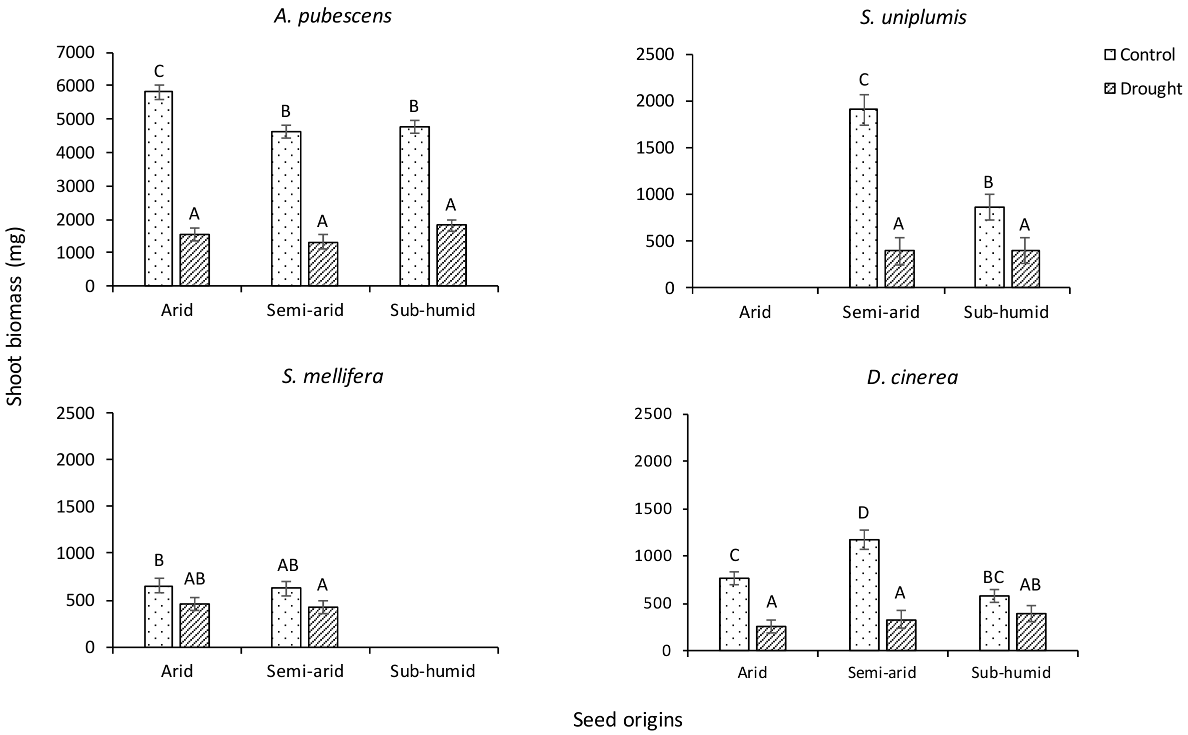
Appendix. 1. Shoot biomass (mean ± SE) showing the response of the grasses (top) to the two shrubs, and the shrubs (bottom) to the two grasses, as neighbours. Different letters represent significant differences and are unique to each species.



Appendix. 2. Shoot biomass (mean ± SE) for the four focal-species (grasses on top and shrubs at the bottom), showing the effect of seed origin and a heterospecific neighbour. The grasses are showing the effect of either *D. cinerea* or *S. mellifera* as a neighbour and the shrubs those of *A. pubescens* and *S. uniplumis*. Different letters represent significant differences and are unique to each graph.

Chapter 3

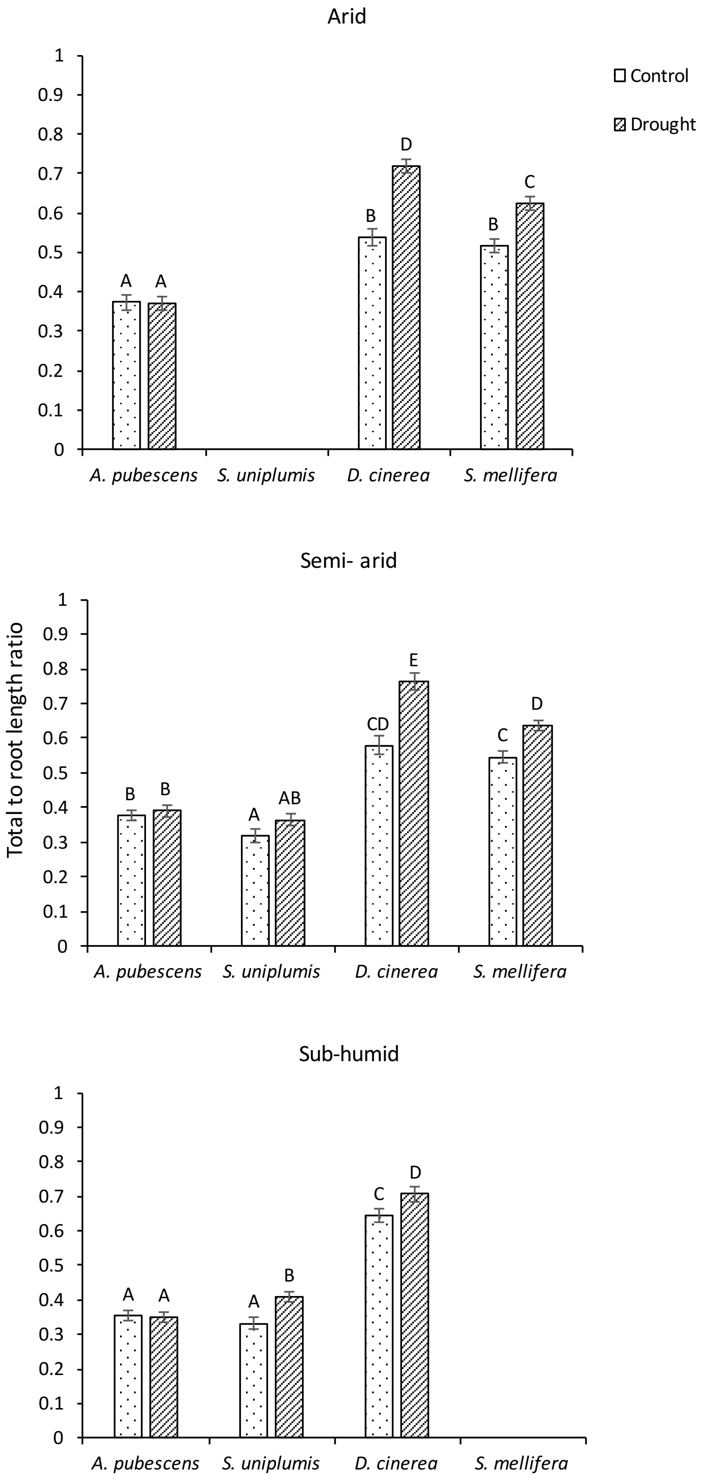
Experiment 1



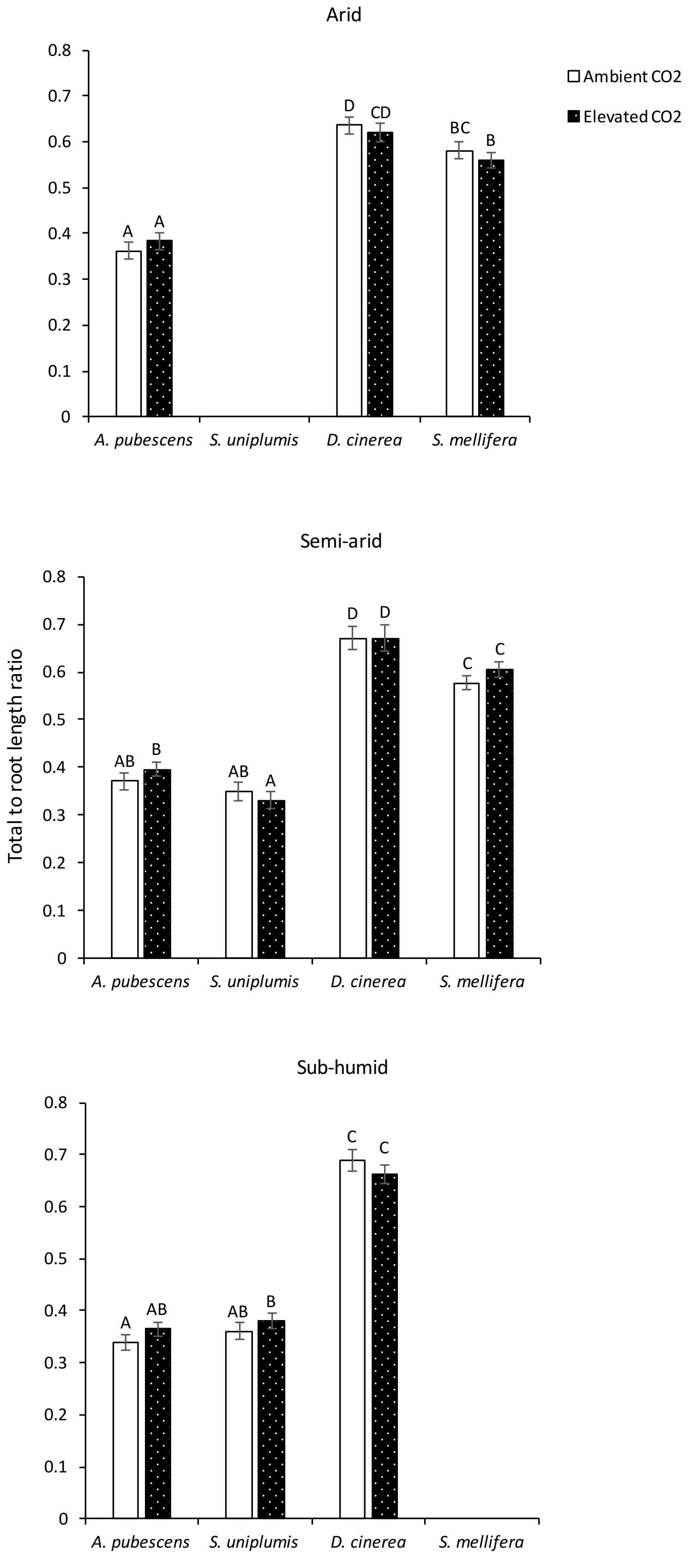
Appendix. 1. Shoot biomass (mean ± SE) for each of the focal species (grasses on top and shrubs at the bottom), showing ecotypic response to drought. Different letters represent significant differences (GLMs), and are unique to each species.

Appendix. 2. Results of GLMs with a Normal distribution and logit link function, constructed to test for the effect of water (H_2O), elevated CO_2 and seed origin (Orig.), on root elongation of the four focal-species. Significant results ($p < 0.05$) are indicated in bold.

	A. pubescens			*S. uniplumis*			*D. cinerea*			*S. mellifera*		
Effects	**F**	**df**	**p-value**	**F**	**df**	**p-value**	**F**	**df**	**p-value**	**F**	**df**	**p-value**
CO_2	6.69	1	**0.010**	0.001	1	0.982	0.34	1	0.559	0.05	1	0.817
H_2O	0.14	1	0.713	18.23	1	**<0.001**	37.92	1	**<0.001**	26.46	1	**<0.001**
Orig.	8.92	2	**0.012**	4.11	1	**0.043**	4.28	2	0.118	1.16	1	0.282
Orig.x CO_2	0.04	2	0.982	1.94	1	0.163	0.16	2	0.923	1.65	1	0.200
Orig. x H_2O	0.68	2	0.713	0.99	1	0.319	6.59	2	**0.037**	0.17	1	0.680
CO_2 x H_2O	7.86	1	**0.005**	1.42	1	0.234	0.14	1	0.713	1.30	1	0.254
Orig. x CO_2 x H_2O	3.68	2	0.159	0.58	1	0.446	2.71	2	0.258	3.60	1	0.058



Appendix. 3. The effects of drought on seedling root to total length allocation to roots for the different ecotypes of our focal-species. Different letters indicate significant differences (GLMs) and are unique to each graph.

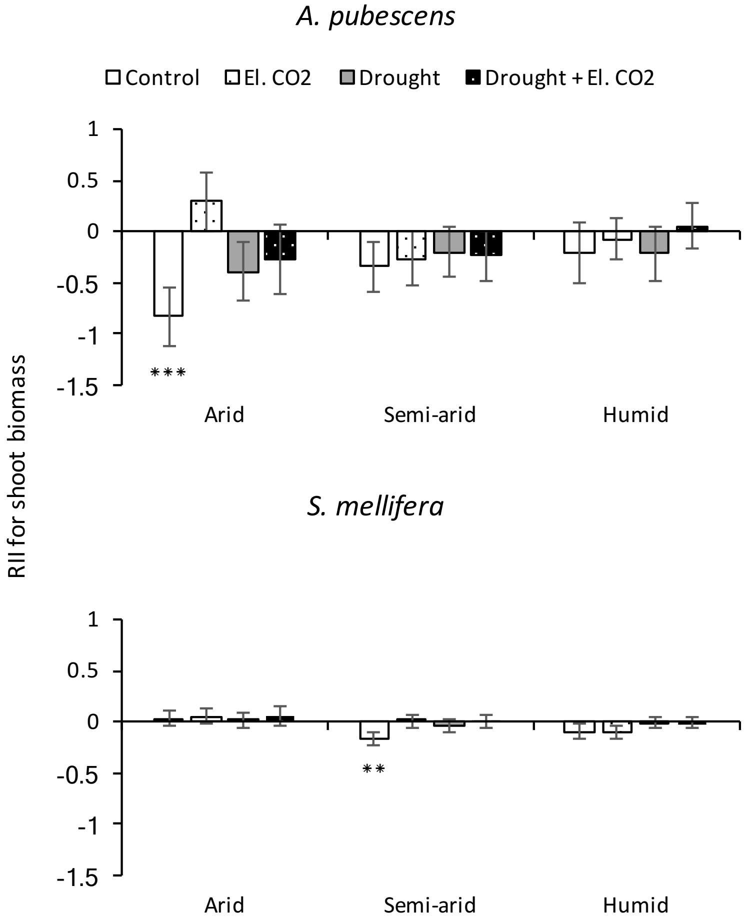


Appendix. 4. The effects of elevated CO_2 on seedling root to total length allocation to roots for the different ecotypes of our focal-species. Different letters indicate significant differences (GLMs) and are unique to each graph.

Experiment 2

Appendix. 5. Results of GLMs with a normal distribution with a Logit link function constructed to test for the effect a heterospecific neighbour on seedling biomass of two of the four focal species, under different treatments. Significant results (P <0.05) are indicated in bold.

	A. pubescens			***S. mellifera***		
Effects	**F**	**df**	**p-value**	**F**	**df**	**p-value**
CO_2	3.36	1	0.067	1.23	1	0.268
H_2O	0.03	1	0.857	1.65	1	0.199
Orig.	1.17	2	0.557	3.61	2	0.165
Orig. x CO_2	2.41	2	0.301	1.72	2	0.422
Orig. x H_2O	0.16	2	0.924	0.97	2	0.616
CO_2 x H_2O	1.19	1	0.275	0.20	1	0.659
Orig. x CO_2 x H_2O	2.23	2	0.328	0.75	2	0.687



Appendix. 6. Relative Interaction Index (RII) showing the effects of a neighbour (*A. pubescens* on *S. mellifera* and *vice-versa*) on shoot biomass of a grass (top) and shrub (bottom), under the different treatments. Positive values suggest facilitation and negative values competition. Asterisks indicate significant differences from zero (*p< 0.05, **p< 0.01 and ***p< 0.001, GLMs).

Curriculum vitae

PERSONAL INFORMATION

Citizenship:	Namibian
Gender:	Female
Date of Birth:	12 January 1981
Place of birth:	Otjiwarongo, Namibia
Marital status:	Married

EDUCATION

2014- present **PhD in Plant Ecology** **University of Tübingen, Germany**

PhD title: The effects of climate change, land-use and elevated carbon dioxide on tree-grass interactions in southern African savannas

2007 – 2008 **Master of Science: Biodiversity Management and Research** **University of Namibia (UNAM) and Humboldt University, Berlin**

Thesis: Impacts of Large herbivores on vegetation and soils around water points in Waterberg Plateau Park, central Namibia

1999 – 2002 **Bachelor of Science (Biology)** **University of Namibia**

Thesis: Enhancing the germination of the Devil's claw (*Harpagophytum procumbens*), using smoke extract (smoke discs)

EMPLOYMENT

2009 **Lecturer** **Department of Biological Sciences, UNAM**

- Preparing and conducting lectures, setting up and marking tests and examinations, preparing practical/ field exercises for students, undertaking field trips, doing research and being a member of university committees and other groups relevant to my work (community service).

2003 **Tutor** **Department of Biological Sciences, UNAM**

- Giving tutorials (extra classes) to first year undergraduate biology students, running practical sessions, handling students marks (entering of practical, test and assignment marks, and calculating final marks), and assisting with marking.

2002 **Student assistant** **Department of Biological Sciences, UNAM**

- Assist first year Biology students with laboratory work and marking/ grading of laboratory reports.

PUBLICATIONS AND CONFERENCES

2017	**Nesongano W. C. (presenter)**, A. Fangmeier, I. Mapaure and K. Tielbörger. Drought and elevated CO_2 effects on shrubs and grasses in southern African savannas. 30th Conference of the Plant Population Biology Section of the Ecological Society of Germany, Austria and Switzerland (GfÖ) in Halle, Germany, 18- 20 May 2017.
2013	Isakov A., D. J. Z. Liabunya, E. Ssekuubwa, I. Soumana, M. W. Bezabeh, M. H. Gabou, O. Chemutai, S. Wahabu, T. Bataa, **W. C. Mukaru (presenter)** and Z. Shigaeva, 2013. Soil carbon and the quest for land quality: Experiences from Africa and Central Asia. Soil Carbon Sequestration: for climate, food security and ecosystem services. International conference, Reykjavík, Iceland, 27-29 May 2013.
2012	**Mukaru W.C.** and I. Mapaure, 2012**.** Impacts of large herbivores on plant species diversity and range condition around water points in waterberg plateau park, central Namibia. Journal of Research in Conservation Biology 1 (1): 41-48.
2011	**Mukaru W. C.**, 2011. Impacts of large herbivores on vegetation and soils around waterpoints: Waterberg Plateau Park, central Namibia. Lambert Academic Publishing.
2011	**Mukaru W. C. (presenter)** and I. Mapaure, 2011. Impacts of large herbivores on vegetation structure, composition and diversity around water points in Waterberg Plateau Park, central Namibia. 46th Annual Congress of Grassland Society of Southern Africa.

SHORT COURSES

2013	UNU-Land Restoration Training programme	Agricultural University of Iceland and Soil Conservation Services of Iceland
2004	Tropical ecology and conservation	Tropical Biology Association (in Kenya)
2003	Conservation Biology and Wildlife Management	University of Namibia and the Cheetah Conservation Fund Research Centre