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Quantifying drought strategies in C_4 perennial Southern African grasses

by

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Dissertation

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Master of Science

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Preface

“I can breathe where there is green. Green grows hope. It keeps my heart beating and helps me remember who I am.”
— Courtney M. Privett

“Get close to grass and you’ll see a star.”
— Dejan Stojanovic

Declaration

I declare that the work in this thesis is my own work, unless specifically mentioned and acknowledged in the text. It is being submitted for a Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any university. The thesis is structured in a format for publishing each research chapter.

04 April 2023

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Dedication

I dedicate this thesis to my fallen angel Onalenna Magabe.

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Abstract

Plants have a range of strategies for surviving drought associated with the morphological and physiological traits that govern water use and loss. Most research has been done on woody species and whether the research applies in C_4 grasses whose above-ground biomass cures annually over a dry season is not clear. In this study I described and quantified drought strategies and recovery patterns of thirteen perennial C_4 grass species found in savanna and grassland ecosystems in South Africa. An experiment was undertaken where the grasses were grown and then exposed to a drought for three months, after which they were watered for one week to simulate a post drought recovery. I monitored stomatal conductance, leaf water potential, osmotic adjustment, leaf senescence and post-drought resprouting to assess whether plants with restricted water availability retained varying proportions of live leaf material or whether a wasteful water strategy during drought was associated with increased leaf death and post-drought recovery. Based on the measurements of the thirteen species, a broad range of strategies emerged related to the trade-offs between being efficient or safe, whereby some grasses control water loss by closing stomata and sacrificing photosynthesis while others continue to lose water while they photosynthesise. Additionally, osmotic adjustment demonstrated in species such *Digitaria eriantha* and *Panicum maximum* demonstrated an alternative drought strategy that is not currently recognised in the literature. Grasses with low stomatal control cured faster than those with a high stomatal control, which might have implications for flammability and leaf phenology in seasonal ecosystems with annual drought.

Keywords: Curing rate, Drought, Physiological drought tolerance, Safe vs Efficient trade-off

Glossary

Cavitation: is when liquid water in the xylem evaporates gas and creates a bubble (embolism), which blocks the flow of water (Choat *et al.*, 2018).

Critical leaf water potential (Ψcrit) (MPa)**:** The water potential where leaf stomatal conductance drops below 50 mmol/m^{-2}/s^{-1}, or the ecological threshold for plant function (Craine *et al*., 2013).

Curing rate: The change in number of dead leaves on the plant as the soil moisture decreases with the drought i.e., the slope of the response of curing to soil moisture.

Drought: insufficient soil water to meet the needs of plants for an extended period

Hydraulic conductance (m/s): The rate/ease at which water flows inside plants

Hydraulic failure: Occurs when vapour pressure deficit and soil moisture trigger the collapse of the plant hydraulic system due to the increase in water demand causing a reduction in the leaf water potential and xylem cavitation and the plant cannot move water from the root to the leaves (Choat *et al*., 2018).

Osmotic potential: (Ψs) (MPa)**:** The potential of water molecules to move from a hypotonic solution to a hypertonic solution across a semipermeable membrane (Madeleine M Jones and Turner, 1978).

Osmotic adjustment: A lowering of osmotic potential due to net solute accumulation in response to water stress, it sustains turgor, relative water content and stomatal conductance at low leaf water potential (Jones and Turner, 1978).

Physiological drought tolerance: The degree to which a plant maintains its biomass production during drought conditions, surviving via physiological mechanisms that enable it

to maintain stomatal conductance at low water potentials. It is measured as the critical leaf water potential (Ψcrit) at which a plant closes its stomata and stops transpiring, which is when stomatal conductance is 5% of maximum (Tucker *et al.*, 2011).

Relative water content (%): This is a measurement of hydration status (actual water content) relative to maximal water holding capacity at full turgidity. It can be used as a dehydration index because it is related to the uptake of water by the roots and loss of water through transpiration (Manzoni *et al*., 2014).

Stomatal conductance (gs) (mmol m^{-2} s^{-1}): The rate of CO_2 entering or water vapor exiting the leaf through stomata (Craine *et al*., 2013).

Stomatal sensitivity: The degree to which stomatal conductance responds to a reduction in water availability. In this thesis it is quantified as the slope of water potential and stomatal conductance. Steeper slopes mean more sensitive stomatal control.

Transpiration: The process of evaporation of water from the leaves of plants (Li *et al*., 2017)

Turgor loss point (Ψtlp) (MPa)**:** The negative water potential at which the leaf cell loses turgor and the leaf wilts, closes its stomata, and ceases gas exchange and growth (Madeleine M Jones and Turner, 1978).

Water potential (Ψ) (MPa): The potential energy of water relative to pure water at the soil surface (Craine *et al*., 2013).

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CHAPTER 1

Introduction and literature review

Abstract

Grasslands span 360,589 km^2 of South Africa and are typically dominated by perennial C_4 grasses. Climate change and the changing patterns of rainfall and drought require a better understanding of the strategies C_4 grass species adopt to deal with drought. Here, is the introduction of the rationale for this thesis, which will by provide a summary of what is known about the drought strategies of C_4 grasses. Perennial C_4 grasses face periods of water limitation during their lifetime and the duration and frequency of these drought conditions affect the strategies used to survive this stress. The extent and severity of drought found in an environment is thought to affect the types of strategies used. Explaining how grasses cope with drought is essential for predicting how grass species will survive and perform during more frequent and extreme droughts that are predicted to come with global change. A lot of research has been done on the different strategies plants use to tolerate droughts, however, C_4 grasses might not conform to these strategies due to their life histories and the seasonal environments they are found in.

The thesis is divided into four Chapters. Chapter One is a literature review chapter. Chapter Two is a data chapter that quantifies the drought and recovery strategies of thirteen C_4 grass species to an experimental drought, and tests for differences between grass clades and grass photosynthetic sub-types. Chapter three tests whether trade-offs and drought trait syndromes identified in trees are also present in C_4 grasses and classifies grass species into drought strategies and Chapter four is a conclusion chapter

Keywords: Curing rate, Drought, Flammability, Stomatal conductance, Water potential

1.1 Introduction

Research into the functional ecology of drought has increased in recent decades as ecologists aim to understand and predict community responses to water stress (Crausbay *et al*., 2020). Consequently, there is a great deal of knowledge of the physiological consequences of drought, and the variety of strategies different plants use to tolerate droughts. However, C_4 grasses present an intriguing challenge because of their unique life histories and the fact that they often occur in seasonally arid environments where they can experience a predictable, 6-month drought every year (Bartlett *et al.*, 2016).

Research on grass eco-physiology has yet to integrate an understanding of how droughts impact aspects of C_4 grass biology (Crausbay *et al*., 2020) . A lack of understanding in grass drought research creates a challenge in trying to accurately predict the impact and interaction plants have with their environment. Experimental research into grass responses to drought will advance understanding and improve modelling of essential physiological and ecological processes that relate to drought e.g., how fire regimes in natural grasslands might change in the future as rainfall patterns and grass community's change (Bartlett *et al.*, 2016).

Plant water use

Water is essential for plant growth (Wang *et al.*, 2016). Water moves from the soil, into a plant through roots by osmosis, and out into the atmosphere via the stomata by evaporation (Wang *et al.*, 2016). Movement of water is facilitated by a water potential gradient, called the soil-plant-atmosphere continuum, in which the atmosphere has a lower water potential than the leaves, which have a lower water potential than the soil (Craine *et al*., 2013). Water potential (Ψ) is defined as the potential energy of water relative to pure water at the soil surface (Craine *et al*., 2013). Water potential is measured in units of pressure (MPa), which is negative in the plant environment (Craine *et al., 2013*).

The maintenance of water potential in a leaf is closely related to 1) stomatal conductance, defined as the rate of CO_2 entering, or water vapour exiting, through stomata and is determined by the movement of water and the number of stomata (stomatal density) (Li *et al.*, 2017) and 2) transpiration, which is the process of water movement through a plant and its evaporation from the leaves (Li *et al.*, 2017). Open stomata are required for diffusion of CO_2 into the leaf and photosynthesis. Opening of stomata will result in a high stomatal conductance and more water being lost through transpiration, however the water is replaced through the process of pulling water from the xylem, and this creates tension (negative pressure) in the water system of the plant (Sack and Scoffoni, 2012).

Drought

A drought occurs when there is not enough water in the soil to meet the needs of plants for an extended period, ranging from a couple of weeks (short term) to a longer period (3-5 years) (Mishra and Singh, 2010). Although droughts result in water stress, plants that are found in environments that experience frequent droughts are adapted to either withstand or quickly recover from this water stress (Fu and Meinzer, 2018). The three distinct characteristics that differentiate droughts from each other are intensity, duration, and distribution (Mishra and Singh, 2011), although from an individual plant perspective intensity and duration are the most important. Plants go through drought stress under two conditions: either the supply of water to the roots is compromised (not enough water in soil) which is driven by rainfall or transpiration rates are too high (high atmospheric demand), which is driven by temperature/humidity. Droughts affect many plants characteristics, including growth, yield, and photosynthetic activity (Anjum *et al.*, 2011).

Relative water content is a measure of water status and can be used as a dehydration index, or drought metric, because it represents the balance between the uptake of water by the

roots and loss of water through transpiration (Manzoni *et al*., 2014). During a drought, a plant's relative water content, leaf water potential, stomatal conductance, and rate of transpiration will all decrease, while leaf temperature will increase (Anjum *et al*., 2011). As a result of these changes, photosynthesis declines as the plant is deprived of CO_2 (Anjum *et al.*, 2011) and chlorophyll content is reduced because of oxidative stress causing a loss of pigmentation and chloroplast membrane content. This process is known as curing (Brodribb *et al*., 2005).

Plant water stress

Distributing water from the soil to parts of the plant under drought stress is difficult because there is a resistance to the water flow through leaves (Brodribb *et al*., 2005) due to cavitation and embolism. Cavitation is when liquid water in the xylem transforms to a gas and creates a bubble (embolism), which blocks the flow of water (Choat *et al.*, 2018). This occurs when there is high water tension – i.e., big water potential gradient between the soil and the atmosphere. The embolism interferes with transportation and regulation of water, and when too many xylem vessels have embolised it can cause hydraulic failure which is the inability to move water from roots to the leaves (Choat *et al*., 2018).

In soil that is drying, the stomata will first regulate the loss of water from the leaves by closing, to try and maintain xylem pressure and avoid cavitation, but as water stress becomes more severe, stomatal conductance can no longer reduce sufficiently and cannot stop the drop in xylem pressure and hydraulic function (Choat, 2013). A drop in xylem pressure also results in a loss of turgor as the plant can no longer support its structure, which is why when a plant is drought stressed, we can observe the rolling and wilting of a leaf (Sterling, 2005). The turgor loss point/wilting point is defined as a negative water potential at which the leaf cells lose turgor and the leaf wilts, closing stomata and ceasing gas exchange and growth (Blackman and Brodribb, 2011; Blackman *et al*., 2014). The turgor loss point represents the leaf water potential

whereby the plant cannot take up enough water to recover from wilting (Bartlett *et al*., 2015). It is estimated from the relationship between the inverse of plant water potential and relative water content which is also known as a pressure-volume curve (see Figure 1.1, where the "turgor loss point" is indicated by the diamond) (Blackman and Brodribb, 2011; Blackman *et al*., 2014).

Turgor leaf potential is the difference between osmotic potential (Ψs) (MPa), (the potential of water molecules to move from a hypotonic solution to a hypertonic solution across a semipermeable membrane) and water potential (Ψ) (MPa) (Jones and Turner, 1978). Turgor leaf potential decreases when the soil dries and the plant experiences water deficit. Plants can avoid reaching Turgor loss point through osmotic adjustment, whereby the plant adjusts its salinity, Osmotic adjustment occurs through the accumulation of ions in cells which can therefore maintain turgor (Jones and Turner, 1978). Plants with a more negative turgor loss point can maintain both stomatal and hydraulic conductance at lower leaf water potentials (Bartlett *et al*., 2015).

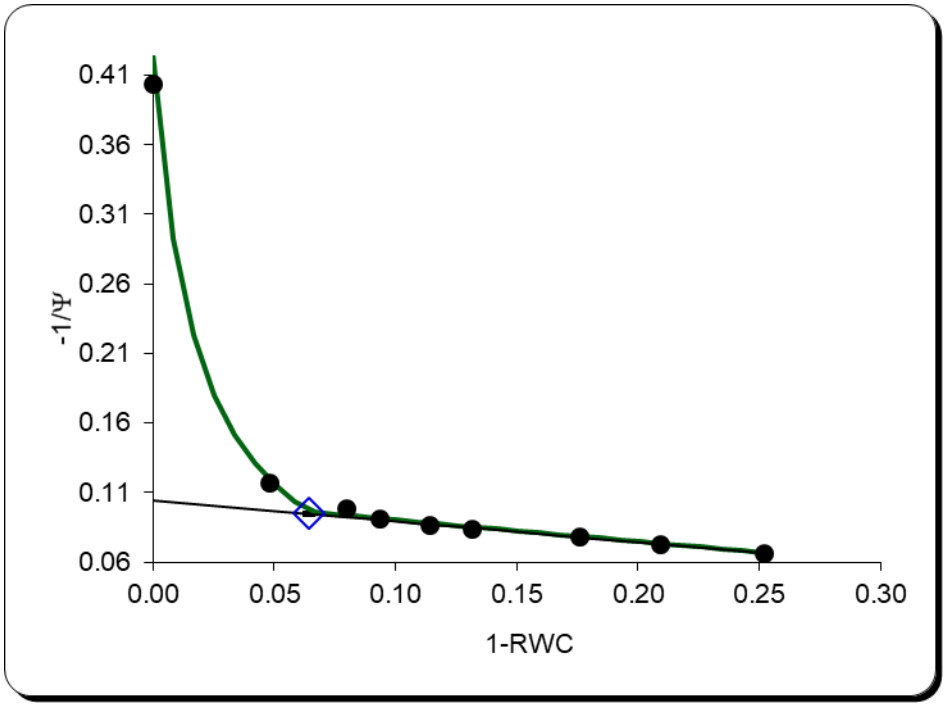


Figure 1.1: Pressure-volume curve shows the relationship between the inverse of water potential (Ψ) and (1 — RWC, RWC is the relative water content.). Beyond the turgor-loss point (◇), the relationship between (1 — RWC) and -1/(Ψ) is linear. The extrapolation of this linear relationship toward the y-axis gives osmotic potential (the black line) of the tissue when the tissue is still turgid.

Physiological drought tolerance

Physiological drought tolerance is a measure of the degree of water stress a plant can endure without losing hydraulic capacity (Ocheltree *et al.*, 2016). There have been various methods/indices proposed in the literature for measuring physiological drought tolerance in trees and grasses. The standard approach for woody plants is to quantify the stem water potential at which 50% loss of hydraulic conductivity occurs (Brodribb and Cochard., 2009). Brodribb and Cochard., (2009) suggest that xylem cavitation can be used as a measure of physiological drought tolerance because drought tolerant plants have smaller xylem vessel diameters that can withstand drought stress without cavitating. Maréchaux *et al.*, (2015) used

tree leaf water potential at wilting or turgor loss point as a determinant of the tolerance of leaves to drought stress and demonstrated that leaf osmotic water potential at full hydration is closely correlated with turgor loss point. Drought tolerance in grasses was investigated by Tucker *et al*., (2011), who identified drought tolerance to be associated with the degree of sclerification of vascular bundles and inversely related to the vessel diameter, both of which help the grass resist xylem cavitation. Tucker *et al.*, (2011) used the water potential at which a plant reduces stomatal conductance to 5% of maximum to define physiological drought tolerance in grasses and linked physiological drought tolerance with other plant functional traits, including root mass, shoot mass, and leaf thickness.

C_4 grasses

Perennial grasses are dormant in the dry season, but instead of dropping their leaves like drought-deciduous trees, they retain their dead leaves in the canopy and create fuel for fire. Some grasses cure much more rapidly and completely than others and it is likely that this can be explained by how these grasses survive droughts. C_4 grasses tend to be more drought tolerant as compared to C_3 because of differences in the mechanisms they use to capture CO_2 during photosynthesis. C_4 grasses have removed the process of photorespiration by concentrating CO_2 in the bundle sheath, which effectively saturates Rubisco at ambient CO_2 concentrations (Ripley *et al*., 2010). Removing the process of photorespiration enables plants to maintain photosynthetic rates at a lower stomatal conductance (i.e., stomata are more closed) which allows them to maintain photosynthesis under drier conditions (Ripley *et al.*, 2010).

C_4 grasses often dominate ecosystems in seasonal tropical environments which always (predictably) have an annual winter drought (Ripley *et al*., 2010), such as savannas. However, within these environments there is much variation in the availability of water during the growing season - with some savannas having much more predictable and high rainfall than

others - as well as variation in the probability of extreme (multiyear) droughts (Farooq *et al*., 2009).

Drought strategies

There is a great deal of discussion in literature about how to define and measure drought strategies, but for grasses the categorising of drought strategies depends on other variables such as hydraulic conductance, soil water potential, soil moisture, and other factors. Plant drought strategies are often characterised as falling between two extremes (McDowell *et al*., 2008): Water saving plants (safety strategy), through closing their stomata, can sustain stable midday leaf water potentials under conditions of deficit in soil water content and an increase in the demand for water (Li *et al*., 2017). In contrast, water spending plants (efficiency strategy) let their leaf water potential change depending on the shifts in water demand and availability by keeping their stomata open (McDowell *et al*., 2008). These plants can maintain stomatal conductance and photosynthesis even at lower soil water potentials, but with increased risk of cavitation (Choat *et al.*, 2018). Therefore, the "physiological drought tolerance" as defined above would represent an efficiency strategy and a water spending plant.

Plants occupying the same environment can have different strategies to balance the trade-off between water loss and growth (Sack and Scoffoni., 2012). Closing stomata will result in the loss of photosynthesis because capturing carbon and releasing water and oxygen can only occur when stomata are open (Wang *et al*., 2016). However, plants avoid embolism by closing their stomata. Water saving strategies run the cost of depleting the plants carbon supply. If plants maintain photosynthetic rates under water stress, they have a higher risk of cavitation and increase the potential to lose their aboveground structure or mortality (McDowell *et al.*, 2008). This is a trade-off between photosynthesis and cavitation during drought, or between being safe or efficient. The particular growth form of a grass, with no long-lived woody

structures, and almost all buds and meristematic tissue at the base, means that hydraulic failure is a lot less costly, so the trade-offs between safety and efficiency might work very differently.

Drought Recovery

A full understanding of the trade-offs and consequences of different drought strategies requires an understanding of the process of recovery (Choat *et al*., 2018). Choat *et al*., (2018) looked at how quickly trees that were affected by drought recovered (i.e., rehydrated). They found that survival and recovery are controlled by the amount of damage to the apical and cambial meristematic tissues, the functioning hydraulic pathway, the general health of the tree, and lastly the water and nutrients available during the recovery phase. Minor drought stress resulted in low level cavitation and was easily reversible through refilling embolised conduits, which can happen daily during overnight rehydration (Choat *et al*., 2018). More intense drought stress resulted in hydraulic recovery via the formation of new wood. The most severe drought stress causes above ground mortality and recovery after this kind of stress only occurs by resprouting of stems (Brodribb and Cochard, 2009; Sack and Scoffoni, 2012; Choat *et al*., 2018).

The rate of recovery after drought via rehydration is primarily determined by how negative the water potential was during the drought (a measure of drought severity) and the plant's traits. Traits like vessel diameter and packing are important for dictating vulnerability to cavitation as well as the ability to recover from embolisms (Choat *et al*., 2018), with smaller vessels making it easier to recover from embolisms as well as reducing the likelihood of embolisms in the first place (Choat *et al*., 2018).

The ability to recover after drought via resprouting is also variable. In Mediterranean environments it has been shown that resprouting is more common among species on the safety-end of the safety-efficiency continuum (Pausas *et al*., 2017). Again, grasses have less above-

ground biomass to lose than woody plants, and as resprouting is also an important adaptation for tolerating fire and herbivory (Archibald *et al.*, 2019), most grasses are prolific resprouters (Simpson *et al.*, 2021). It is therefore likely that grass recovery from drought via resprouting will be quite common. However, it is not known whether resprouting in grasses is linked to drought strategy as has been shown in trees: i.e., whether grasses using a "safe" drought strategy (closing stomata during a drought) show a lower resprouting response after the drought than "efficient" species (those that continue to photosynthesise).

The degree to which grasses recover from drought via resprouting vs rehydration will affect how much dead biomass remains after a drought. McGranahan *et al*., (2016) highlights that certain grass species cure much more slowly than others, and that this can feed back to affect environmental processes like fire. Since fire is one of the most important processes structuring C_4 grassy ecosystems, it is important to understand drought strategy in C_4 grasses. To do this, one must move beyond physiological drought tolerance and understand the whole plant life history strategy associated with drought (Choat *et al*., 2018).

Understanding plant adaptations to drought involves not only assessing their performance during drought, but also the degree to which they recover and the mechanism by which they do so once water is no longer limiting. How plants recover from drought can have an impact on their distribution, for example those that recover well tend to be found in dry or drought prone environments (Brodribb and Cochard, 2009).

It is important to determine the range of drought response strategies shown by C_4 grasses because it will assist in assessing the risks of different types of droughts to grass communities and help answer questions such as "During a drought which species will survive and how will they survive?" and "How severe should a drought be to cause mortality?". The answers to these questions have far reaching implications for grassy ecosystem ecology.

Aim

To understand the response patterns of drought and recovery in African C_4 grasses and the implications for grass ecosystem ecology.

Objectives

1. To describe and quantify the diversity in functional responses to drought, and recovery from drought in thirteen perennial C_4 grass species.
2. To investigate whether photosynthetic sub-type or grass evolutionary history is associated with drought responses.
3. To evaluate whether safety-efficiency trade-offs are present in C_4 grasses and whether drought responses can predict drought recovery responses.
4. To identify different drought strategies in C_4 grasses.

CHAPTER 2

To describe and quantify the diversity in functional responses to drought and recovery from drought in thirteen perennial C_4 grass species.

Abstract

Changes in temperature and rainfall linked to global climate change are predicted to cause fluctuations in the duration and severity of droughts, particularly in Southern Africa. Plants show a range of strategies for surviving drought and have been conceptualised to align along a safety- efficiency trade-off, where some species reduce water loss to prevent xylem damage and maintain biomass, while others continue to photosynthesise, potentially risking cavitation and loss of above-ground biomass. C_4 grasses in savannas are intriguing in that their above-ground biomass senesces and cures every year during the long dry season, therefore the safety-efficiency trade-off might function very differently in this plant group, with implications for other life history characteristics. In this chapter, I describe and quantify the range of drought and recovery strategies shown by some representative Southern African C_4 grass species. To do this, a greenhouse drought experiment was conducted on thirteen species of perennial C_4 grasses, which were replicated twenty-one times making a total of 273 individual pots. Plants were exposed to an experimental drought in a greenhouse where they did not receive any water for a period of 3 months. Physiological drought tolerance of grasses – measured as the leaf water potential at which the stomatal conductance fell below an ecological threshold ($50 mmol/m^2/s^{-1}$) for functioning and leaf senescence was monitored. Osmotic adjustment was also quantified using pressure-volume curves. Post-drought plants were re-watered and monitored for 1 week as they recovered. During this time, the number of new leaves, number of rehydrated leaves, leaf fluorometry and chlorophyll content of all leaves was measured. The results indicated that species that were able to recover from drought (i.e., did not die) either did it by rehydration or by resprouting or both.

Keywords: Drought strategy, Curing, Efficient Vs. Safe, Trade off

2.1 Introduction

Savannas are ecosystems known for the coexistence of trees and grasses where moisture availability is strongly seasonal and alternates between warm wet and cool dry seasons (Govender *et al.*, 2006). Moisture availability is an important determinant of plant growth in savannas (Farooq *et al*., 2009), and grass growth is strongly seasonal. Many savanna grass species are perennial, suggesting that they can survive repeated dry seasons (droughts). This has led to the assumption that most C_4 grass species have quite similar, simple plant water use strategies i.e., that they tend to use water when it is available until it is depleted after which they cure or go dormant (Higgins *et al*., 2010).

A common theme in plant physiological research is the trade-off between stress tolerance and growth; an example of this trade-off at the tissue level is the safe-efficient trade-off, which suggests that plants with the greatest resistance to hydraulic failure should have low maximum water usage (Ocheltree *et al*., 2016). C_4 plants are considered drought tolerant due to their ability to concentrate CO_2 and reduce stomatal conductance. However, different C_4 grass clades do organise themselves along gradients of water availability (Visser *et al.*, 2012), experimental data demonstrate wide variation in drought strategy across grasses globally (Ocheltree *et al*., 2016) and observational data of C_4 grass communities in droughts show shifts in species composition Wigley-Coetzee (2020) as well as an experiment by Donaldson *et al*., (2018).

Drought

Droughts have been seen as natural disasters and occur in all climates. Droughts can be defined in several ways in different disciplines but are associated with a decrease in moisture availability over an extended which can be exacerbated by elevated temperatures and low relative humidity (Mishra and Singh, 2010).

The three distinctive characteristics of droughts are intensity, duration, and spatial extent (Farooq *et al*., 2009). Permanent droughts are characterised as periods of 3-5 years or more of continued moisture shortage (Farooq *et al*., 2009). Seasonal droughts occur in climates with well-defined wet and dry seasons. Additionally, droughts can occur in the middle of the wet season, these droughts are unpredictable, usually short (20-25 days), and are irregular (Farooq *et al.*, 2009).

A drought can be defined using relative terms (e.g., dry, or long) or it can be defined by attempting to identify the duration, severity, and frequency of it. Physiologists, ecologists, agronomists, and geographers all define droughts differently. For this study, I will be defined as "insufficient soil water to meet the needs of plants for an extended period" (Farooq *et al.*, 2009), which is a definition that allows the drought to be a recurring feature of the climate.

Plant response to water stress

Water stress occurs when soil water availability falls below what a plant needs to optimally photosynthesise. The extent (amount of time) and severity (degree of water stress) of drought will affect the types of strategies that plants can use to survive or cope with this stress (Baniwal *et al.*, 2004). Plants go through drought stress when the water supply to the roots is compromised (e.g., decreased precipitation) or when transpiration rates are high (e.g., heat stress) (Anjum *et al.*, 2011).

Hydraulic failure in plants occurs when vapour pressure deficit and soil moisture trigger the collapse of the plant hydraulic system due to the increase in water demand causing a reduction in the leaf water potential and xylem cavitation (Manzoni *et al*., 2014). The two traits that control how fast plant tissues lose water during a drought and the thresholds at which the loss of water leads to hydraulic failure are 1. The physical limits of the vascular system and 2. The capacity to maintain plant water function within these functional limits (Choat *et al*., 2018).

If plants do not close their stomata they run the risk of cavitation, loss of above-ground biomass, and even death if resprouting is not possible.

Different strategies plants use to deal with water stress

Some plants tolerate drought stress by reducing photosynthetic activity and conserving water. In these plants we observe a reduction in stomatal conductance as soil water potential decreases due to dry atmospheric conditions. While stomatal conductance is lowered the plants maintain a high leaf water potential (McDowell *et al.*, 2008). This results in a reduction of photosynthetic rates but allows the plants to maintain a functioning leaf canopy so that when the drought ends, they can quickly start photosynthesising again. This is referred to as the "Safe strategy" (Bartlett *et al*., 2015). An alternative strategy is to maintain high stomatal conductance and allow leaf water potential to decline as soil water potential declines. This enables plants to maintain high photosynthetic rates, but the cost is that they might run out of water, resulting in cavitation, potential losses of above ground structures, and even death (Sanders and Arndt, 2015). This is referred to as the "Efficient strategy" and is often also associated with osmotic adjustment (Sanders and Arndt, 2015).

Osmotic adjustment can ease the effects of drought stress by altering the relationship between leaf water potential and leaf turgor potential for stomatal closure (Farooq *et al*., 2009) i.e., they can continue leaf function under water stressed conditions (Bartlett *et al*., 2015). Plants that osmotically adjust generally grow under drier soil moisture conditions and in dry environments (Bartlett *et al*., 2015). Plants that osmotically adjust can maintain both stomatal and hydraulic conductance because they have a more negative turgor loss point. They accumulate solutes in their leaves, and this allows them to have a lower water potential in their leaves, and a more negative turgor loss point.

The drought-induced trade-off between reduced photosynthesis due to stomatal closure and increased risk of top-kill through continued transpiration has been called the "Safety-Efficiency" trade off (De Guzman *et al*., 2017). It was first identified in woody plants by Pockman and Sperry., (2000) and it has been shown that plants vary in their ability to resist water stress, and that this can be predicted by the intensity of water stress experienced in their distribution range as well as their evolutionary histories (McDowell 2008; Bartlett *et al*., 2016; Choat *et al*., 2018). It has also been shown that a variety of drought strategies can be apparent within one community (De Guzman *et al*., 2017). Plants therefore show a range of drought strategies, which depend strongly on the types of droughts they experience and have evolved with.

Recovery from drought

Tree species exposed to several types of water stress recover differently. A mild drought results in a quick overnight recovery because trees can refill embolised conduits in the leaf which allow them to fully rehydrate overnight (Broadribb and Cochard, 2009). A severe drought can result in above-ground mortality, or top kill (Choat *et al*., 2018) (Broadribb and Cochard, 2009) if the water transport system of the plants is damaged beyond repair. Hydraulic limitation is the process controlling recovery from drought in tree samples (Broadribb and Cochard, 2009).

Resprouting refers to the ability of some plants to form new shoots after destruction of living tissues. It is common in many plants worldwide and thought to be common in ancient floras (Zeppel *et al*., 2015). Some people have proposed that resprouting was originally an adaptation to drought, which has also proved successful for tolerating fire and grazing (López-Soria and Castell, 1992). Resprouting may occur from apical, epicormic, basal or below ground buds, and occurs in response to tissue loss through fire, herbivory, and drought (Zeppel *et al*.,

2015). This means that grasses that are adapted to recover from frequent defoliation by herbivory and fire, might have strong resprouting responses to drought. Even plants that cannot recover damaged biomass can still survive drought if they are able to resprout (Zeppel *et al*., 2015).

Droughts and evolutionary history & photosynthetic pathways of C_4 grasses

Drought is often discussed as a major concern in savanna ecosystems, associated with lasting shifts in savanna structure and functioning (Simpson *et al*., 2016). Droughts are common in savannas and C_4 grasses tend to be able to maintain higher photosynthetic rates than C_3 species during drought conditions (Ripley *et al*., 2010). C_4 grasses have a CO_2 concentrating mechanism, which allows them to maintain high internal CO_2 concentrations with low stomatal conductance.

As a result of this, C_4 species are generally more successful than C_3 grasses in arid environments because they have a greater regulation of light, water and nitrogen when exposed to elevated temperatures and decreasing stomatal conductance (Ripley *et al*., 2010). However, C_4 grasses cover the entire tropics, growing in very dry and very wet regions, and are very variable across this range, so they have a range of different drought strategies (Fish *et al*., 2015; Craine *et al.*, 2012). Within the C_4 grassy tropics the probability of an extended drought during the growing season, as well as the consequences of loss of above-ground biomass will vary widely and might select for different drought strategies across the range of habitats that C_4 grasses occupy. Previous research by Visser *et al.* (2012) has shown that grasslands in arid parts of South Africa are dominated by species from Chloridoideae clade which are associated with low rainfall whilst Andropogoneae grasses are associated with higher rainfall and found in more mesic environments. Lehmann *et al*., (2019) found the same pattern globally.

There is also evidence that the C_4 photosynthetic pathway used by grasses affects their drought strategy in the field. During droughts C_4 grasses decrease in biomass and recover quickly after rain, however Wigley-Coetsee and Staver (2020) demonstrated that the rates at which species recovered was closely related to photosynthetic machinery. Species with NADP-ME photosynthesis declined in abundance during drought, whereas species with PEP-ck and NAD-ME photosynthesis either increased or were unaffected by drought. The physiological mechanisms causing this were not clear but could be linked to bundle sheath morphology: XyMS− bundle sheaths are associated with NADP-ME whereas XyMS+ bundle sheaths are associated with PEP-ck and NAD-ME species. Thus, C_4 photosynthetic type appears to be an under-recognised driver of drought sensitivity: the common wisdom is that C_4 grasses have a photosynthetic advantage over C_3 during drought due to their stomatal regulation abilities, however Ripley *et al.,* (2010) showed that C_4 species using NADP-ME photosynthesis are metabolically more sensitive and recover more slowly from drought than C_3 species

Most of the drought literature (e.g., McDowell *et al.*, 2008; Choat *et al.*, 2018) was developed in forest ecosystems using woody plants. However, because grasses lack a permanent above-ground woody structure that needs to be maintained, the costs of cavitation and embolism would be lower, and this might alter the trade-offs discussed above.

To understand the range of drought strategies within grasslands worldwide, Craine *et al.*, (2012) defined physiological drought tolerance as the critical leaf water potential at which stomatal conductance fell below an ecological threshold for functioning. This was further explored by Ocheltree *et al.*, (2016) who identified a Safety- Efficiency trade off in C_4 grasses within one community in the tall-grass prairie ecosystem. However, they did not link this up with information on the recovery patterns of the grasses, so the degree to which grass drought strategies drive resprouting vs rehydration is yet to be considered. Physiological factors controlling cellular hydration and cell integrity may play critical roles (Zeppel *et al.*, 2015), but

major physiological factors that control for the ability of resprouting and rehydration of cured leaves in perennial grass species are unknown. Most work on recovery has been done on woody species and little work has been applied to grass species.

Aim

To improve our understanding of drought response and recovery strategies of C_4 grass species and test whether these align with expected patterns based on phylogenetic-environment relationships and photosynthetic attributes.

Objective

To describe and quantify the diversity in functional responses to drought of thirteen perennial C_4 grass species relate them to patterns of drought recovery.

Research questions

a. At what plant water potential do different grass species close their stomata during a drought?

b. Do some grass species cure faster than others when exposed to similar water stress?

c. Do different grass species osmotically adjust during a drought?

d. Do grass species have different recovery strategies?

e. Is there any evidence that grass phylogenetic history (grass clade), photosynthetic subtype, or environmental envelope are associated with these drought response traits?

Hypotheses

H1: There will be a range of physiological drought responses in the C_4 species studied, and these will be associated with major differences in the rate at which different species cure.

H2: Most grasses will show osmotic adjustment to some degree.

H3: Grasses from more arid environments (Chloridoideae) will have a more conservative water use strategy (safe).

H4: Not all of the grass species will survive the drought and within those that do some will resprout more vigorously than others.

H5: New resprouted leaves will have higher functionality than the old leaves that are rehydrated.

2.2 Methods

Study site

The study was conducted in a greenhouse at the University of the Pretoria Agricultural Experimental Farm in Pretoria, South Africa (25°44'49"S 28°15'40"E), from January to June 2020 (i.e., during the austral growing season).

Study species

Thirteen species of perennial, tufted, C_4 tropical grasses that are widespread throughout South African savannas were selected (Table 2.1). Species were chosen to represent the major grass subfamilies: Panicoideae, Aristidoideae and Chloridoideae, as well as the C_4 photosynthetic pathways shown in grasses: C_4 NADP-ME, C_4 NAD-ME and C_4 PCK and distributed along a rainfall gradient (Arid, Mesic, and Humid) which is between 400-1300mm/year. The mean annual rainfall (MAR) was calculated for each species by extracting data from GBIF (GBIF.org (09 August 2021) GBIF Occurrence Download https://doi.org/10.15468/dl.ggahs4) using the r package rgbif) cleaned and mapped using the raster, mapview and Coordinate Cleaner r package. Rainfall was reported as median and confidence interval (0.25, 0.75). See table 2.1 for the summery for each species

Table 2. 1 Drought experiment species names, habitat they are found in, rainfall reported as median and confidence intervals and information, data was collected from a literature search for each species

Genus	Species	Abbreviation	Habitat	Rainfall (mm/year)	Sub Family	Tribe	Photosynthetic pathway	Reference
Cenchrus	*ciliaris*	Cen cil	Arid	260 (260; 493)	Panicoideae	Paniceae	C_4 NADP-ME	Ghannoum, O., Evans, J. R., Chow, W. S., Andrews, T. J., Conroy, J. P., & von Caemmerer, S. (2005). Faster Rubisco is the key to superior nitrogen-use efficiency in NADP-malic enzyme relative to NAD-malic enzyme C4 grasses. *Plant physiology*, *137*(2), 638-650.
Digitaria	*eriantha*	Dig eri	Mesic	614 (614, 786)	Panicoideae	Paniceae	C_4 NADP-ME	Wigley-Coetsee, C., & Staver, A. C. (2020). Grass community responses to drought in an African savanna. *African Journal of Range & Forage Science*, *37*(1), 43-52.
Panicum	*maximum*	Pan max	Humid	990 (990-1018)	Panicoideae	Paniceae	C_4 PCK	Wigley-Coetsee, C., & Staver, A. C. (2020). Grass community responses to drought in an African savanna. *African Journal of Range & Forage Science*, *37*(1), 43-52.
Hyparrhenia	*hirta*	Hyp hir	Mesic	730 (588,862)	Panicoideae	Andropogoneae	C_4 NADP-ME	Wolfson, M. M., Amory, A. M., & Cresswell, C. F. (1982). The effect of night temperature and leaf inorganic nitrogen status on the C4 pathway enzymes in selected C4 photosynthetic grasses. *Proceedings of the Annual Congresses of the Grassland Society of Southern Africa*, *17*(1), 106-111.
Schmidtia	*pappophoroides*	Sch pap	Arid	461 (358, 570)	Chloridoideae	Eragrostideae	C_4 PCK	Wigley-Coetsee, C., & Staver, A. C. (2020). Grass community responses to drought in an African savanna. *African Journal of Range & Forage Science*, *37*(1), 43-52.
Urochloa	*mosambicensis*	Uro mos	Mesic	808 (614, 1154)	Panicoideae	Paniceae	C_4 PCK	Wigley-Coetsee, C., & Staver, A. C. (2020). Grass community responses to drought in an African savanna. *African Journal of Range & Forage Science*, *37*(1), 43-52.
Eustachys	*paspaloides*	Eus pas	Mesic	623 (623 ,776)	Chloridoideae	Cynodonteae	C_4 PCK	Hattersley, P. W., & Watson, L. (1976). C4 grasses: an anatomical criterion for distinguishing between NADP-malic enzyme species and PCK or NAD-malic enzyme species. *Australian Journal of Botany*, *24*(2), 297-308.
Loudetia	*simplex*	Lou sim	Humid	1112 (967, 1336)	Panicoideae	Tristachydeae	C_4 NADP-ME	Bourel, B., & Novello, A. (2020). Bilobate phytolith size matters for taxonomical and ecological identification of Chad

								grasses: A case study on 15 species. *Review of Palaeobotany and Palynology*, *275*, 104114
Setaria	*sphacelata*	Set sph	Humid	1263 (1040, 1121)	Panicoideae	Paniceae	C_4 NADP-ME	Da Silva, J. M., & Arrabaca, M. C. (2004). Photosynthesis in the water-stressed C4 grass Setaria sphacelata is mainly limited by stomata with both rapidly and slowly imposed water deficits. *Physiologia Plantarum*, *121*(3), 409-420.
Sporobolus	*pyramidalis*	Spo pyr	Humid	1059 (1059,1121)	Chloridoideae	Zoysieae	C_4 PCK	Wand, S. J., Midgley, G. F., & Stock, W. D. (2001). Growth responses to elevated CO2 in NADP-ME, NAD-ME and PCK C4 grasses and a C3 grass from South Africa. *Functional Plant Biology*, *28*(1), 13-25.
Melinis	*repens*	Mel rep	Humid	881 (648, 1400)	Panicoideae	Paniceae	C_4 PCK	Wand, S. J., Midgley, G. F., & Stock, W. D. (2001). Growth responses to elevated CO2 in NADP-ME, NAD-ME and PCK C4 grasses and a C3 grass from South Africa. *Functional Plant Biology*, *28*(1), 13-25.
Aristida	*junciformis*	Ari jun	Mesic	792 (792, 982)	Aristidoideae	Aristideae	C_4 NADP-ME	Schulze, E. D., Ellis, R., Schulze, W., Trimborn, P., & Ziegler, H. (1996). Diversity, metabolic types and δ 13 C carbon isotope ratios in the grass flora of Namibia in relation to growth form, precipitation and habitat conditions. *Oecologia*, *106*, 352-369.
Eragrostis	*superba*	Era sup	Mesic	692 (692, 848)	Chloridoideae	Eragrostideae	C_4 NAD-ME	Schulze, E. D., Ellis, R., Schulze, W., Trimborn, P., & Ziegler, H. (1996). Diversity, metabolic types and δ 13 C carbon isotope ratios in the grass flora of Namibia in relation to growth form, precipitation and habitat conditions. *Oecologia*, *106*, 352-369.

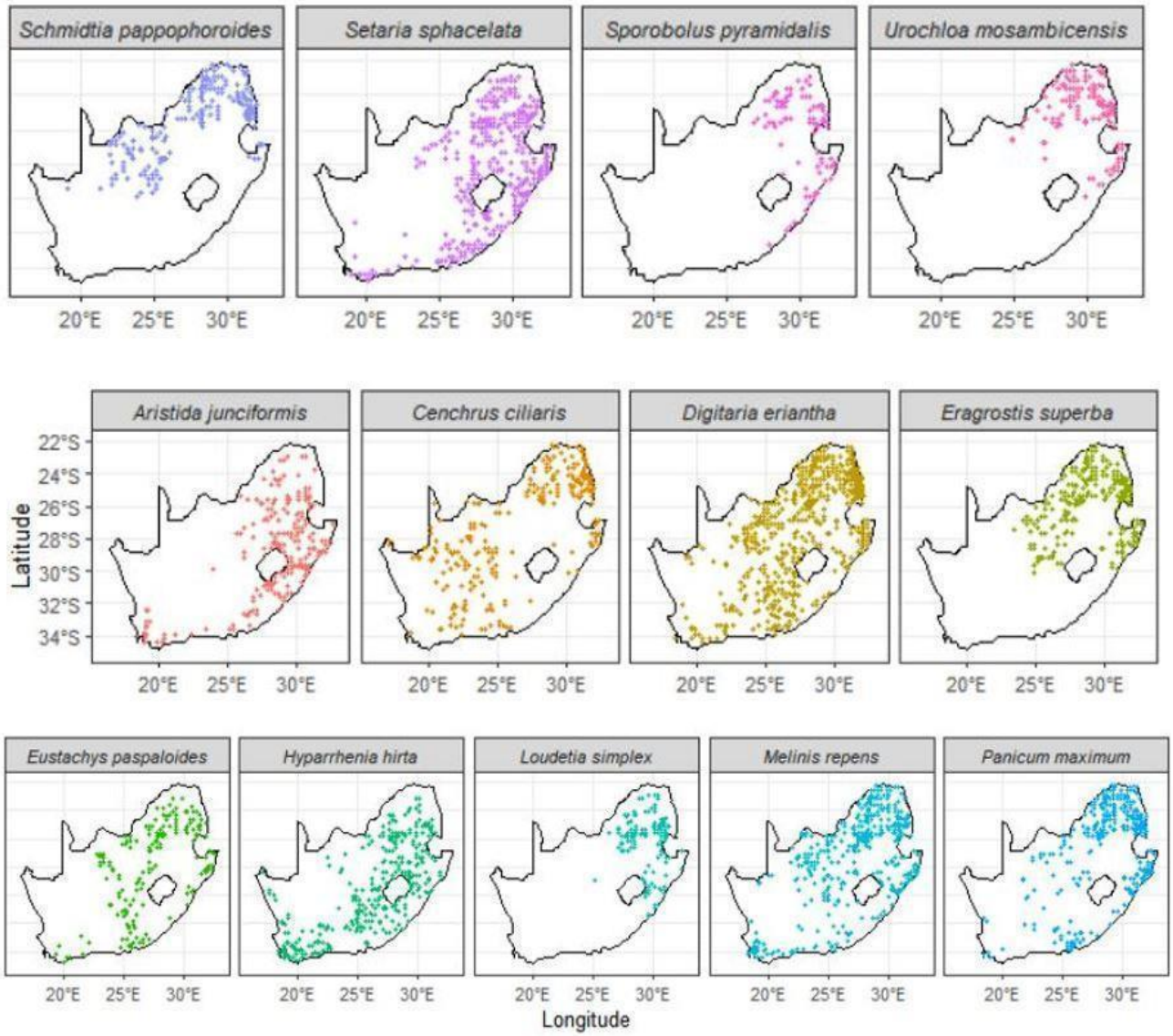


Figure 2. 1: Distribution of the selected grass species across South Africa and the mean annual rainfall (MAR), Location records for each species were extracted from GBIF.

Experimental design

The seeds for this experiment were collected in 2019 from study sites across South Africa. Seeds were germinated in December 2019 in seedling trays in a greenhouse and when they were ~2 weeks old they were transplanted into bigger pots. 21 individuals of each of the thirteen species were planted into free draining pots. The pots were 25cm tall and 12.5 cm wide and filled with a mixture of 70% soil (made up of 80% sand, 12% silt and 8% clay) and 30% compost. Each plant was labelled and given an abbreviation (see species Table 2.1) and number ranging from 1-25 e.g., Pan max 1. The grasses were placed in the greenhouse in rows in such a way that the species and measurement groups are spread throughout the greenhouse (see Figure 2.2) The pots were first watered up to a point of saturation, then they were watered regularly for 15 minutes once a day using a drip irrigation system.

Once they were well established (2-3 months old) on the 17th of March 2020 a drought was applied by stopping the irrigation, temperature and humidity were measured using a data logger. The twenty-one individuals from each species were divided into 3 treatments, the first treatment (fully cured) was droughted until their above-ground biomass was fully cured. The second treatment (drought stressed) was droughted until 80 % of them had closed their stomata (<20% of maximum) and were showing characteristics of drought stress (wilting, leaf rolling, change from green to brown leaf colour). The third treatment were droughted to the same extent as the drought-stress plants, but then rewatered to assess recovery after the drought.

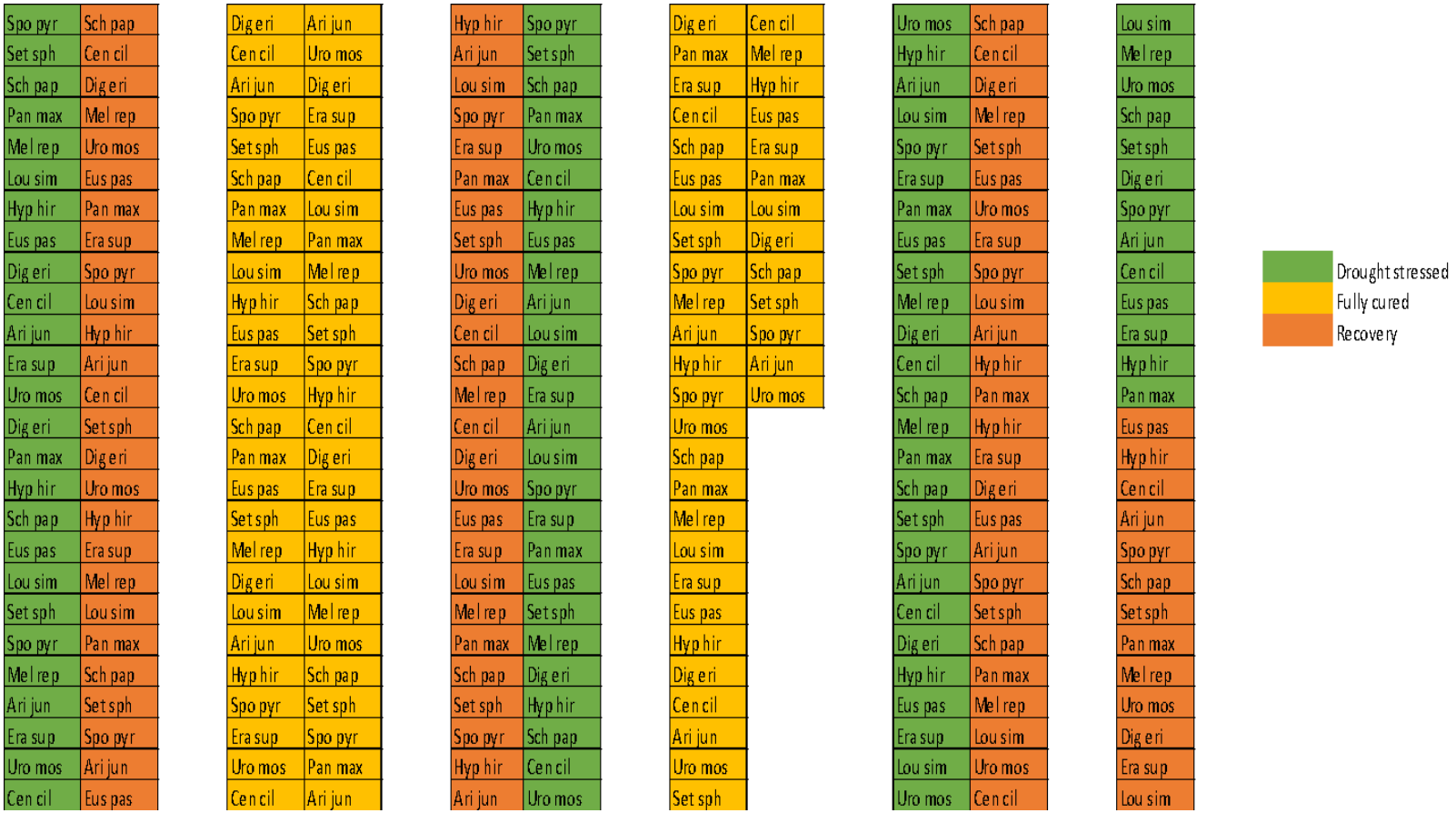


Figure 2. 2: Selected grass species greenhouse setup for the experimental drought which fits 2x31 pots with interspace of 60cm and row space of 1m (Species codes in Table 2.1).

Measurements

Soil Moisture

Soil moisture content (m^3m^{-3}) was measured using a 20 cm soil moisture probe (manufactured by Campbell science, HydroSense II Handheld Soil Moisture and Temperature Sensor). Each pot in the experiment was measured daily between 7-8am, to quantify the rate the plants lose water.

Stomatal Conductance and Water potential

To quantify differences in drought strategies between species the "physiological drought tolerance" index of (Tucker *et al*., 2011) was modified. This required knowing the leaf water potential at which plants close their stomata. Stomatal conductance daily was measured daily and leaf water potential every second day (because it is a destructive measurement and biomass of the plant needed to be conserved) on all the individuals in the fully cured group (7 individuals of each species). Stomatal conductance was measured on one healthy mature leaf per plant between 9-11am using a leaf porometer (Decagon Devices, Inc. SC-1, 2005). Midday leaf water potential was measured between 12 and 2pm on one healthy mature leaf per plant using a Scholander pressure bomb (PMS instrument Model 1505D-EXP). These measurements were used to quantify the stomatal sensitivity to water stress, which is translated to a measure of physiological drought tolerance (Tucker *et al*., 2011).

Pressure-Volume curves

To determine osmotic adjustment pressure-volume curves (PV curves) were measured (Blackman and Brodribb, 2011) for each grass species before the drought was initiated and also halfway through the drought when 80% of plants had closed their stomata, which was indicated by stomatal conductance values which were 20% of the maximum value measured, and the plants were showing characteristics of being drought stressed such as wilting, leaf rolling, and

curing (change from green to brown leaf colour). PV curves were performed on five leaves per species from individuals randomly sampled from the drought stressed group using the most recent mature fully expanded leaf. Leaves were cut at the ligule and the leaf then immediately placed in a pressure bomb, with pressure applied until water came out of the cut surface (Xingdong *et al*., 2007). The pressure was maintained until the leaf reached equilibrium with the pressure in the chamber and that was taken as the point when water stopped coming out of the leaf. The process was repeated by increasing the pressure by increments of 2.5MPa at least ten times (Xingdong *et al*., 2007). The leaves were also weighed between each pressure measurements to get the volume. Newly matured leaves which looked similar, were the same size and in good condition (no curing or holes) from all seven individuals of a species were clipped and the relative water content was calculated:

$$\text{RWC} = \frac{fresh\ weight - dry\ weight}{turgid\ weight - dry\ weight} \times 100$$

The fresh mass was obtained by first removing the leaf from the stem with tweezers and cutting the leaf base with a sharp razor blade; the leaves were then immediately weighed to determine fresh weight. To obtain the turgid mass, the leaves were allowed to float in distilled water inside a closed petri dish for 5 mins and then gently wiped with tissue paper and weighed again. For the dry mass leaf samples were placed in a preheated oven at 80°C, for 48hrs. All the mass measurements were taken on a four decimal point scale (Jones and Turner, 1978). This was done for all species except *Aristida junciformis* because it had not grown large enough and this sampling method is destructive.

Chlorophyll content, fluorescence and curing rate

Both chlorophyll content (using a chlorophyll content meter- Opti-science, CCM-300) and fluorescence (using a handheld chlorophyll fluorometer- Opti-science, OS30P+) were measured at a leaf level to get an independent measure of the health of the leaves and their

photosynthetic capacity. This was only done on the drought stressed and fully cured treatments. Each day (alternating between the groups) The healthiest, new, fully developed leaf was identified, and measurements were taken at the same time (from 1pm-2pm). The number of dead leaves accumulated, leaves for small plants and number of shoots for bigger, leafier plants were counted, these measurements were taken from the drought stressed treatment, every two days throughout the drought

Recovery

On the 5th of May 2020 (56 days of drought), 80% of the grasses were drought stressed and showing signs of stomatal closure, leaf rolling, and curing, and they had all fallen below the stomatal conductance threshold 50 mmol/m^2/s^{-1}, Water was then introduced to the grasses in the recovery treatment. Each of the individuals in the recovery treatment were re-watered to field capacity on the first day, then these individuals were watered twice a day using the drip irrigation for 15 minutes for 8 days. Each day the number of live green leaves on each plant was counted and they were categorised as either new/resprouts or recovered/rehydrated leaves. Recover/rehydration occurs when leaves that are considered dead (cured, wilted and no sign of chlorophyll) come back to life (turn green and are turgid), but this number also includes leaves that remained green throughout the drought treatment. If these leaves rehydrate then they cannot increase over time - but the observation that they are alive increases. i.e., if the proportion of rehydrated leaves increases, it is because the leaves that were assumed to be dead have now rehydrated and are alive. During the recovery daily chlorophyll content of a new (resprouted new shoot) and old (greenest, rehydrated) leaf on each plant (if they were present) were measured.

2.3 Statistical analysis

Data were analysed using R statistical software (RStudio 1.2.5001). Data were assessed for normality using a Shapiro-Wilks test and histograms. If data were not normal, it was log-transformed the data and checked that they were normal before running statistical tests.

Throughout the course of the drought, the distributions of all traits were plotted using both raw (individual plants) and summarised data (species-level averages) to visually describe data and understand the range of traits measured (stomatal conductance, water potential, number of dead leaves and chlorophyll content).

To determine the plant water potential at which different grass species close their stomata during a drought (question a) because stomatal conductance was measured daily, and water potential was measured every second day. The stomatal conductance was matched to the day where water potential was measured. The matched dataset was then used to fit a linear model with stomatal conductance as a response variable, and plant water potential and grass species as predictor variables with an interaction term between these (i.e., assessed whether the slopes of the relationship differed between species). From this linear model water potential where each species reached a stomatal conductance of 50 $mmol/m^2/s^{-1}$ was Extracted. This was the index for **stomatal sensitivity.**

To determine whether curing rates differed between grass species (question b) a regression with number of dead leaves as the response variable and soil moisture as the predictor variable, with species as a fixed effect was ran. To decide on which models to use by using Akaike's information criterion (AIC), selecting as the 'best' model the one that was simplest and had an AIC value within ΔAIC of two of the lowest AIC values obtained. The slope of the response of curing to soil moisture was used to define the species-specific trait:

curing rate. This was also done for mean chlorophyll content and fluorometry against soil moisture with species as a fixed effect.

To find out to what degree different grass species osmotically adjust during drought (question c) pressure volume curves were calculated for hydrated and drought stressed leaves of each species. First, the linear portion of each PV-curve where water potential changes with osmotic pressure and turgor pressure is zero was isolated. Then a linear function to this curve was fitted and the x-intercept (water potential at 100% water content) extracted out. Lastly, the x-intercept of the hydrated leaf was subtracted from that of the drought stressed leaf to identify the trait **osmotic adjustment** for each species (Jones and Turner, 1978).

For a visual description on how the grass species responded to the recovery. Firstly, the **proportion of live** and **proportion of resprouted leaves** of each grass species was calculated. The proportion of live leaves was quantified as the total number of green leaves/the total number of leaves on the plant. The proportion resprouted was quantified as the total number of resprouted/total number of live leaves. Proportions were used instead of counts because each individual pot varied in size, some species had more leaves than others. i.e., the recovery was assessed relative to the leaf area of the plant before the drought. Then the data was summarised by the mean of each species (question d).

To assess whether leaves that had gone through the drought had been damaged in any way or were still fully functional a t-test of chlorophyll content as the response variable, and leaf type (old and new) as the predictor variable for each species was ran.

For each species in the experiment, the mean and standard deviation of six important metrics of drought response: 1. Stomatal sensitivity (slope of the regression with leaf water potential), 2. Curing Rate (slope of the regression with soil moisture), 3. Osmotic Adjustment (Average difference between hydrated and drought stressed leaves), 4. Median proportion Live

leaves at the end of 8 days rehydration, 5. Median proportion of these leaves that were resprouts were calculated.

To assess whether these species-level drought response traits showed any pattern in relation to grass clade, photosynthetic sub-type, or rainfall (hypothesis e). each species was classified accordingly in terms of whether they are (Arid, Mesic, Humid) using the average rainfall in its distribution range, Arid areas have a MAR less than 400mm/year and Mesic areas have a MAR between 600 -800 mm/year and Humid areas have a MAR above 800mm/year (see Table 2.1 for MAR values, (Higgins *et al*., 2010)). As well as the species sub-families (Panicoideae, Chloridoideae, Aristidoideae), and photosynthetic pathways (C_4 PCK C_4, NADP-ME and C_4 NAD-ME), Aristidoideae and the NAD-ME were excluded from the analysis because there was only one Aristidoideae species and one NAD-ME species. ANOVAs were then run, where the response variable was the species-specific drought trait (13 data points in total) and did Tukey post-hoc tests to determine differences between groups (question e).

2.4 Results

At what plant water potential do different grass species close their stomata during a drought?

As soil moisture decreases during the drought stomatal conductance also decreases (Figure 2.3), but there is variation in the rate of decrease of each species. Some species, such as *Panicum maximum*, closed their stomata earlier as compared to species such as *Hyparrhenia hirta* and *Urochloa mosambicensis*, which maintained high stomatal conductance for longer.

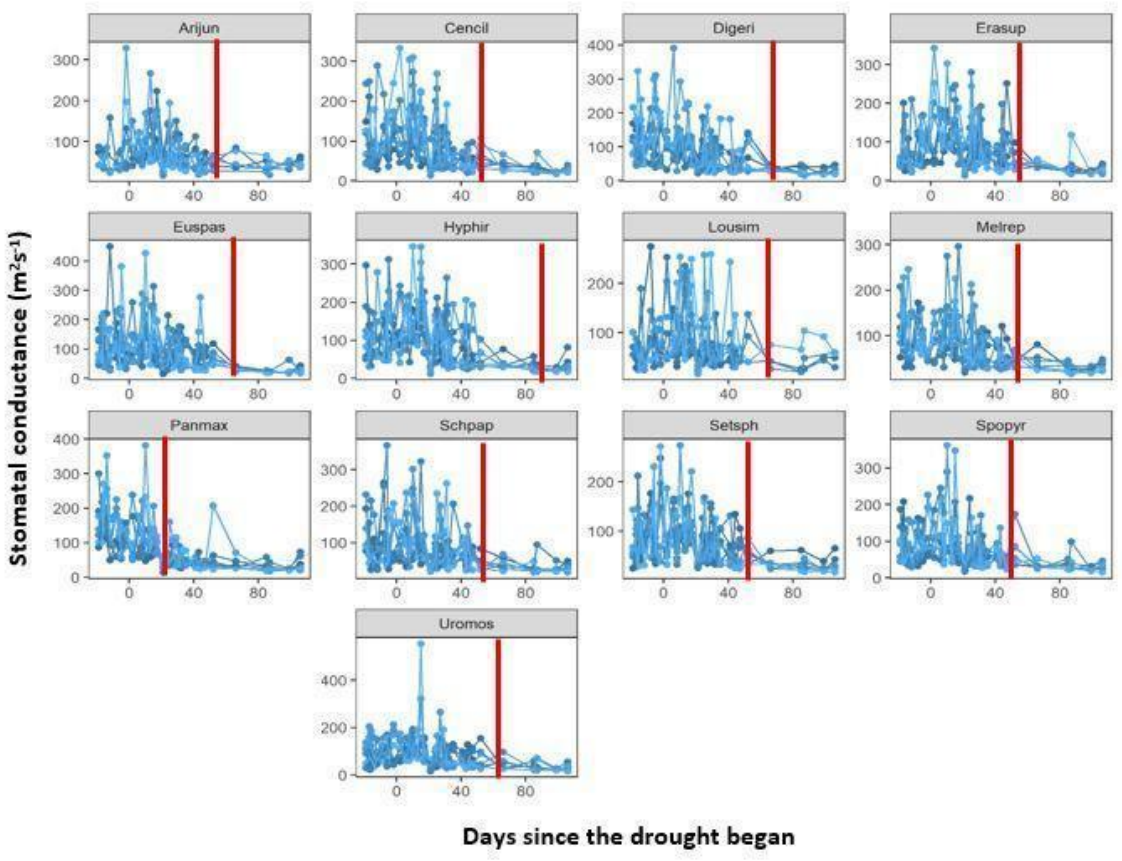


Figure 2. 3: Stomatal conductance values of the droughted plants throughout the experimental drought. Points represent the values of individual leaves from the replicates for each species. The red line represents the day of stomatal closure where the average stomatal conductance dropped below 50 mmol/m^2/s^{-1}. (For full species names represented in the boxes see Table 2.1)

During the drought, water potential decreased over time as the soil dried, but the rate at which it decreased was not the same for all species (Figure 2.4). Species such as *Aristida*

junciformis, *Loudetia simplex* and *Sporobolus pyramidalis* maintained high water potentials for longer (water potential only drops later during the drought) whilst species such as *Panicum maximum* and *Urochloa mosambicensis* reduced their water potential quickly. There was also some variation between individuals of the same species.

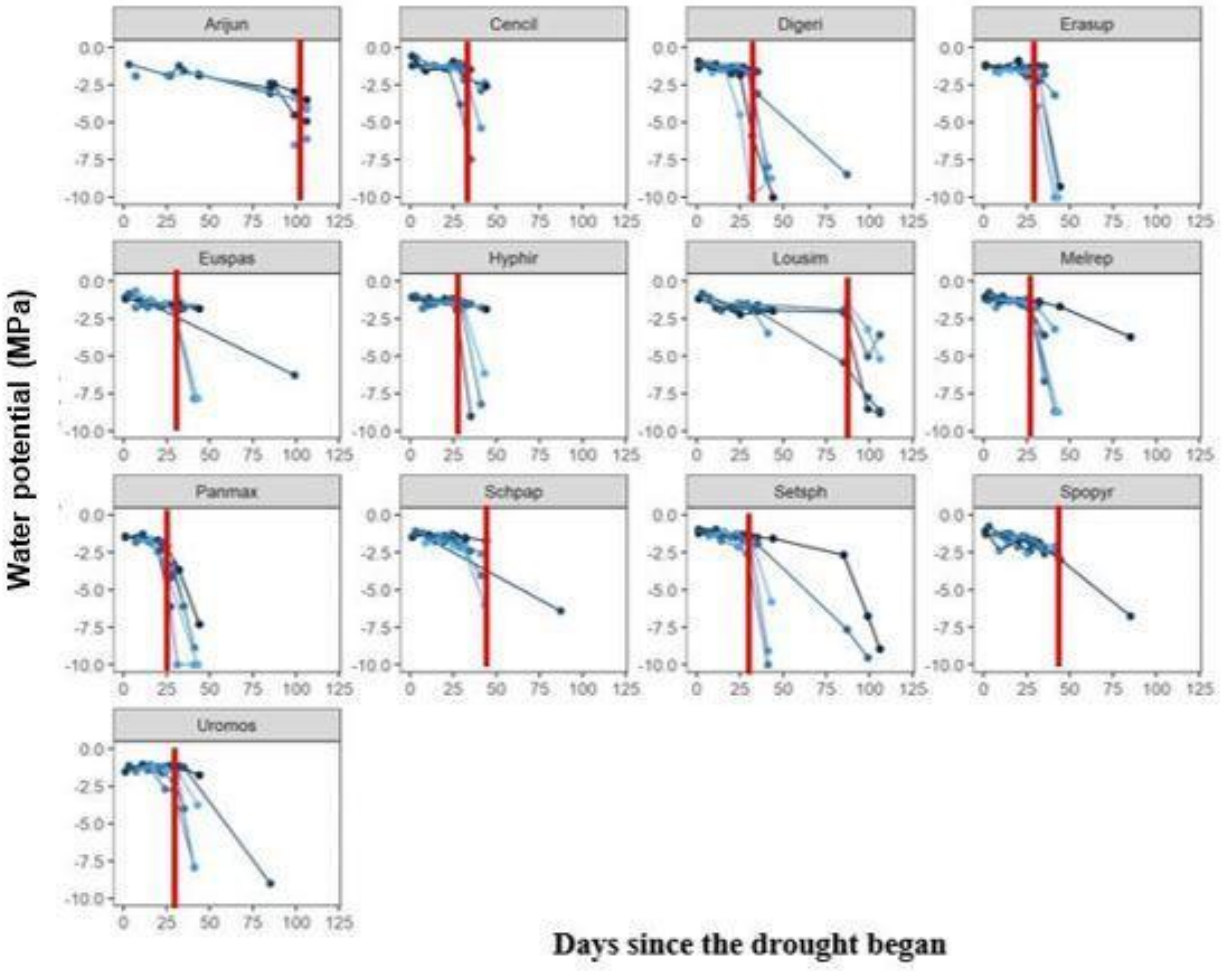


Figure 2. 4: Water potential values of the droughted plants throughout the experimental drought. Points represent the values of individual leaves from the replicates for each species each day. The red line represents the average day of stomatal closure (For full species names represented in the boxes see Table 2.1)

According to a linear model of the response of stomatal conductance to water potential with species as an interaction term, there was a significant difference between species in how their stomatal conductance responded to changes in water potential (Figure 2.5). Some species

had steeper slopes as compared to others, indicating that they have high stomatal sensitivity. Additionally, the point (intercept) at which each species reached the threshold (closed their stomata) 50mmol/m^2/s^{-1} varied between species, with some reaching this threshold after fewer days of drought than others (Multiple R-squared= 0.27, Adjusted R-squared= 0.19, RSE= 0.22, F=0.19, $p<0.001$). See appendix for the model results

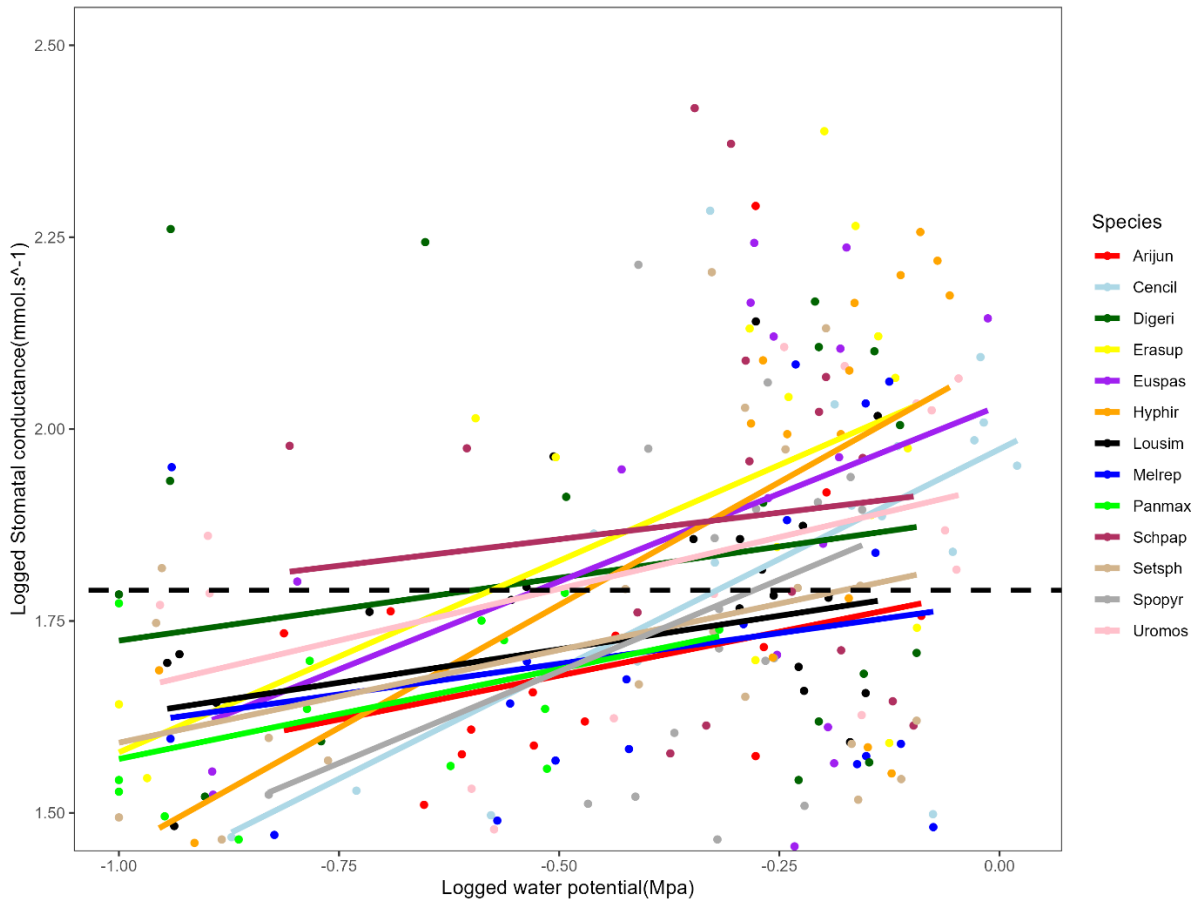


Figure 2. 5: Regression relationship between logged stomatal conductance and logged water potential values of the droughted plants throughout the experimental drought. The dotted line represents 50 mmol/m^2/s^{-1}Points represent the individual values across all 13 droughted species each day. For full species names see Table 2.1.

Do different grass species cure faster than others when exposed to a drought?

Based on a linear model on the relationship between the number of dead leaves and soil moisture with species as an interaction there is a significant difference in the curing rates of species (Figure 2.6) (Multiple R-squared=0.59, Adjusted R-squared=0.57, F=34.22, p<0.001). See appendix for the model results.

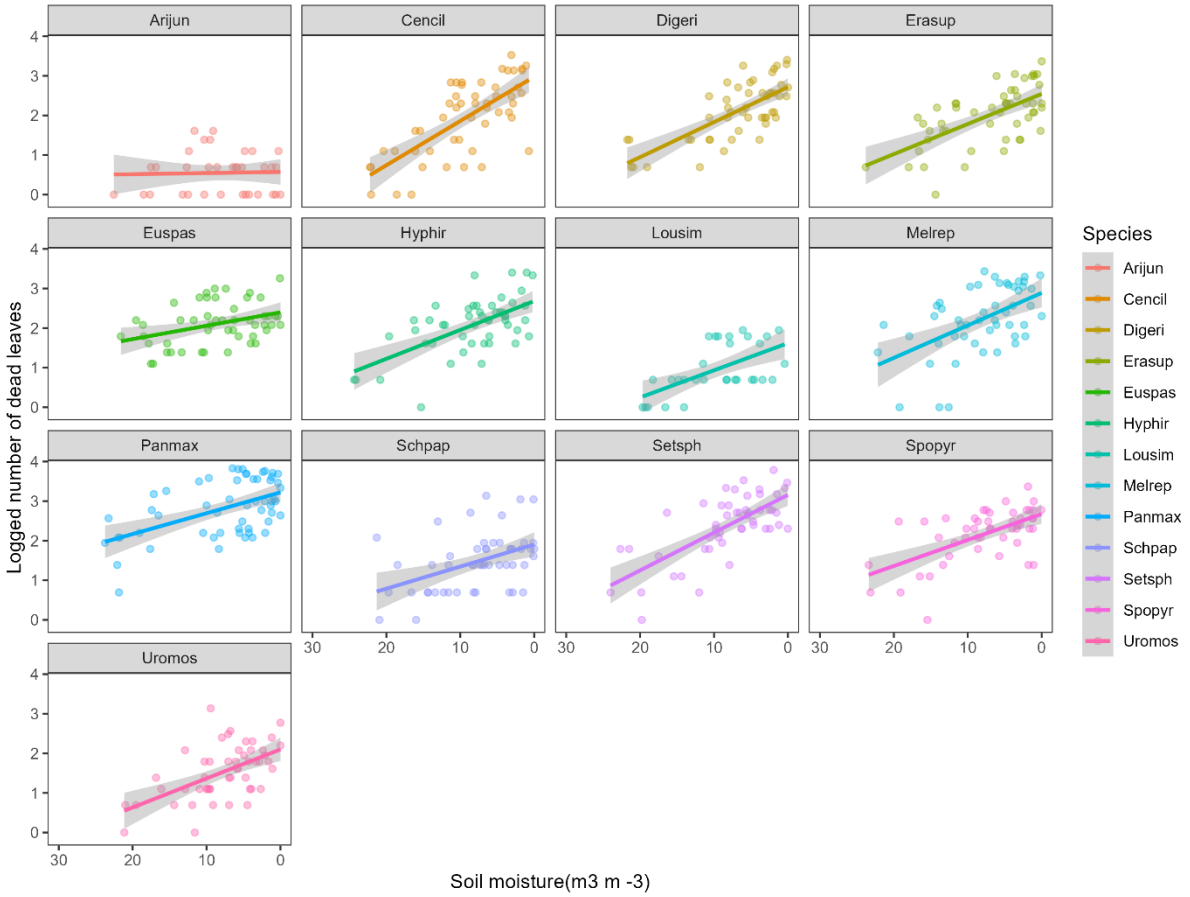


Figure 2. 6: The relationship between the logged average number of dead leaves and soil moisture. Points represent the mean value across all 13 individuals of each droughted species on each day (For full species names represented in the boxes see Table 2.1)

Changes in the health or chlorophyll content of the remaining green leaves on the plant were assessed using a linear model of the relationship between chlorophyll content and soil moisture (Fig 2.7). Although most species managed to maintain at least some leaves with high

chlorophyll content throughout the drought (and some like *Urochloa mosambicensis* even increased chlorophyll content of their live leaves) there was a significant difference in the rate chlorophyll was lost between the species (Multiple R-squared= 0.09, Adjusted R-squared= 0.08, RSE= 53.06, F = 6.34, p<0.001). See appendix for model results.

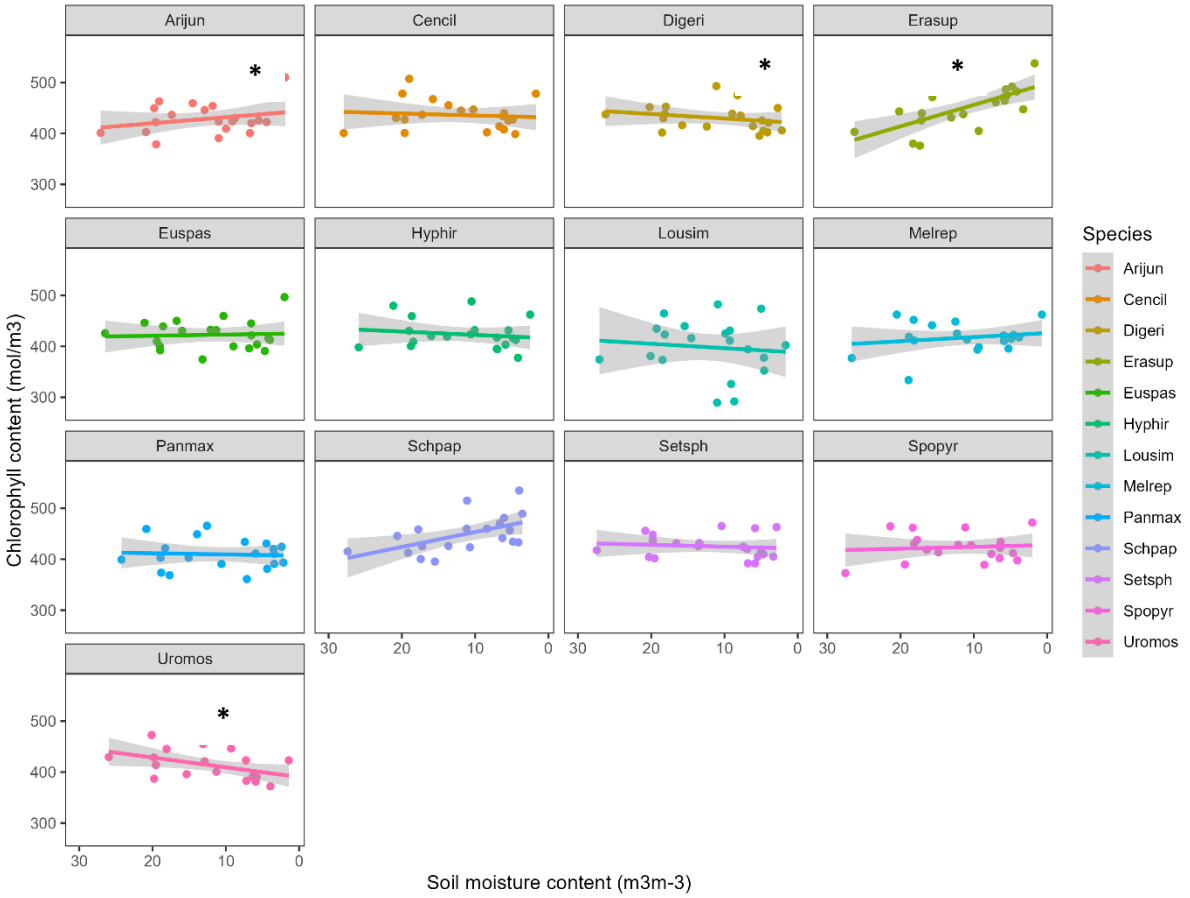


Figure 2. 7: Chlorophyll content and soil moisture values of the droughted plants throughout the experimental drought. Points represent the mean value across all 13 individuals of each droughted species on each day. * Represent significantly different species. (For full species names represented in the boxes see Table 2.1).

There is evidence for a reduction in performance of the photosynthetic systems of the remaining green leaves through the drought (Figure 2.8), based on a linear model between fluorometry and soil moisture. Fluorometry decreased with soil moisture, this indicates a

change in the performance/health of the leaves (Multiple R-squared= 0.079, Adjusted R-squared= 0.07, F= 15.33, RSE =0.046, p <0.001).

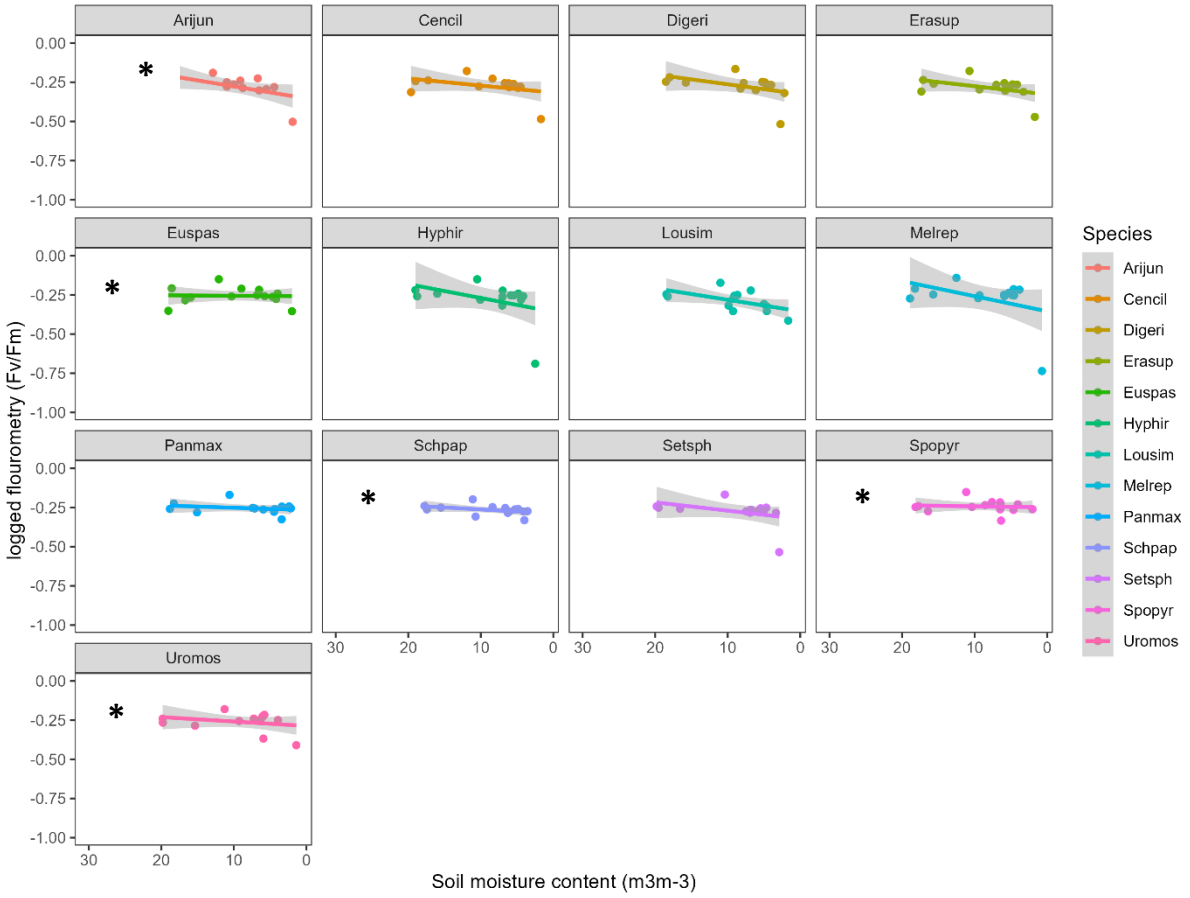


Figure 2. 8: Fluorometry and soil moisture values of the droughted plants throughout the experimental drought. Points represent the mean value across all 13 individuals of each droughted species on each day. * Represent significantly different species. (For full species names represented in the boxes see Table 2.1).

To what degree do different grass species osmotically adjust during a drought?

Figure 2.9 shows the osmotic adjustment of the 13 different grass species. Some species, such as *Eragrostis superba, Schmidtia pappophoroides* and *Panicum maximum* adjust more, as is shown by the significant difference in the relationship between -1/water potential and relative water content of the well-watered grasses (pre-drought) compared to drought stressed grasses. Other species, such as *Melinis repens, Loudetia simplex* and *Cenchrus ciliaris,* do not osmotically adjust as much, as is shown by the overlapping nature of this relationship.

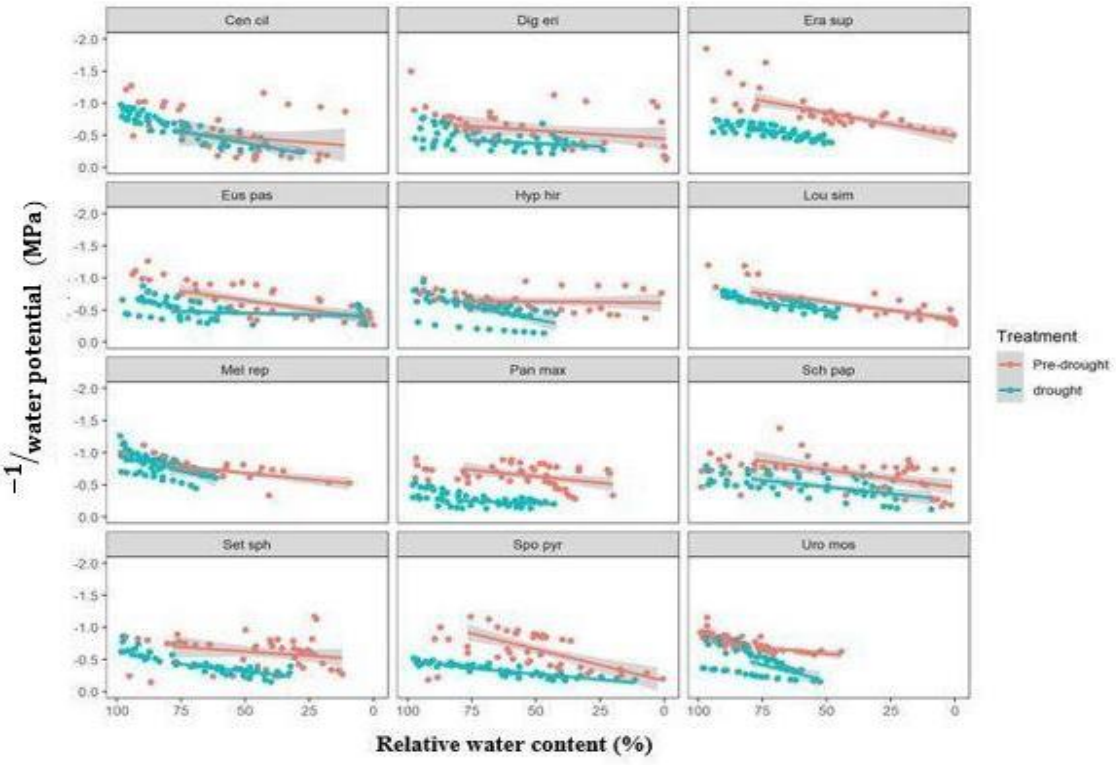


Figure 2. 9: Osmotic adjustment indicated by the difference in relationship between -1/water potential and relative water content of the well-watered grasses (pre-drought) and drought-stressed grasses. Points represent the individual values of a pressure volume curve for 5 different individuals of each species. (For full species names represented in the boxes see Table 2.1).

Do grass species have different recovery strategies? - Resprout VS Rehydrate

None of the individual plants in the experiment died during the 8-week drought that was imposed. As the plants recovered after watering started again the mean proportion of live leaves (rehydrated leaves) as a fraction of all leaves on the plant increases and so does the chlorophyll content of the rehydrated leaves (Figure 2.10). However, there is variability between species, with some species such as *Sporobolus pyramidalis* and *Aristida junciformis*, ending up with a higher proportion of rehydrated leaves when compared to other species such as *Melinis repens*, *Setaria sphacelata* and *Panicum maximum* (Figure 2.10).

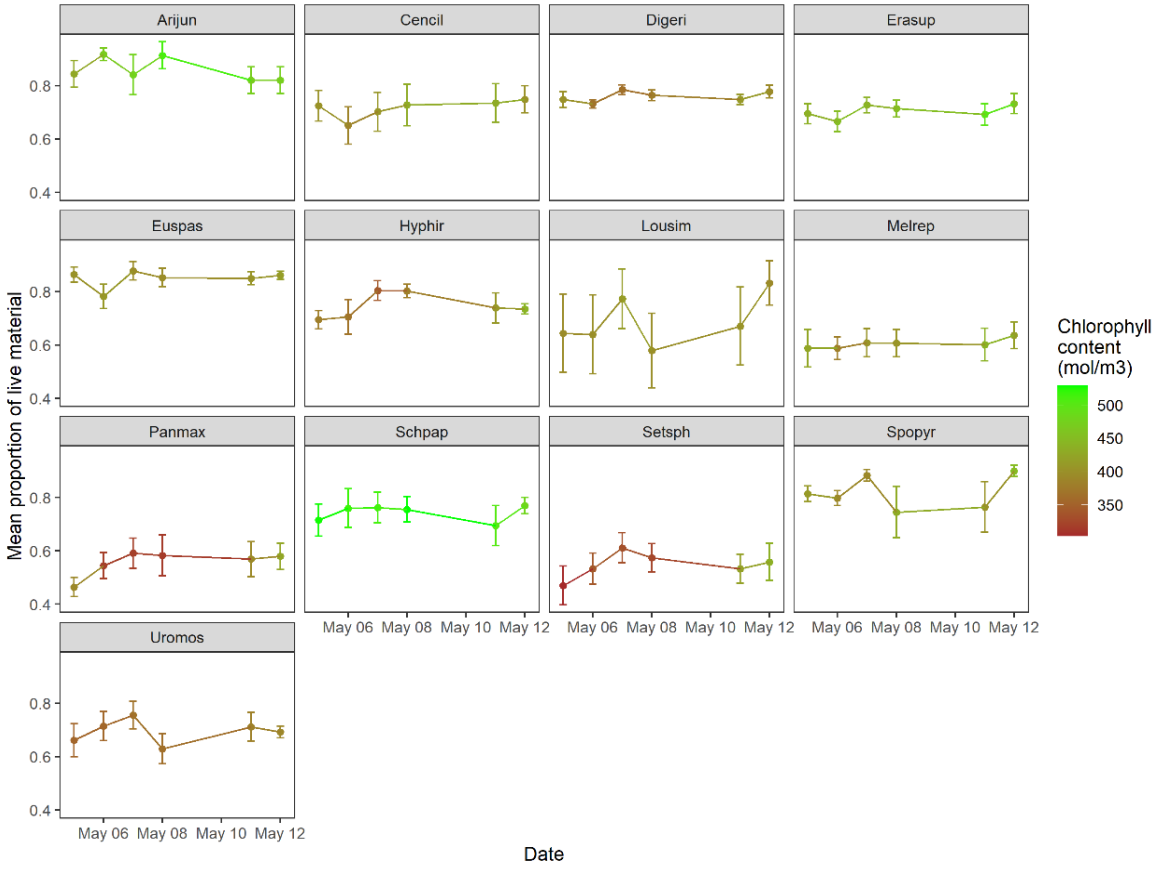


Figure 2. 10: Line graph between mean proportion of live leaves (rehydrated) and time throughout the experimental recovery with chlorophyll content as a gradient. Error bars represent the standard error value across all 13 individuals of each droughted species on each day (For full species names see Table 2.1).

Many individuals also sprouted new green leaves as well as rehydrating existing leaves, however there is variability between species. Some species, such as *Cenchrus ciliaris* and *Digitaria eriantha*, have a higher mean proportion of resprouted leaves as compared to other species, such as *Aristida junciformis and Setaria sphacelata* (Figure 2.11). Some species also resprout more quickly than others e.g., *Eustachys paspaloides* compared with *Hyparrhenia hirta*.

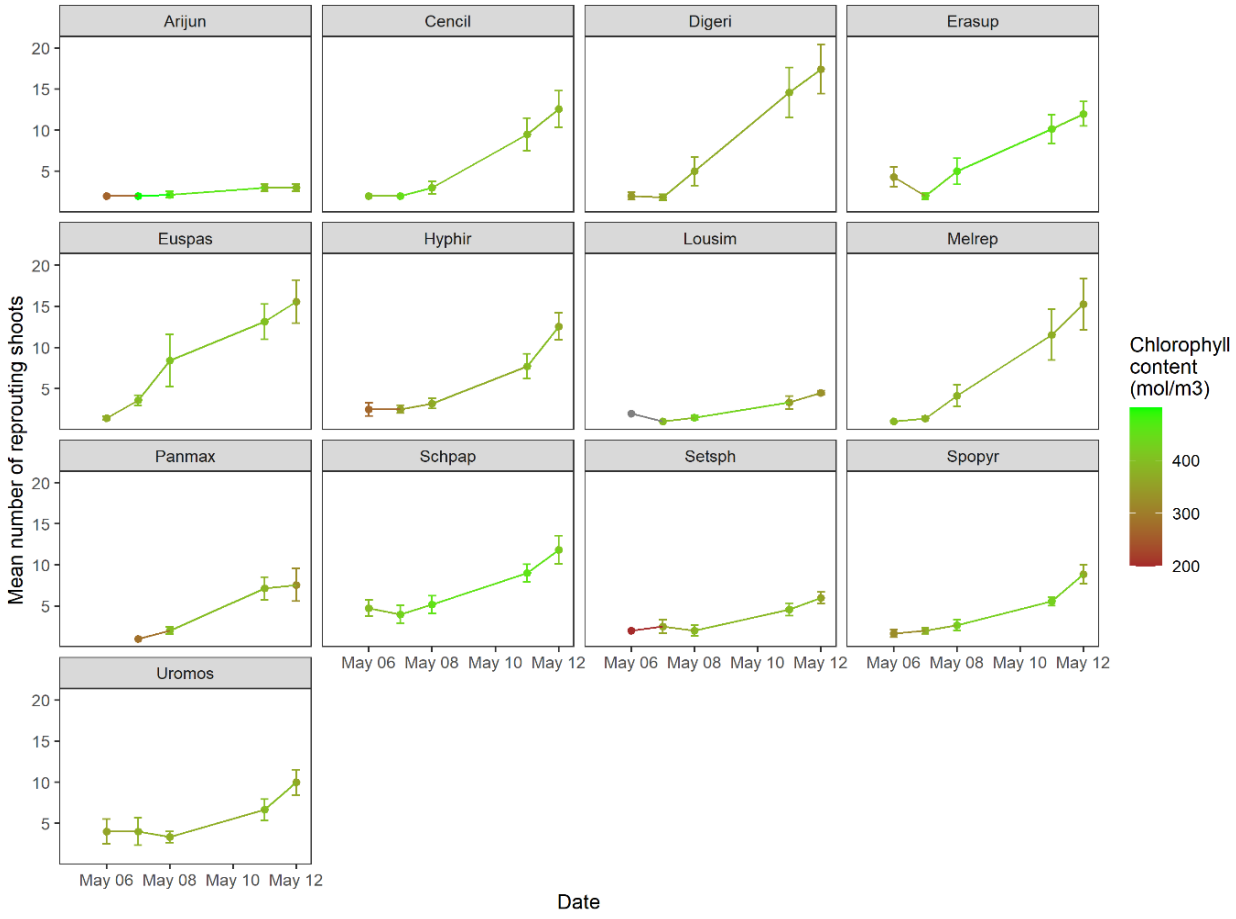


Figure 2. 11: Line graph showing the mean number of resprouted leaves over time throughout the experimental recovery with chlorophyll content as a gradient. Error bars represent the standard error value across all 13 individuals of each droughted species on each day (For full species name, see Table 2.1).

There is a significant difference in the chlorophyll content between the 13 grass species and between the new and old leaves in each species, with old leaves on average having 60

mol/m^3 more chlorophyll than new leaves (Figure 2.12). The effect of species was also significant (Multiple R-squared= 0.46, Adjusted R-squared= 0.36, F= 4.85, p= 5.152e-10), See model in appendix.

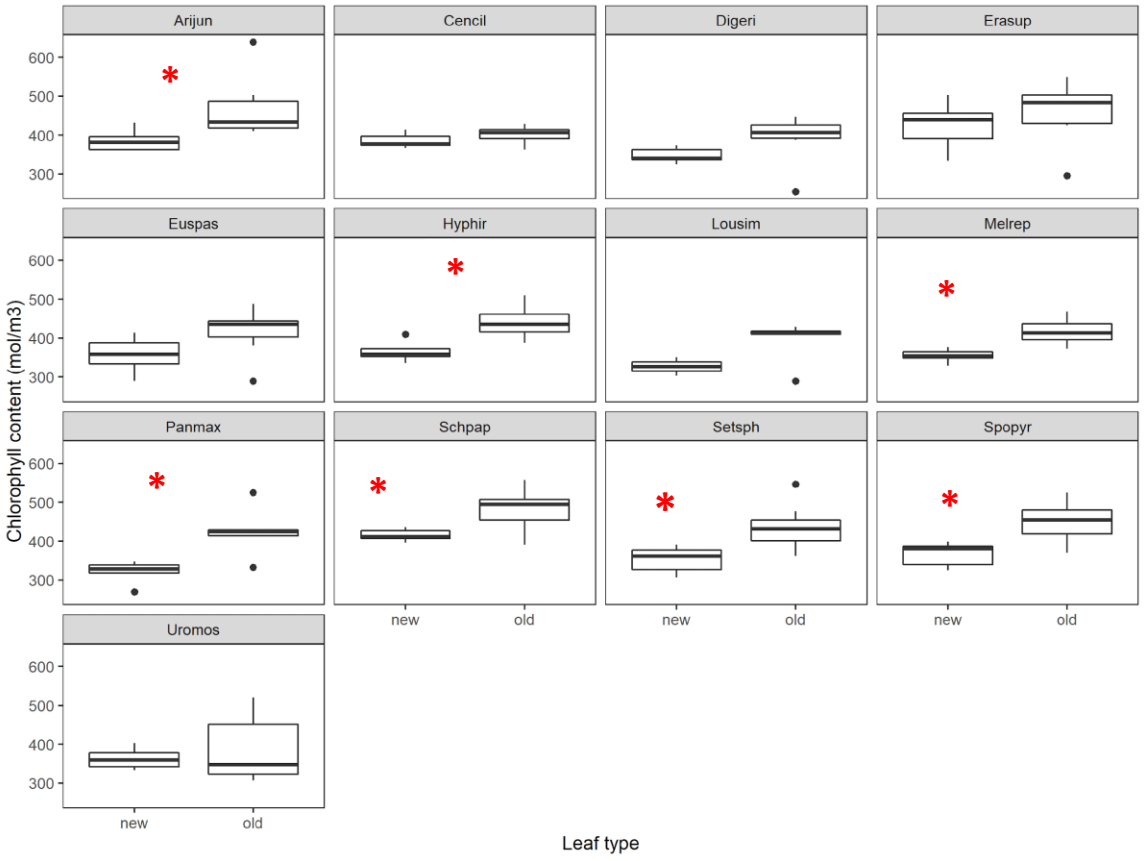


Figure 2. 12: Box plot between the chlorophyll content of a new (resprouted) leaf against the chlorophyll content of an old (rehydrated) leaf at the end of the experimental recovery. * Represent species for which this difference was significant. (For full species names see Table 2.1).

Table 2.2 is a summary of the average value for each species of the traits related to physiological drought response and recovery collected in this chapter. Values vary between species.

Table 2. 2: Summary of the drought response traits measured in chapter 2. The traits are Osmotic adjustment (OA), Stomatal sensitivity (ScSlope), Curing rate (Curing slope), Proportion of rehydrated leaves (PropLive) and the proportion of resprouted leaves (PropRes). The values in brackets represent the standard deviations or confidence intervals.

Species	OA	ScSlope	CuringSlope	PropLive	PropRes
Aristida junciformis		**1.79**	**-0.001**	**0.85**	**0.15**
		(-0.27±0.73)	(-0.08±-0.06)	(0.13)	(0.01)
Cenchrus ciliaris	**-0.26**	**1.97**	**-0.049**	**0.83**	**0.17**
	(0.54)	(-0.57±1.71)	(1.13±1.67)	(0.14)	(0.07)
Digitaria eriantha	**-3.74**	**1.89**	**-0.085**	**0.87**	**0.13**
	(8.52)	(-0.91±1.24)	(1.13±1.68)	(0.06)	(0.08)
Eragrostis superba	**-0.57**	**2.08**	**-0.069**	**0.77**	**0.23**
	(0.54)	(-0.6±1.6)	(1.04±1.59)	(0.10)	(0.11)
Eustachys paspaloides	**-0.46**	**2.03**	**-0.045**	**0.90**	**0.10**
	(0.29)	(-0.68±1.6)	(1.25±1.79)	(0.04)	(0.04)
Hyparrhenia hirta	**-0.12**	**2.09**	**-0.044**	**0.87**	**0.13**
	(0.29)	(-0.48±1.76)	(1.2±1.74)	(0.05)	(0.05)
Loudetia simplex	**-0.58**	**1.8**	**-0.059**	**0.90**	**0.10**
	(0.72)	(-0.92±1.27)	(1.14±1.75)	(0.22)	(0.004)
Melinis repens	**0.19**	**1.77**	**-0.063**	**0.71**	**0.29**
	(0.11)	(-0.95±1.28)	(1.33±1.87)	(0.13)	(0.16)
Panicum maximum	**-1.65**	**1.8**	**-0.056**	**0.75**	**0.25**
	(2.01)	(-0.99±1.46)	(1.9±2.43)	(0.13)	(0.07)
Schmidtia pappophoroides	**-2.5**	**1.9**	**-0.045**	**0.83**	**0.17**
	(4.46)	(-1.07±1.35)	(1.55±1.1)	(0.08)	(0.08)
Setaria sphacelata	**-0.22**	**1.83**	**-0.063**	**0.91**	**0.09**
	(0.38)	(-0.83±1.32)	(1.49±2.03)	(0.19)	(0.03)
Sporobolus pyramidalis	**-0.57**	**1.92**	**-0.068**	**0.95**	**0.05**
	(0.29)	(-0.86±1.82)	(1.25±1.79)	(0.06)	(0.05)
Urochloa mosambicensis	**-0.25**	**1.92**	**-0.058**	**0.78**	**0.22**
	(0.28)	(-0.83±1.38)	(0.61±1.16)	(0.06)	(0.09)

Is there any evidence that grass phylogenetic history (grass clade), photosynthetic subtype, or environmental envelope are associated with these drought response traits?

When examining osmotic adjustment between the grass subfamilies, determination of osmotic adjustment between the grass subfamilies showed that Panicoideae did not osmotically adjust more than Chloridoideae (Figures 2.13 a, t-test, t = 0.23, df = 7.7, p = 0.83) or between species with NADP-ME and PCK photosynthetic pathways (Figure 2.12 b, t-test, t = 0.06, df = 6.3, p = 0.95). However, there is significant statistical difference that grass species from arid regions showed more osmotic adjustment (lower values) than those in mesic and humid regions (Figures 2.13 c, ANOVA, F = 0.37, p = 0.02).

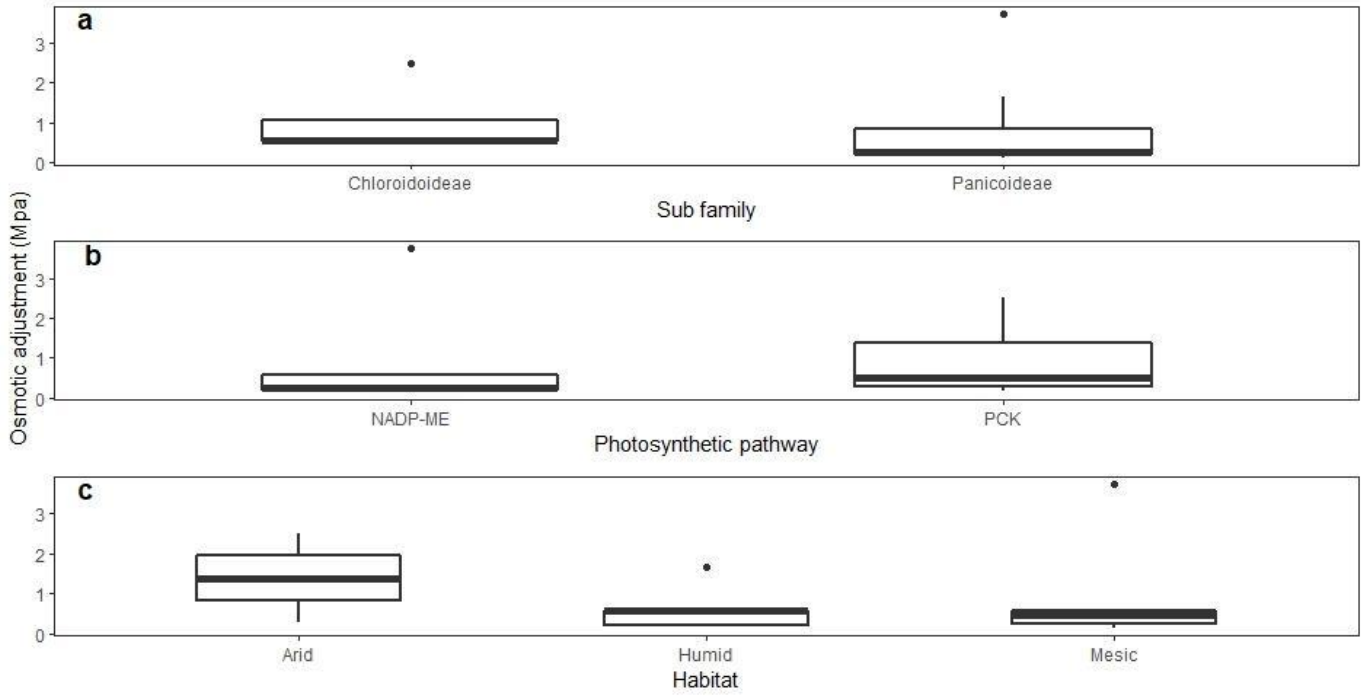


Figure 2. 13: Boxplot of the osmotic adjustments a) sub families b) photosynthetic pathways and c) habitats the 13 grass species are found in. Boxes represent the medians, 25 and 75th confidence intervals.

When examining days to stomatal closure the difference between Chloridoideae and Panicoideae was not significant (Figure 2.14 a, t-test; t = 1.89, df = 8.2618, p = 0.09) , there was also no evidence for statistical differences between species with NADP and PCK photosynthetic pathways (Figure 2.14 b, t-test; t = -0.016, df = 9.56, p = 0.99) or between species from arid, mesic and humid environments (Figure 2.14 c, ANOVA, F =2.71 , p =0.12).

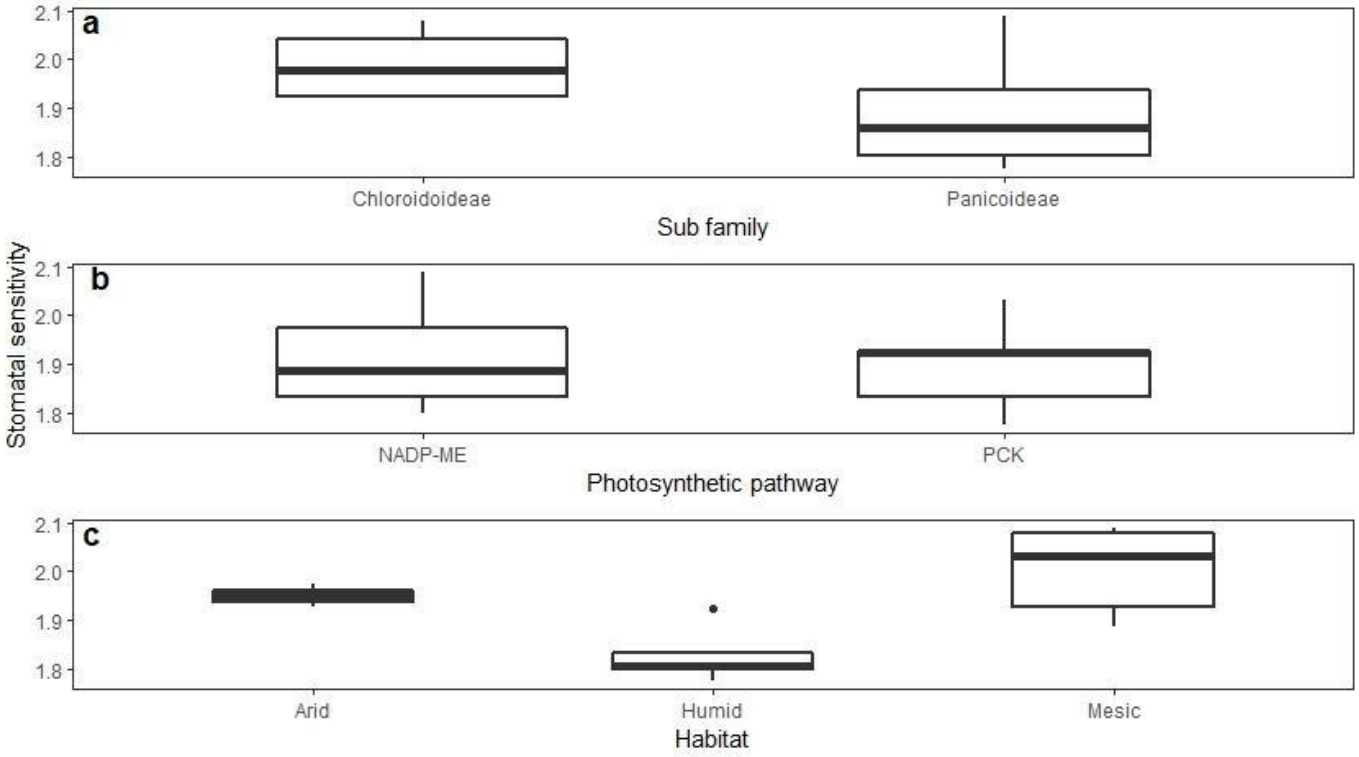


Figure 2. 14: Boxplot of stomatal sensitivity between a) sub-families, b) photosynthetic pathways and c) habitats of the grass species. Boxes represent the medians, 25 and 75th confidence intervals

When examining curing rates and sub-families, Panicoideae grasses do not have a higher curing rate as compared to Chloridoideae (Figure 2.15 a, t = 0.36, df = 5.53, p = 0.73). There was also no statistical difference in curing rates between photosynthetic pathways (NADP and PCK) (Figure 2.15 b, t-test, t = 0.47, df = 6.09, p = 0.65), or between plants from the three habitats (Arid, Mesic, Humid) (Figure 2.15 c, ANOVA, F = 0.81, p = 0.48).

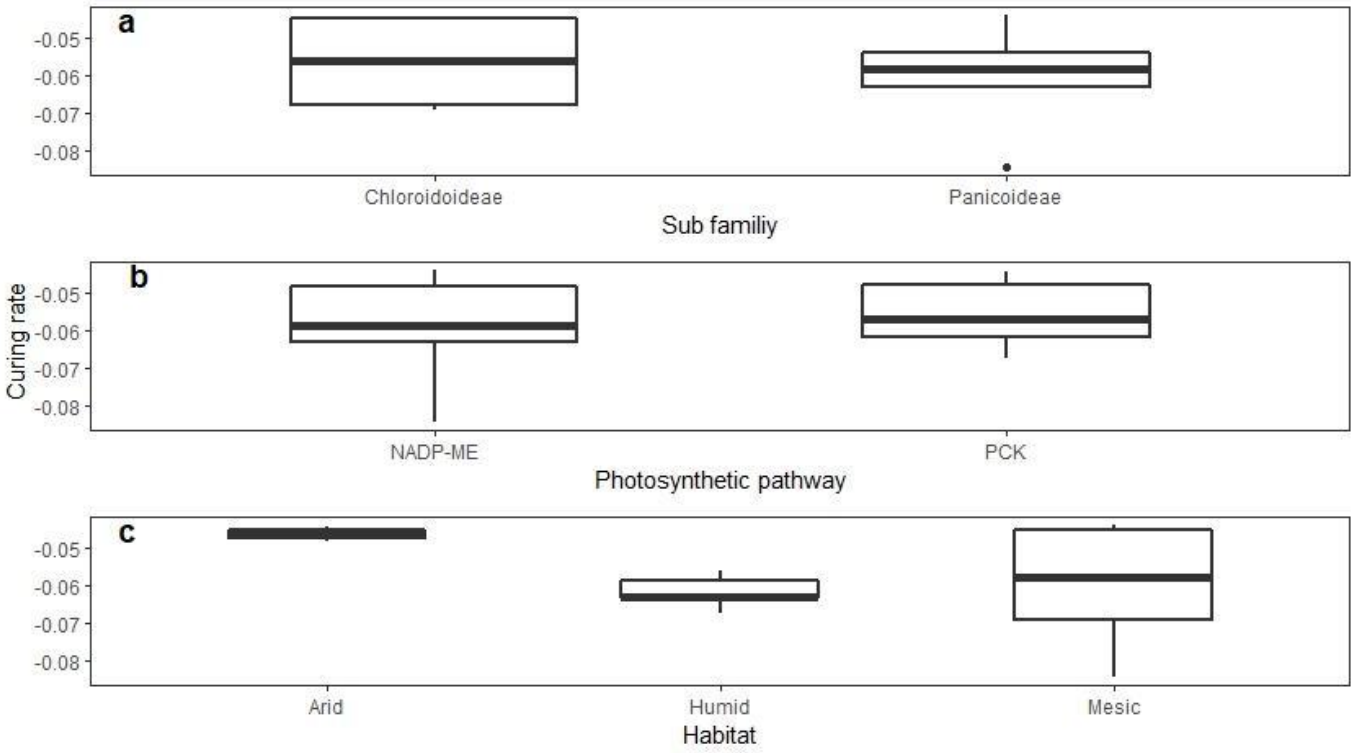


Figure 2. 15: Boxplot of the curing rate between a) sub families, b) photosynthetic pathways, and c) habitats of the grass species. Boxes represent the medians, 25 and 75th confidence intervals

Likewise, the significant statistical ere was no evidence of differences in resprouting between sub-families (Figure 2.16 a, t = -0.74, df = 5.7, p-value = 0.49). photosynthetic pathways (NADP and PCK) (Figure 2.16 b, t = -1.3, df = 6.05, p-value = 0.24), or between plants from the three habitats (Arid, Mesic, Humid) (Figure 2.16 c, F = 0.07, p= 0.93).

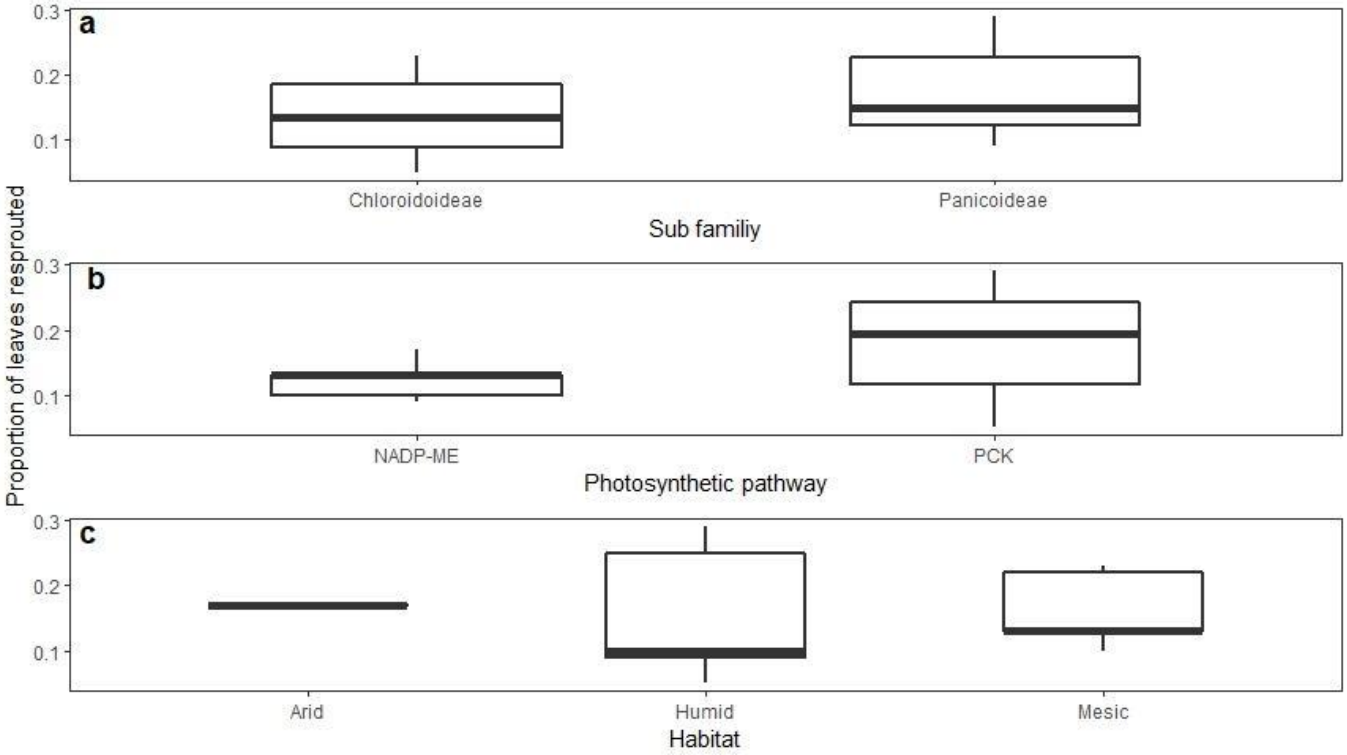


Figure 2. 16: Boxplot of the proportion of leaves resprouted between a) sub-families, b) photosynthetic pathways and c) different habitats of the grass species. Boxes represent the medians, 25 and 75th confidence intervals.

2.5 Discussion

Grass species had a variety of responses to the drought. As soils dried, the plants closed their stomata, osmotically adjusted, and sometimes showed a reduction in the chlorophyll content of the live leaves. Each species also cured during the drought (the number of dead leaves increased as soil moisture decreased), but this also varied significantly between species. The variation here can be explored in terms of the safety-efficiency trade-off.

Stomatal closure is a common response to a water deficit. My first research question looked to quantify when (at which water potential) this loss of conductance occurred in each species, with the assumption that plants that maintained stomatal conductance at low water potentials were putting their water transport systems at higher risk of cavitation and irreparable damage (Tucker *et al*., 2011). Specifically, some species have a higher stomatal sensitivity (close their stomata earlier) as compared to others, such as *Panicum maximum* and *Loudetia simplex* (Fig 2.5), this indicates that these species are sacrificing photosynthesis for hydraulic safety (i.e., the "safe" side of the safety-efficiency trade-off). Some species have a shallow slope/lower stomatal sensitivity (close their stomata later) for example *Aristida junciformis* and *Cenchrus ciliaris*, this indicates that they are sacrificing hydraulic safety for growth (i.e., the "efficient" side of the safety-efficiency trade-off) (Fig 2.5).

The variability that was observed between species could be because some species had denser, smaller stomata that are more likely to respond quickly to water stress resulting in a greater tolerance to drought ("safe" species) (Henry *et al*., 2019). As the plants closed their stomata with decreasing water potential there is a decline of stomatal conductance and reduced photosynthesis. Therefore, it would be preferable for plants to maintain high stomatal conductance through a drought, but only if they can maintain live leaves. In physiological responses to drought, it is important to quantify the degree to which the leaf area of the plant

is maintained in a functioning state, this is an indication that a plant is no longer able to maintain a fully functioning leaf canopy and is reducing its leaf area.

The second research question was to determine whether different grass species cure faster than others. It was expected that there would be examples of grass species with a drought strategy associated with the safe side of the safety-efficiency trade-off: these would have a low curing rate because they close their stomata during the drought to retain their soil moisture content and use water sparingly to maintain high turgor pressure to keep the leaves standing. In contrast, also there was an expectation to find C_4 grasses with a drought strategy associated with the efficient side of the safe-efficient trade-off – which would maintain high stomatal conductance at the risk of losing hydraulic conductivity. higher curing rates. Expected in grasses.

There was a significant difference in the rates of curing between the species, with species such as *Panicum maximum, Melinis repens* and *Schmidtia pappophoroides* curing much faster than species such as *Aristida junciformis* and *Loudetia simplex* (Fig 2.6). During the curing process, water loss results in a decrease in chlorophyll content in drought stressed plants (Wang *et al*., 2016). Interestingly, although there was significant curing, the chlorophyll content of the remaining live leaves remained quite high, or, in some cases, even increased. This is an indication that all the plants are still working to maintain a functioning canopy of leaves, and, in the case where the chlorophyll content increases, reallocating nitrogen resources from the cured leaves to the remaining functional leaves.

The third research question was to determine whether grass species osmotically adjust. Certain grass species do, but this varies across species. Leaves that osmotically adjust remain turgid at more negative water potentials and can maintain stomatal conductance and photosynthetic rates in water limited conditions (Sack and Scoffoni, 2012; Ocheltree *et al*.,

2016). Some species such as *Panicum maximum* and *Eragrostis superba* adjusted more than others such as *Aristida junciformis* and *Cenchrus ciliaris* (Fig 2.9).

To fully assess drought strategies, how grass species recovered needed to be assessed. Every individual exposed to the drought survived – i.e., there was no drought-induced mortality. This is to be expected: although the drought treatment persisted for three months, it is still shorter than the ~6-month drought these species are exposed to annually, during the dry season. Despite no mortality, there were big differences in how each species recovered from the drought. During the recovery there was a general increase in the number of both resprouted and rehydrated leaves for all the species, and when looking at the chlorophyll analysis there was no evidence to show that rehydrated leaves showed any damage from the drought experience and are just as functional as the resprouted leaves (sometimes more so (Fig 2.12)), this was very surprising as there were expectations that rehydrated leaves would be less functional as compared to resprouted leaves because during the rainy season water potential is usually higher for resprouters than for non-resprouters, resulting in resprouters being more resistance to drought (Ackerly 2004). However, Paulas and Pausas., (2006) found that non-resprouters have higher drought resistance at leaf level due to having a higher water-use efficiency because of a higher leaf mass area.

In answering the fourth research question of whether grass species recover more by resprouting or by rehydrating. Species can be ranked in terms of their resprouting vs rehydrating response. Some species are recovering through resprouting (high proportion of resprouted leaves), such as *Panicum maximum, Melinis repens, Urochloa mosambicensis* and *Eragrostis superba* (Fig 2.10). Other species, such as *Eustachys paspaloides, Loudetia simplex, Sporobolus pyramidalis,* are recovering through rehydrating. *Setaria sphacelata* appears not to recover well – i.e., it is not drought tolerant, and some species recover through a combination

of resprouting and rehydrating (*Cenchrus ciliaris, Schmidtia pappophoroides*, *Digitaria eriantha*, *Hyparrhenia hirta* and *Aristida junciformis*) (Fig 2.11).

It is interesting to consider these results in terms of the natural environments my selected species are found in, and their phylogenetic relationships because the trade-off between growth and the ability to deal with stress might not only be related to precipitation but to biogeography because plants adapt to different growing environments (Ocheltree *et al*., 2016).

There is likely a link between resprouting and dry regions because arid and semi-arid ecosystems are dominated by plants that regenerate by resprouting since traits such as resprouting evolved with drier conditions so plants would be able to survive droughts and fires (López-Soria and Castell, 1992). Plants found in dry environments have a higher water-use efficiency due to having high leaf nitrogen content which allows them to reach lower stomatal conductance and enhancing water conservation during photosynthesis (Ackerly., 2004). It is likely that Chloridoideae species would resprout more as compared to Panicoideae because Chloridoideae species cure faster and close their stomata later. However, I was surprising that the responses to drought varied predictably between sub-family groups.

Based on the findings from Visser *et al*., (2012), who found that Chloridoideae species tend to be associated with more drought-prone environments. It is likely that species from the Chloridoideae sub-family closed their Stomata earlier than species from Panicoideae sub-families. However, there was no clear relationship for stomatal closure and environmental niches, and it could be because many of the species I measured have exceptionally large ranges, so mean rainfall in their distribution in South Africa does not really reflect their true environmental niche.

A relationship between Chloridoideae species and early stomatal closure would have meant that species from Panicoideae family would cure faster than Chloridoideae species, indicating that there is a trade-off between maintaining high transpiration rates and maintaining a full leaf canopy (safe-efficient). Despite a poor association between drought response traits and environmental niche, there was evidence that plants from arid environments osmotically adjusted more, this is to be expected based on the study by Bartlett *et al*., (2015). When grasses that occur across arid regions reach low stomatal conductance, they lose the ability to conduct water at higher leaf water potentials resulting in them having to osmotically adjust (Ocheltree *et al*., 2016). Again, there was no evidence that species with PCK photosynthetic pathway were less affected by drought as compared to species with NADP-ME photosynthetic pathway. This could be because of the unevenness between the number of species in each category (Wigley-Coetsee and Staver, 2020).

In conclusion, a large variation in a range of important drought responses within the thirteen grasses studied, which implies that the safety-efficiency trade-off might be important in C_4 grasses, even though they are all exposed to predictable long seasonal droughts. However, it would be important to look more closely at these trade-offs and determine whether the C_4 grasses studied can be grouped into plants with similar drought syndromes. This is the focus of the next chapter.

CHAPTER 3

To evaluate whether safety-efficiency trade-offs are present in C_4 grasses, whether drought responses can predict drought recovery responses and identify drought syndromes in C_4 grasses.

Abstract

Perennial grasses face periods of water limitation (drought) over a lifetime, and where drought extent and severity impact diverse life history strategies of grasses in diverse ways. C_4 grasses are deciduous: they senesce their leaf material in the dry season. Unlike trees, and where the aboveground architecture of grasses is predominantly leaves, grasses do not drop-dead leaves, rather they are retained on a plant. Deciduousness in grasses due to seasonal moisture deficit impacts how grasses perform in and recover from drought events. It is important to understand the drought strategies and recovery process in C_4 grasses that survive drought to assess overall fitness and the effectiveness of different drought strategies. Here, a conceptual framework using four traits related to drought tolerance - stomatal sensitivity, osmotic adjustment, curing rates and recovery- to define diversity drought tolerance strategies was developed. This chapter demonstrates trait co-ordination based on a safety-efficiency trade-off that operates as a continuum. Overall, there are five safe syndromes and five efficient syndromes related to quantitative variation in drought response traits. The implications of these findings are that, once there is an understanding of the diverse drought strategies, future ecosystem function and resilience can be predicted.

Keywords: Drought strategies, Recovery, Safety-Efficiency trade off

3.1 Introduction

Environmental constraints of physiological drought tolerance

Water availability is a main driver of savanna vegetation structure and function (Blackman *et al*., 2014). With changing rainfall regimes and increases in temperature, an increase in droughts is predicted to occur across the globe (Blackman *et al*., 2014). Savannas are characterised by seasonal rainfall and extensive dry seasons. The responses of savanna ecosystems to climate change will be influenced by species composition (Blackman *et al*., 2014). Savanna plant species (C_4 grasses and C_3 trees and shrubs) have evolved a range of strategies to tolerate seasonal climates. For example, C_4 grasses typically use water more efficiently and are more physiologically responsive to intermittent rainfall events, compared to C_3 trees and shrubs, especially during periods where the plant water availability is lower than average levels required for growth (Hoover *et al*., 2019).

Trees have invested in carbon-rich above-ground structures. Cavitation and loss of above-ground parts can be costly, therefore most woody species operate within well-defined safety margins to prevent cavitation (Wigley-Coetsee and Staver, 2020). For grasses, cavitation and loss of above-ground biomass could be less costly as it is easier to regrow, but in highly competitive grassland environments there might still be selection for conservative water use strategies that maintain functioning above-ground biomass through droughts.

The predominant form of drought species experience should determine the competitive life history strategy in any given geographic area by acting as an environmental filter (whether it is a lengthy term drought or an intermittent drought) (Wigley-Coetsee and Staver.,2020). There are variations on how these species respond to drought such as maintaining living biomass and rehydrating it could be because of being able to tolerate the mid rainy season droughts (Zeppel *et al*., 2015). Maintaining functioning photosynthetic material to take

advantage of subsequent rain events would confer a competitive advantage for species that are frequently exposed to short-term droughts throughout the growing season. All the grass species in this study experience an extended and intense drought every year (during the dry season). However, some grass species in arid environments with variable rainfall may also experience mid-growing season droughts, which also function as a selective force.

Safety-Efficiency trade off

Grasses display a diversity of water use strategies (Tucker *et al.*, 2011; Craine *et al*., 2013; Ocheltree *et al.*, 2016), but it is not clear whether the safety-efficiency trade-offs observed in trees are apparent in grasses. The safety-efficiency trade-off is an important axis of plant strategy variation which can be seen as a selection response for drought strategies (Ackerley., 2004). The safety-efficiency trade-off suggests that during a drought, plants either maintain high rates of hydraulic conductance that supports continued growth but at a risk of hydraulic failure via cavitation or hydraulic conductance is reduced (the rates at which water flows inside plants) at a cost to growth but providing resistance to cavitation (Manzoni *et al*., 2013).

Stomatal function is constrained by a safety-efficiency trade-off, such that woody species with greater stomatal conductance under high water availability have greater sensitivity to closure. This trade-off is exemplified by experiments on leaves of Californian woody species that describe that the stomata is the first defence against drought and that the trade-off constrains the rates of water use and the drought sensitivity of leaves (Henry *et al.*, 2019). González de Andrés *et al*., (2021) demonstrated that the capacity of trees to withstand and recover from drought depends on the ability to transport water to leaves to maintain photosynthetic activity during and after drought.

Physiological drought tolerance and drought strategies

Plant functional traits are measurable and inheritable characteristics of plants which can be physiological, morphological, anatomical, and structural which can be used to predict and understand plant composition and success (González de Andrés *et al*., 2021). Plant functional traits related to water can drive plant performance during a drought. Quantifying the physiological traits that link with drought strategies and environmental constraints, such as the leaf water potentials at stomatal closure, wilting and hydraulic failure is essential in improving predictions in the changes of species' distributions and community composition (Bartlett *et al*., 2014). Some of the key physiological traits are estimated from the relationship between the leaf water potential and leaf water volume, known as the pressure–volume (PV curve) i.e., Turgor loss point, osmotic adjustment (Bartlett *et al*., 2014). However, little is known about the extent of traits related to drought strategies across C_4 grass species and ecosystems such as savannas and grasslands.

Classic theory on drought tolerance focuses mostly on the physiological responses during the drought, it is important to look at the whole plant life history. Particularly characteristics that affect recovery after the drought in order for us to better understand what syndromes of plant traits are effective in different environments (Choat *et al*., 2018). Plants with different drought strategies will be differentially affected by drought (McDowell *et al*., 2008). "Efficient" species have low stomatal control, which allows for continued gas exchange during drought and a quick response to pulses of moisture, but (unless they have access to stored water or deep rooting systems) this comes at the expense of greater tissue dehydration (Brodribb *et al*., 2014). During an extended drought, these species are likely to lose above-ground biomass through hydraulic failure and will need to resprout new leaves to recover when

the drought breaks. “Safe” species tightly regulate their water status within a narrow range through high stomatal control and are therefore less likely to lose above-ground biomass.

Understanding the traits that enable perennial grasses to survive and grow during periods of drought and recovery is also important in grass system management, especially in water-limiting environments. Grasses are particularly interesting in this regard because they do not have any perennial above-ground structures like tree trunks that they need to protect from cavitation which provides capacity for resprouting and recovering from above-ground damage (Chai *et al*., 2010, Zeppel *et al*., 2015). This capacity might affect the degree to which recovery from drought is achieved either by rehydration of existing biomass or resprouting of new biomass from basal bud banks (Zeppel *et al*., 2015).

Plant life histories and Plant syndromes

Plant life histories are shaped by multiple trade-offs due to the requirement to allocate scarce resources across a range of plant functions (Sankaran, 2019). These are usually constrained in terms of the plant functional traits that make up an effective life history, for instance it is not possible to have high investment in growth while also investing in reproduction (Hoover *et al*., 2019). A useful aspect of functional ecology is identifying trait combinations (syndromes) competitive in multiple environmental contexts and in response to different forms of stress and disturbance (Sankaran, 2019).

Trait syndromes, such as drought response strategies, arise from trait trade-offs (Hoover *et al*., 2019). Trait syndromes can be classified based on photosynthetic pathways, growth forms, morphology, phenology, and life history e.g., evergreen plants have syndrome of traits such as tough leaves, low surface leaf area (SLA) (thick leaves), low water content, and high C:N ratio (low nitrogen), because to have an evergreen leaf requires certain carbon investment whilst deciduous plants have a syndrome of traits such as fragile leaves, high SLA, high water

content, and low C:N, because they need to maximise C-uptake over the short period of time they are functional (Pringle *et al*., 2011). These syndromes have also been related to disturbances such as herbivory or fire (Archibald *et al*., 2019). In the case of drought plants trait syndromes can be classified by stomatal conductance, water potential, soil moisture content, curing rate and osmotic adjustment, e.g., in terms of the safety vs efficiency trade of the traits for safe species are high stomatal conductance and low water potential, resulting in lower curing rate.

Chapter two assessed the response of thirteen tropical C_4 grass species to an eight-week drought event by measuring stomatal conductance, water potential, chlorophyll content, number of dead leaves, number of live leaves, number of resprouted shoots and relative water content. These measurements enabled quantification of species drought tolerance and recovery. However, functional traits are part of broader plant strategies and therefore do not act independently. To identify grass species drought strategies, traits that provide information on the physiological responses of the grasses to drought and rewatering were identified. These were based on their physiological drought response traits measured in chapter two. Chapter three, I assess whether grasses with different traits values reflect different strategies in drought by examining five key drought response traits: stomatal sensitivity, the degree of osmotic adjustment (OA), curing rate and recovery after the drought (resprouted and rehydrated):

Understanding plant adaptations to drought involves not only assessing their performance during drought, but also the degree to which they recover and the mechanism by which they do so once water is no longer limiting.

Aim

The aim of this chapter is to assess whether C_4 grass drought responses and recovery traits are correlated and identify plants with similar drought strategies.

Objectives

a. Test for expected relationships between

    1. Stomatal sensitivity, curing rates and osmotic adjustment

    2. Recovery-resprouting and rehydrating

    3. Curing rates, osmotic adjustment, and recovery

b. Group species according to drought traits.

c. Identify the different drought strategies represented in the data collected.

3.2 Methods

In the previous chapter five traits that characterise the drought and recovery responses of tropical grasses were identified and quantified Table 3.1.

Table 3. 1 Summary of traits explored for plant drought performance.

Trait	Abbreviation	Definition	Function
Stomatal sensitivity	Sc Slope	The rate at which stomata close in response to reduced water potential(mmol/m^2/s^{-1}/MPa)	The relationship between stomatal conductance and Water Potential. A steep slope indicates a focus on safety, while a shallow slope indicates efficiency.
Curing rate	Curing slope	The accumulated number of dead leaves as the soil moisture declines (dead leaves/day)	It indicates the degree of leaf senescence ('top kill') in response to water stress. Plants which have a steep slope have more leaf senescence, and fewer live leaves at the end of the drought.
P**roportion of rehydrated leaves**	PropLive	The proportion of all live leaves that rehydrated and leaves that did not cure at the end of an 8-day recovery $1 - \frac{resprouted}{resprouted + live\ leaves}$ Species average is the median of 7 individuals	The degree to which plants can recover by rehydrating existing leaf material .
Proportion of resprouted leaves	PropRes	The proportion of all live leaves that had resprouted at the end of an 8-day recovery $\frac{resprouted}{resprouted + live\ leaves}$ Species average is the median of 7 individuals.	It indicates the degree to which plants can recover by resprouting to replace lost biomass
Osmotic adjustment	OA	The difference between osmotic potential at full turgor (y intercept of the pressure volume curve) for a hydrated vs a drought stressed leaf.	It indicates functional adjustment to maintain turgid leaves. plants that are efficient adjust more. A larger value indicates more osmotic adjustment and due to net solute accumulation in response to water stress.

3.3 Statistical Analysis

To assess for expected relationships between the variables (curing rate, stomatal sensitivity, osmotic adjustment, and recovery) and the test hypotheses (Table 3.2) (objective a). Linear models were ran. All models met the assumptions.

Table 3. 2: Table of hypotheses linked to analysis

Variables	Hypothesis
Curing rate and stomatal sensitivity	H1. Grass species with high stomatal sensitivity cure slower than those with lower sensitivity.
Osmotic adjustment and Curing rate	H2. Grass species that osmotically adjust cure more slowly than grass species that do not adjust.
Stomatal sensitivity and Osmotic adjustment	H3. Grass species with high stomatal sensitivity do not osmotically adjust.
Recovery and curing rate	H4. Species that retain living leaf material and rehydrate at the end of the drought have a less pronounced resprouting response than species that cure and discard above-ground biomass in response to drought.
Recovery and Stomatal sensitivity	H5. Grasses with high stomatal sensitivity cure slowly and rehydrate their leaves whilst those with low stomatal sensitivity resprout, since their above-ground biomass will have senesced.
Recovery and Osmotic adjustment	H6. Species that osmotically adjust recover from drought by rehydrating.

A linear regression between the mean proportion of rehydrated live leaves against the mean proportion of resprouted leaves for each species on the last day of the recovery. To

determine whether species that retained a lot of living biomass at the end of the drought had less of a resprouting response than those that lost more above-ground biomass. The proportion was calculated over all leaves (including cured leaves) and therefore does not sum to 1.

To look at which traits were correlated with each other and identify drought strategies (objective b) a Principal Components Analysis was performed on all trait data collected during the drought: curing rate, osmotic adjustment, recovery (median of proportion resprouted and median of the proportion rehydrated leaves) and the slope of water potential and stomatal conductance with decreasing soil moisture (stomatal closure) which will be referred to as "stomatal sensitivity". It was confirmed that all the variables were not correlated, and data was normalised and centred.

To identify the different drought strategies represented in the data collected (Objective c) the data collected in chapter two was used to code a decision tree to determine whether the expected strategies are represented in the data collected. A decision tree based on the expectations of the important trade-offs and correlated traits discussed above was created. Species were into categories based on 1. stomatal sensitivity, 2. osmotic adjustment, 3. curing rates, and 4. recovery response in that order, and set up expectations for the possible range of drought strategies present in grasses (some of these combinations should not be possible). Then each species was based on whether they were above or below the median value. The decision tree was constructed by taking each species-level trait, ordering the species according to their values from low to high and dividing them according to their median as "high" or "low."

3.4 Results

Relationship between curing rate, Osmotic adjustment, and days to stomatal closure

There was no statistically significant difference that grass species that closed their stomata earlier in the drought cured slower than those that closed their stomata later in the drought, as demonstrated by a linear model of the relationship between days to stomatal closure and curing rate (Figure 3.1 a, Multiple R-squared = 0.09, Adjusted R-squared = -0.01, F = 0.89, p = 0.37). There was also no evidence that grass species that osmotically adjust closed their stomata later, as shown by the linear model of the relationship between logged osmotic adjustment and stomatal conductance (Figure 3.1 b, RSE = 0.01264, Multiple R-squared = 0.09, Adjusted R-squared= -0.01, F= 0.89, p = 0.37). Additionally, no evidence that grasses that osmotically adjust will cure less was found, as shown by the linear model of the relationship between the logged osmotic adjustment and curing rate (Figure 3.1 c, RSE= 1.1, Multiple R-squared= 0.1936, Adjusted R-squared= 0.104, F = 2.16, p = 0.18), although plants with high stomatal sensitivity were never found to osmotically adjust (Figure 3.1c).

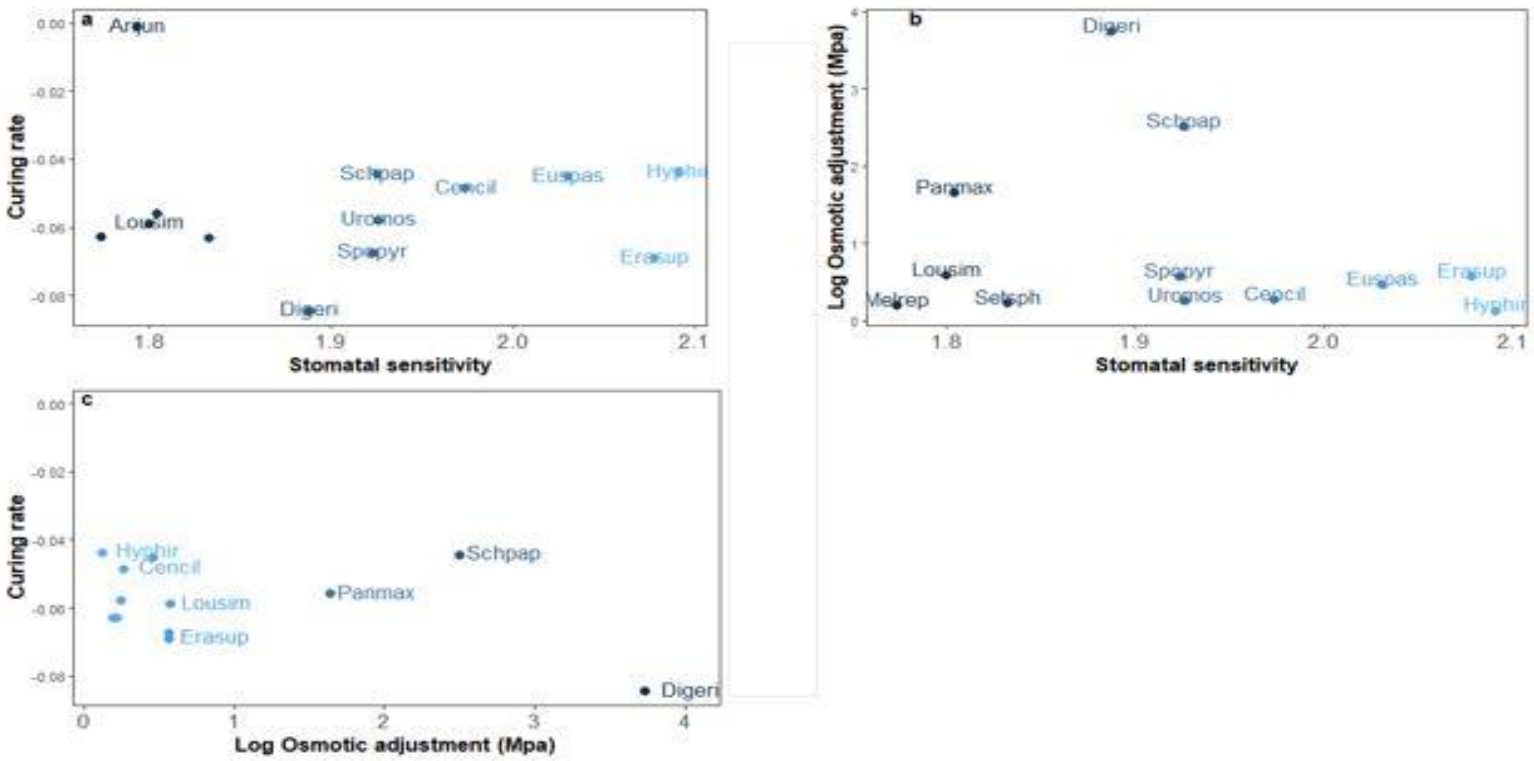


Figure 3. 1: a) Relationship between stomatal sensitivity and curing rate values of the droughted plants throughout the experimental drought. b) Relationship between logged osmotic adjustment and stomatal sensitivity. c) Relationship between curing rate and osmotic adjustment of the droughted plants throughout the experimental drought. Points represent the mean value across all 13 individuals of each droughted species on each day (For full species name see Table 2.1). Curing rate: high value means low curing, Stomatal sensitivity: high value means high stomatal sensitivity, Osmotic adjustment: High value means high osmotic *adjustment*.

Do curing rates, osmotic adjustment and days to stomatal closure influence the method of recovery?

The relationship between the mean proportion of resprouted leaves and rehydrated leaves was marginally significant (Figure 3.2, RSE= 0.09, Multiple R-squared= 0.23, Adjusted R-squared = 0.16, F = 3.34, p = 0.09). Of all 13 grass species, only one (*Setaria sphacelata*) did not rehydrate or resprout vigorously, and therefore would be considered not tolerant of drought (although these individuals did not die). The other 12 species all showed some ability to recover from drought. The species which resprouted more than they rehydrated were *Panicum maximum, Melinis repens, Urochloa mosambicensis and Eragrostis superba* and those that rehydrated more than they resprouted were *Eustachys paspaloides, Loudetia simplex and Sporobolus pyramidalis*. Species that can do both: *Cenchrus ciliaris, Schmidtia pappophoroides, Digitaria eriantha, Hyparrhenia hirta and Aristida junciformis)* can also be seen.

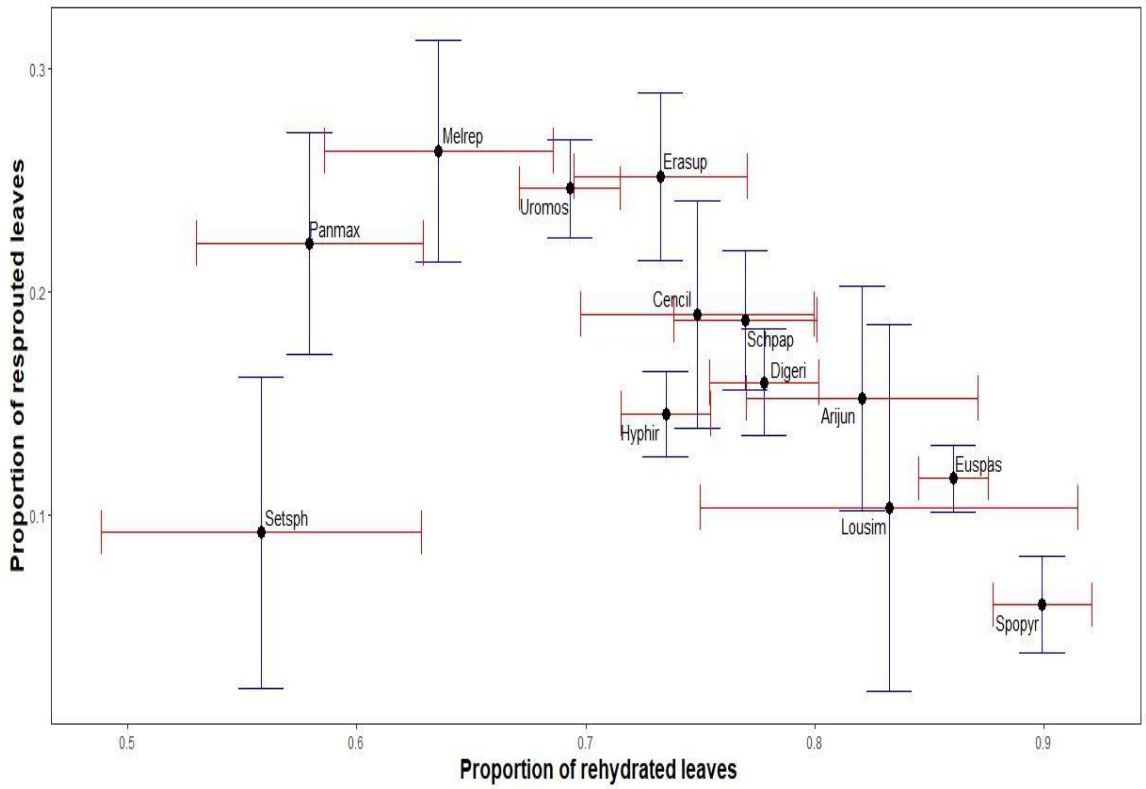


Figure 3. 2: Scatter plot between proportion of above ground biomass that consists of rehydrated leaves vs resprouted leaves of the recovered plants at the end of the rehydration period. Points represent the mean value across all 13 individuals of each recovered species, red error bars represent the standard error for the proportion of rehydrated leaves and the blue error bars represent the standard error for the proportion of resprouted leaves. The proportion was calculated over all leaves (including cured leaves) and therefore does not sum to 1 (For full species names see Table 2.1).

Although we expected plants that cured quickly and fully to have more resprouting leaves than those that kept their leaves alive during the drought, we did not find this (Figure 3.5, RSE= 0.07, Multiple R-squared= 0.007, Adjusted R-squared = -0.10, F = 0.07, p = 0.8). There was no significant statistical evidence that species with low osmotic adjustment, resprouted more than species that had a higher osmotic adjustment (Figure 3.3 b, RSE= 0.07, Multiple R-squared= 0.02, Adjusted R-squared = -0.09, F = 0.14, p = 0.7). Lastly there was no statistical evidence that species that closed their stomata earlier recovered more by rehydrating than resprouting (Figure 3.3 c, Multiple R-squared= 0.007, Adjusted R-squared= -0.1, F= 0.06, p = 0.81)

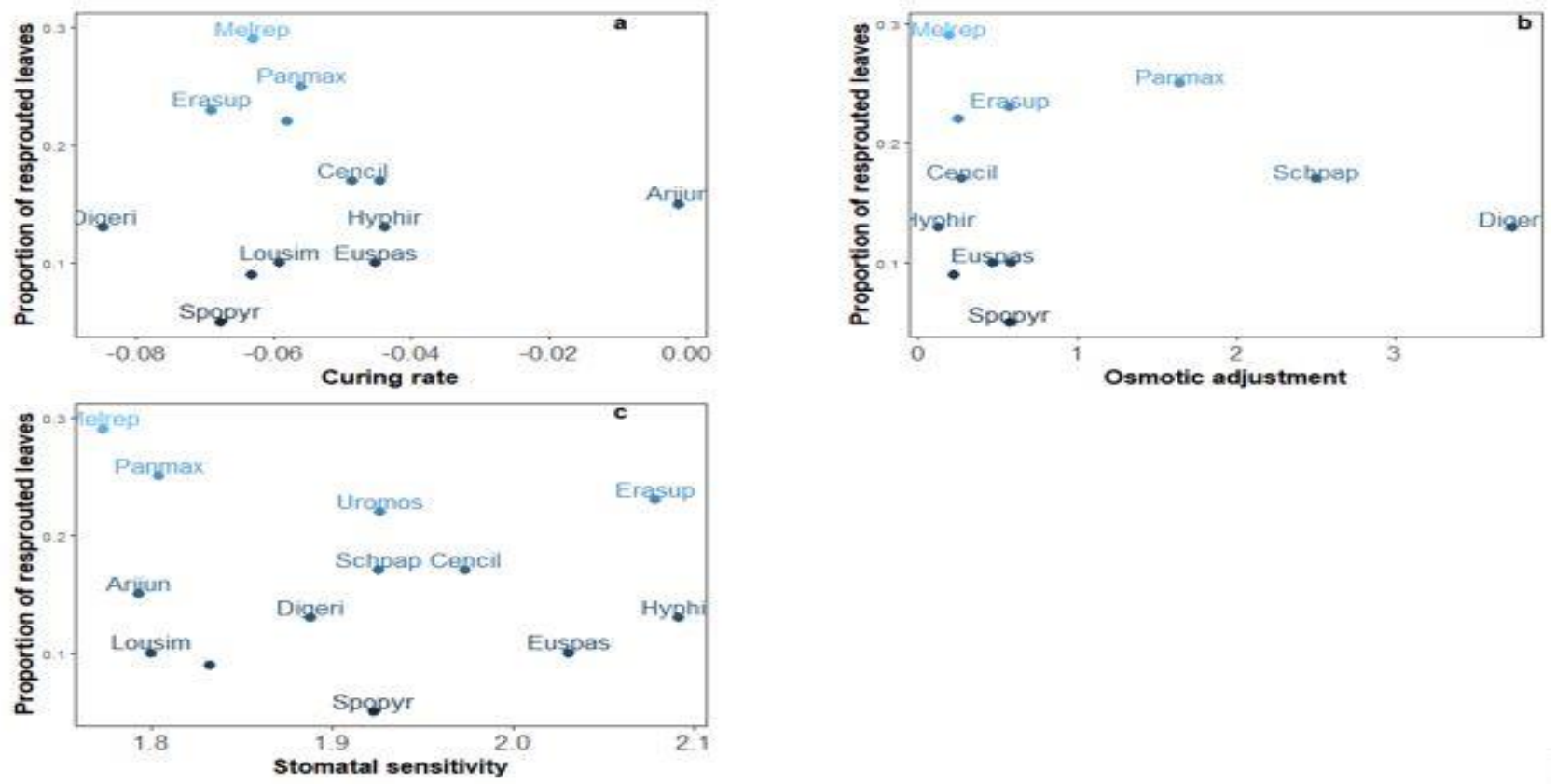


Figure 3. 3: Relationship between a) curing rate as soil moisture decreases, b) osmotic adjustment, and c) days to stomatal closure and the median proportion of resprouted leaves of the recovered plants throughout the experimental drought. Points represent the mean value across all 13 individuals of each droughted species on each day (For full species name see Table 2.1). Curing rate: high value means low curing, Stomatal sensitivity: high value means high stomatal sensitivity, Osmotic adjustment: High value means high osmotic adjustment.

Grouping species according to drought traits

Even though the individual biplots seldom showed the expected relationships the principal components analysis pulled out two important axes of variation. The first axis of principal components accounted for 41 % of the variance, the second axis accounted for 32 % of the variance in the data and adding the third axis accounts for 89 % of the accumulated variance. Axis PC1 is a gradient from resprouting and rehydrating. Axis PC2 is a gradient from high osmotic adjustment and a high curing rate to a low osmotic adjustment, high stomatal sensitivity, and low curing. Stomatal sensitivity can be better explained by PC3 (see appendix).

There are two distinct strategies. The first being a drought strategy with high stomatal sensitivity, low osmotic adjustment, and low curing rates, this is indicated by species such as *Eustachys paspaloides, Hyparrhenia hirta, Eragrostis superba, Urochloa mosambicensis* and *Cenchrus ciliaris*. The second strategy is having low stomatal sensitivity, high osmotic adjustment, and high curing rates seen in species such as *Digitaria eriantha, Schmidtia pappophoroides, Loudetia simplex* and *Sporobolus pyramidalis*. However how these species recover is differs, some species resprout e.g., *Melinis repens, Panicum maximum, Eragrostis superba,* and *Urochloa mosambicensis* whilst other rehydrate e.g., *Setaria sphacelata, Loudetia simplex* and *Sporobolus pyramidalis*. Then we have Intermediary species such as *Schmidtia pappophoroides* (Figure 3.4).

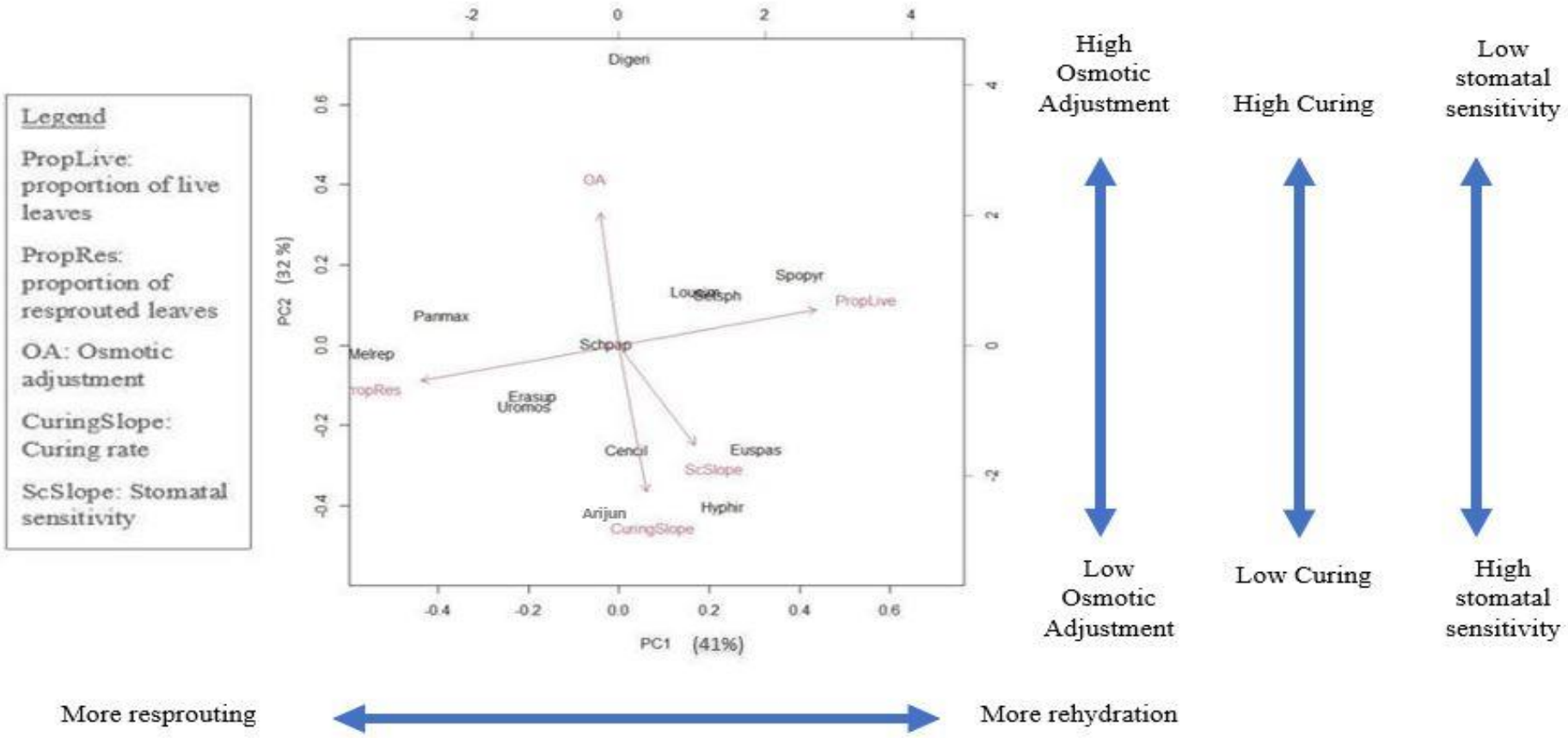


Figure 3. 1: Biplot showing results of a PCA for drought strategy traits. Drought traits are plotted on the first two PCs of the trait data collected. The relative magnitude and direction of trait correlations with the PCs are shown with red arrows and all 13 recovered species on each day (For full species name see Table 2.1) (For drought strategy traits see legend box and for definitions see table 3.1). Curing slope: high (-) value means low curing, ScSlope: high value means high stomatal sensitivity, Osmotic adjustment: high value means high osmotic adjustment.

Are the expected strategies represented in the data collected?

Traits that play a role in physiological drought response, recovery and plant performance were used to categorise the drought strategies of C_4 grasses using data collected on the experiment done in chapter two (Figure 3.5). Groupings were interpreted in terms of the safety-efficiency trade off, but with recognition that there are several ways to achieve the same outcome (safe vs efficient). Table 3.3 is a summary of the drought strategies each species uses. Species such as *Aristida junciformis* (Safe 1), which has high stomatal sensitivity and a low curing rate and *Digitaria eriantha* (Efficient 4) which had a low stomatal sensitivity, high curing and high osmotic adjustment are on the extreme ends of the safety-efficiency continuum, whilst the rest of the species were placed somewhere in between.

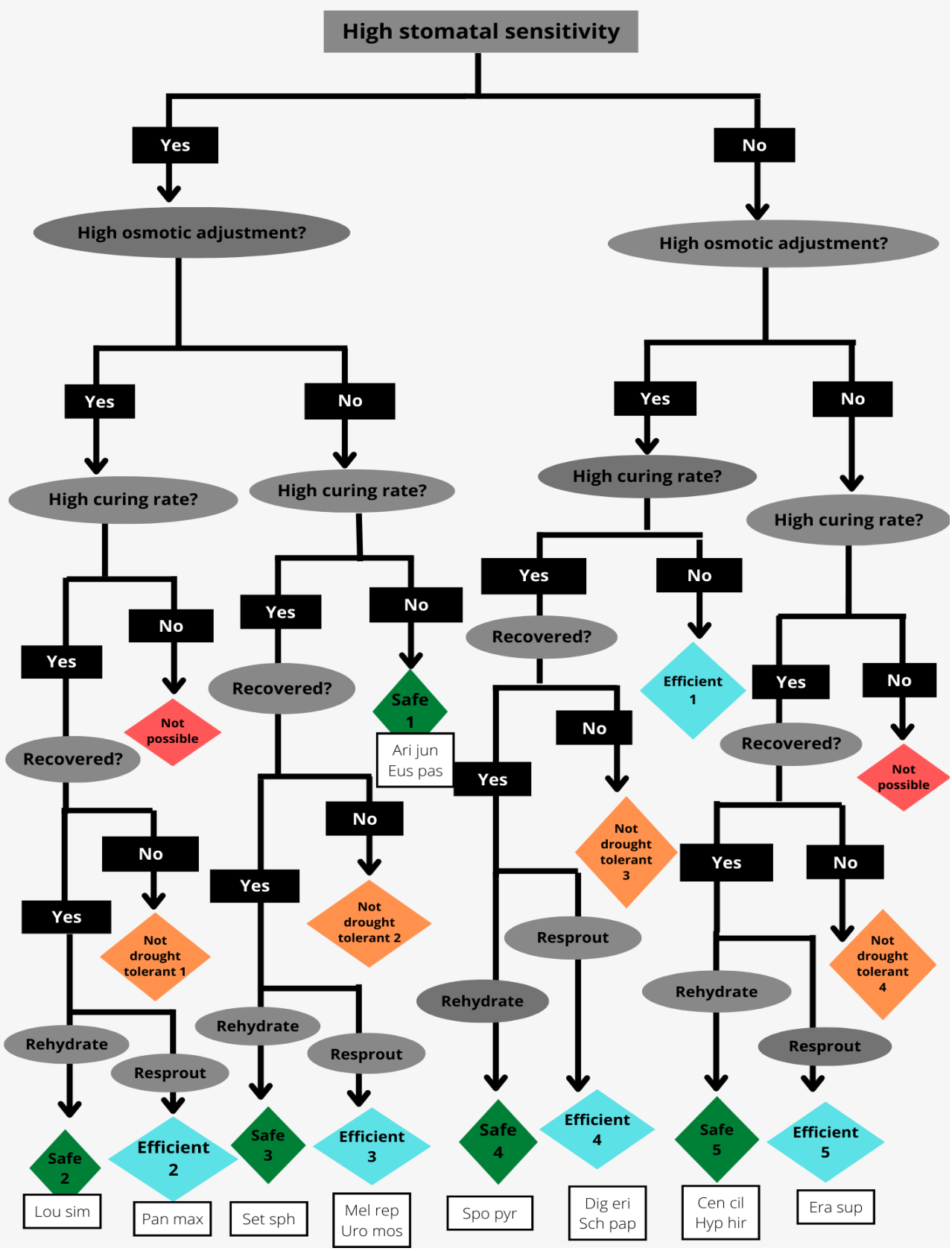


Figure 3. 5: Conceptual model for grouping species on where they lie on the safety Vs efficiency continuum.

Table 3. 3: A summary of drought strategy classification of drought species summary.

Drought strategy	Traits	Species
Safe 1	High stomatal sensitivity, no osmotic adjustment, low curing rate and rehydrators	*Aristida junciformis* *Eustachys paspaloides*
Safe 2	High stomatal sensitivity, osmotic adjustment, high curing rate and rehydrators	*Loudetia simplex*
Safe 3	High stomatal sensitivity, high curing rate, no osmotic adjustment and rehydrators	*Setaria sphacelata*
Safe 4	Low stomatal sensitivity, osmotic adjustment, high curing rate and rehydrators	*Sporobolus pyramidalis*
Safe 5	Low stomatal sensitivity, no osmotic adjustment, low curing rate and rehydrators	*Cenchrus ciliaris* *Hyparrhenia hirta*
Efficient 1	Low stomatal sensitivity, osmotic adjustment, low curing rate and resprouters	No example for this in my species choice
Efficient 2	High stomatal sensitivity, high curing rate, osmotic adjustment and resprouters	*Panicum maximum*
Efficient 3	High stomatal sensitivity, high curing rate, no osmotic adjustment and resprouters	*Melinis repens* *Urochloa mosambicensis*
Efficient 4	Low stomatal sensitivity, osmotic adjustment, high curing rate and resprouters	*Digitaria eriantha* *Schmidtia pappophoroides*
Efficient 5	Low stomatal sensitivity, no osmotic adjustment, high curing rate and resprouters	*Eragrostis superba*

3.6 Discussion

Examining how the five key traits (stomatal sensitivity, osmotic adjustment, curing rate and recovery-resprouting and recovery) for physiological drought tolerance varied with one another indicated that there was a trade-off between growth or photosynthetic rate and the ability to deal with stress that each species made when they were experiencing drought (Fig 3.4).

A relationship between stomatal sensitivity and curing rate was observed. There are grass species that had a higher stomatal sensitivity (closed their stomata earlier in the drought) and cured slower such as *Hyparrhenia hirta, Eustachys paspaloides and Cenchrus ciliaris*, this was an example of a species employing a "safe" strategy. Closing stomata earlier during the drought facilitates the retention of soil moisture through sparing water use, thus allowing the plant to maintain high turgor pressure. Some species kept their stomata open (low stomatal sensitivity) and had higher curing rates such as *Digitaria eriantha*, *Eragrostis Superba* and *Sporobolus pyramidalis*.

Osmotic adjustment adds complexity to this safety-efficiency trade off by providing plants with an alternative way to maintain high stomatal conductance. When looking at the traits, I found that grass species that had a low stomatal sensitivity (closed their stomata later) also osmotically adjusted e.g., *Digitaria eriantha*. Osmotic adjustment allowed them to remain turgid at more negative water potentials and maintained stomatal conductance and photosynthetic rates during drought (Sanders and Arndt, 2015). However osmotic adjustment is costly, due to metabolic constraints and plants may instead depend more on other morphological and physiological traits to survive drought, such as root water use efficiency (Bartlett *et al.*, 2014). Surprisingly osmotic adjustment is not only found in species with low stomatal sensitivity and high curing rates (efficient strategy). Species such as *Panicum*

maximum and *Melinis repens* which closed their stomata early were also able to osmotically adjust, this indicates that the traits function together to form a syndrome and a continuum for the safety-efficiency trade off.

Another big trade off that was found was, how grass species recover. It was noticeably clear from Fig 3. 2 as well as Fig 3.4 that grasses either rehydrate or resprout. This trade-off is because resprouting requires stored reserves, and new leaves might be shaded by the old dead ones. Species that retain living leaf material and rehydrate at the end of the drought have a less pronounced resprouting response than species that cure and discard above-ground biomass in response to drought. Therefore, overall, because old leaves that rehydrated were as healthy, or more healthy than new leaves (Fig 2.12) rehydrating could be a better option. Therefore, the fact that many species opted for the resprout option implies that there could be a serious negative impact of having a safe strategy during the drought (lower photosynthesis).

These findings imply that the range of drought strategies shown by these grasses affects their recovery process after drought. Drought recovery is dependent on the recovery of existing leaf tissue (rehydration) and the regeneration of new tissue from crowns, stolons, and rhizomes of grass plants (resprouting) (Chai *et al.*, 2010). In grasses, rehydrating might have more benefits as compared to resprouting because resprouting requires stored reserves, and new leaves might be shaded by the old dead ones. Also, there is no evidence that the resprouting leaves are healthier than the rehydrated leaves (in fact the opposite). However, some of the species that rehydrate are also able to resprout.

Another interesting relationship was between recovery and osmotic adjustment. It was expected that species that osmotically adjust recover from drought by resprouting because species that retain living leaf material and rehydrate at the end of the drought have a less pronounced resprouting response than species that cure and discard above-ground biomass in

response to drought (Zeppel *et al*., 2015). However, this was not the case as osmotic adjustment was possible in both rehydrators and resprouters.

Species such as *Eustachys paspaloides* and *Aristida junciformis* had a low curing rate and closed stomata early (high stomatal sensitivity) recovered by rehydration after the drought (Fig 3.3 and Fig 3.4). This was expected, because this safe strategy would have prevented cavitation and allowed the leaves to remain functional. In contrast species such as *Hyparrhenia hirta, Cenchrus ciliaris* and *Schmidtia pappophoroides* which took a long time to close their stomata ended up losing more above ground biomass and had to resprout after the drought (Fig 3.3 and Fig 3.4). As they did not prevent water loss, as the drought continued these plants were unable to maintain their full canopy, and thus photosynthetic tissue needed to be replaced once the drought ended. However, some species with high curing rate were able to resprout only because they osmotically adjusted such as *Panicum maximum* and *Digitaria eriantha.*

The type of drought strategy might also provide insight into the types of droughts that varied species usually experience. A safe strategy would be most appropriate when the droughts are of short duration because the moment the drought breaks, the living leaves are able to continue photosynthesising. An efficient strategy would be most appropriate when the droughts are of a longer duration - several months or years. In this case, maintaining a living above-ground biomass is impossible, so the most effective strategy is to use up all available water and then senesce until the rains come back (Fig 3.6).

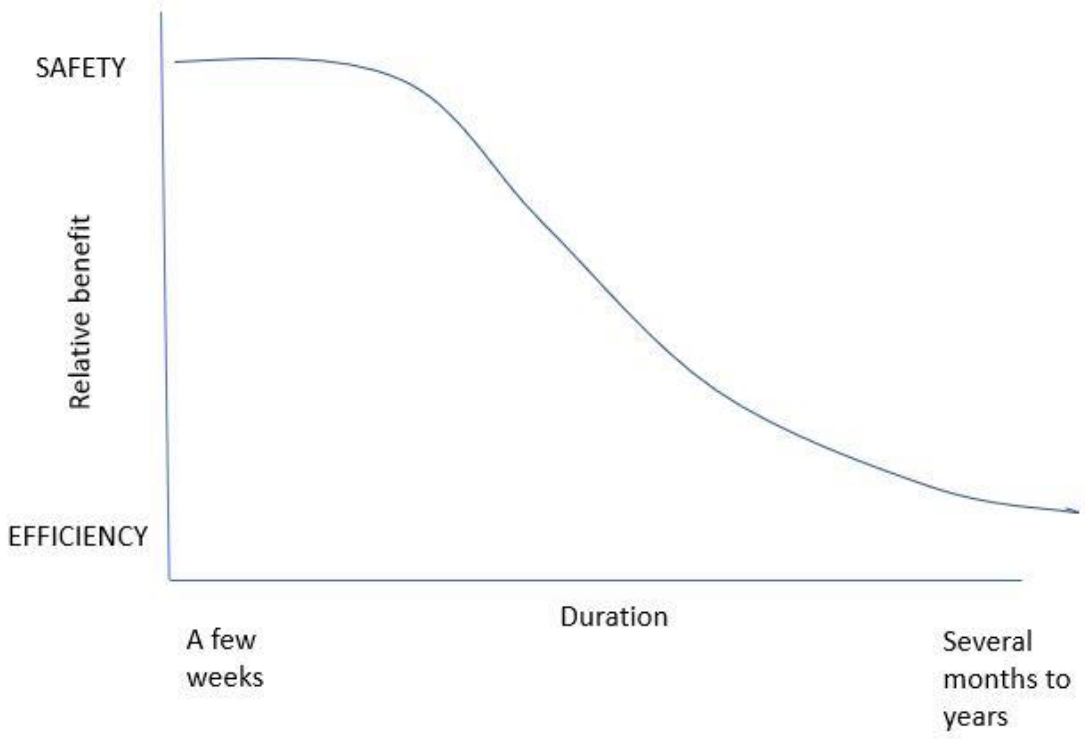


Figure 3. 2: Conceptual model of the relationship between the type of drought strategy that is most effective and the type/duration of drought a plant experiences.

Five key physiological drought tolerance traits (stomatal sensitivity, osmotic adjustment, curing rate and recovery (resprouting/rehydrating)) were used to construct a decision tree to work out where species lie on the safety-efficiency trade-off. The safety-efficiency trade off forms a continuum along which is structured by the physiological traits. An efficient strategy is correlated with resprouting and a safe strategy with rehydrating. Where a species lies on the continuum is based on their stomatal sensitivity (how quickly they close their stomata). Species that have a low stomatal sensitivity can survive by osmotically adjusting but from the PCA (Fig 3.4). Species that have a high stomatal sensitivity are also able to osmotically adjust and this can influence whether they have high or low curing rates.

This study has added to the work that Tucker *et al*., (2011) and Ocheltree *et al*., (2016;) have done on physiological drought tolerance, by including a novel index for stomatal

sensitivity as well as looking at how these grass species recover. Osmotic adjustment is also a trait that previously has not been studied when it comes to the concept of physiological drought tolerance. However, what separates this study from previous work is how it looks at drought traits as syndromes and not only as single traits or bivariate relationships. The bivariate analysis between traits did not have significant relationships, and this is because these traits work together to explain the safety-efficiency trade-offs. I expected to find correlations between these traits, and groups of species showing similar combinations of traits. It was clear that these correlations were there, and when looking at these traits together they built a clear syndrome for safety-efficiency trade- offs.

To conclude, when plants close their stomata due to decreasing water potential it results in a decline of stomatal conductance, and reduced photosynthesis however this means species will retain their live leaf materials (i.e., be safe), and be ready to photosynthesise faster than plants that senesce and need to resprout. In contrast species that keep their stomata open and optimise on photosynthesis (i.e., be efficient) let leaves die. Whether a species is safe or efficient can be classified by how they recover from drought, resprouters are efficient and rehydrators are safe. Using these drought strategies C_4 grasses use can be predicted using the conceptual model (Fig 3.5). Ten strategies were identified, forming a continuum ranging from safest (Safe 1) to most efficient (Efficient 1) strategy.

CHAPTER 4

Conclusion

Physiological drought tolerance (Stomatal sensitivity) is the ability of a plant to maintain its biomass production during drought conditions using physiological mechanisms that sustain stomatal conductance at low water potentials (Tucker *et al*., 2011). It is measured as the critical leaf water potential at which a plant closes its stomata and loses hydraulic functioning (Tucker *et al*., 2011). Although closing stomata early during drought reduces photosynthetic rates, it also avoids damage to the hydraulic system and allows growth to be reinitiated after drought release (Brodribb, 2009). As discussed in this thesis, the trade-offs that create the safety-efficiency continuum result in plants that are likely to suffer in diverse ways from a drought: safe plants will be carbon-stressed, and efficient plants risk losing above-ground biomass.

The aim of this MSc thesis was to understand the patterns of drought and recovery in African C_4 grasses and the implications for grass ecosystem ecology. African C_4 grasses are perennial and tend to lose their aboveground biomass annually during a ~6- month dry season. This thesis, had the following objectives:

1. To describe and quantify the diversity in functional responses to drought and recovery from drought in thirteen perennial C_4 grass species.
2. To investigate whether photosynthetic sub-type or grass evolutionary history is associated with drought responses.
3. To evaluate whether safety-efficiency trade-offs are present in C_4 grasses and whether drought responses can predict drought recovery responses.
4. To identify different drought strategies in C_4 grasses.

The first chapter reviewed the literature on this topic and elaborated on the reasoning behind my research questions. The second chapter described how different grass species varied in terms of: stomatal sensitivity (physiological drought tolerance), curing rates, osmotic

adjustment and recovery abilities as well as evaluated whether there are any patterns between sub-families, photosynthetic sub-types, or plants from different environments and these drought response traits. The third chapter assessed whether these traits were correlated and aimed to identify plants with similar trait syndromes and drought strategies. This Chapter will give the conclusion to this research.

C_4 grass species have different stomatal sensitivities (reaching the 50 $mmol/m^2. s^{-1}$ threshold at different rates), some species close their stomata faster than others. Grass species also varied in curing rates and osmotic adjustments when experiencing a drought. There was also variation in how they recovered from the drought, species either resprouted or rehydrated, indicating variability in drought responses.

The variability in drought responses of these grass species indicated that the traits (stomatal sensitivity, osmotic adjustment, curing and how they recovered) together to form drought strategies. The PCA showed that there is a trade-off between maintaining photosynthetic activity and maintaining above-ground biomass. Species that had a high stomatal sensitivity had low curing and species with low stomatal sensitivity, had a higher curing rate and were able to maintain functionality because they osmotically adjusted. However, the PCA also showed that there is a trade-off between resprouting and rehydrating leaves.

Recovery is another major functional response to drought. Being safe or efficient is related to resprouting or rehydrating. Efficient species recover by resprouting, and safe species recover by rehydrating. Recovery strategies could be particularly useful in explaining and managing grass community changes in response to droughts of different intensities. To understand the range of drought strategies within grasslands, It is essential to identify the safety-efficiency trade-offs in C_4 grasses. However, previous studies did not associate the safety-efficiency trade-off with information on the recovery patterns of the grasses. When mapped these traits

out on a decision tree it emerges that there are five separate ways a grass can be safe and 5 other ways it can be efficient, because both efficient and safe species can have either high or low stomatal sensitivity, curing rate and osmotic adjustment.

It is difficult to expand the results beyond my study because there were a few significant patterns between clades, photosynthetic types, or plants from different rainfall regimes. This was surprising because previous studies such as Visser *et al*., (2012) and Wigley-Coetsee and Staver (2020) show that the biogeography of the different grass clades differ in response to rainfall (Visser *et al.,* 2012) and that grasses with the NADP-ME photosynthetic types were more affected by an extreme drought (Widley-Coetsee & Staver (2020). There was a marginally significant response of stomatal sensitivity where Chloridoideae tend to close their stomata and conserve water, and this would fit with the findings of Visser *et al* (2012). Possibly if there were more species this result would have been stronger: thirteen species is a lot for a greenhouse experiment, but still provides very few degrees of freedom for the statistical tests. One clear pattern was that plants from dry areas show more osmotic adjustment. It has been shown before that plants that osmotically adjust are found to grow under drier soil moisture conditions (Bartlett *et al.,* 2015). This study is the first to my knowledge to relate osmotic adjustment to recovery patterns and the safety-efficiency trade-off.

This study has focused on drought responses and drought strategies, but these drought strategies are likely also to affect other aspects of plant life-history, such as the likelihood that grass species using an efficient strategy will have a larger/deeper below ground system as compared to safe species allowing them to prolong closing their stomata because they can tap into more water in the soil. Such a system will require a lot more carbon and nutrients to build. It is also likely that the below ground system of the plant is what protects it from carbon starvation.

It is very essential that we consider how resprouting links to other ecological drivers such as fire. Plants that are found in ecosystems that are fire prone tend to have traits such as resprouting that allow them to survive in these areas. Resprouting is also linked to an efficient strategy. Moreover, curing (which occurs when droughted grasses cannot maintain their above-ground leaf area) can affect the flammability of plants because the amount of dead material influences the ignition of fire and increases combustibility. Fires have been burning grasslands for a long time to shape the distribution whilst also maintaining the structure and function of grasslands (Bond and Keeley., 2005). Therefore, there are predictions that the variation in flammability of grasses will be associated with their drought response strategies and curing rates. This will in turn impact grass species distribution and the environmental conditions in which they occur (Simpson *et al*., 2016). Although there was no link sub families and environments clearly in this thesis, I suspect that looking at the flammability traits is very essential.

The variation in flammability of grasses from different clades and different life histories is associated with their biogeographic distribution (Simpson *et al*., 2016). Andropogoneae species which are found in mesic savannas and grasslands that burn frequently, tend to be resprouters and are prone to use an efficient strategy (Simpson *et al*., 2016). In contrast, Chloridoideae are not tolerant, they are mostly found in either arid or mesic grasslands that have low fire intensities have been found to be associated with drought prone environments (Visser *et al.,* 2012). Chloridoideae species have lower curing rates, higher stomatal sensitivity, tend to be rehydrators and are prone to use a safe strategy. Aristidoideae species richness is highest in hot, arid grasslands (Visser *et al.,* 2012). However, they are tolerant of drought but not disturbance which is why they both occur in low fire frequencies and increase in the absence of fire (Ripley *et al*., 2015).

There are also the ideas about how osmotic adjustment and being palatable requires nutrients and how that might make plants that osmotically adjust more attractive to grazers, which would play into the flammability-palatability trade off (Hempson *et al*., 2019) i.e., grass species that osmotically adjust might be less flammable as compared to those that do not. The flammability-palatability trade off could also be related to the C:N ratio, species with more carbon are more flammable as compared to species with more nitrogen. Therefore, a higher C:N ratio could mean more osmotic adjustment (species that osmotically adjust burn more) and this also means they might have traits which allow for resprouting after the fire. Higher leaf nitrogen could result in more stomatal regulation to prevent at the risk of embolism (Safety) which may also decrease carbohydrates reserve to boost growth once water (photosynthetic and stomatal conductance is restored) (González de Andrés *et al.*, 2021).

To conclude, research into drought responses in grasses has yet to integrate understanding of its impact on other aspects of C_4 grass biology. It has been argued above that drought strategies in different C_4 grasses show are likely to impact their curing rates, and therefore, the overall flammability of the environments that they occur in. This is a substantial challenge, and assessing these ideas is crucial to accurately predicting the impact and interaction plants have with their environment. It will also allow us to understand and improve modelling of essential physiological and ecological processes that relate to drought e.g., how fire regimes in natural grasslands might change in the future, as rainfall patterns and grass communities change especially with the change in climate which result in more frequent droughts and fires

For future work results can be improved by adding more species, as well as having an equal and considerable number of different photosynthetic pathways and subfamilies. It is also essential to add measurements of stored reserves to determine whether some species are suffering carbon starvation, look. looking at the below ground biomass, as well as leaf

chemistry is also very essential. An unstressed control treatment would be useful, to take account of normal leaf turnover, and provide a baseline for quantifying senescence due to drought. Also, for future, the recovery monitoring period should be longer, to allow for plants that may take longer to resprout after moisture stress is reduced.

Further exploration of the framework developed in this thesis is needed. It is necessary to consider including the environment these species are found in because a drought strategy that is effective is linked to the type of drought (how long it lasts) found in the system. Safe species tend to be distributed in more drought-prone habitats, they will be exposed to short droughts as compared to efficient species which will be distributed in habitats that do not have frequent droughts, and therefore they will be exposed long term droughts (Cardoso *et al.*, 2015). However, this thesis was able to describe and quantify functional responses to drought and recovery from drought in thirteen perennial C_4 grass species and showed that safety-efficiency trade-offs are present in C_4 grasses, as well as identify different drought strategies in C_4 grasses.

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Appendices

Table: Summary of results for linear mixed effects model between stomatal conductance and water potential (WP) at the threshold 50, with species as a random effect. Pr (>|t|) column represents the p-value associated with the value in the t value column. (For full species name see table 2.1).

	Estimate	Std. Error	t value	Pr(>|t|)
LogWP	0.228765	0.253298	0.903	0.3675
Cencil	0.180535	0.150176	1.202	0.2306
Digeri	0.094628	0.155742	0.608	0.5441
Erasup	0.284763	0.152438	1.868	0.0631.
Euspas	0.237262	0.156571	1.515	0.1312
Hyphir	0.297600	0.150760	1.974	0.0497 *
Lousim	0.007118	0.153422	0.046	0.9630
Melrep	-0.019092	0.156470	-0.122	0.9030
Panmax	0.011523	0.233892	0.049	0.9608
Schpap	0.132385	0.165276	0.801	0.4240
Setsph	0.039741	0.154205	0.258	0.7969
Spopyr	0.130016	0.181242	0.717	0.4739
Uromos	0.133259	0.154197	0.864	0.3884
WPCencil	0.343666	0.324520	1.059	0.2908
WPDigeri	-0.065304	0.289655		
WPErasup	0.270379	0.303062	0.892	0.3733
WPEuspas	0.229277	0.326514	0.702	0.4833
WPHyphir	0.411231	0.314661	1.307	0.1926
WPLousim	-0.054445	0.300462	-0.181	0.8564
WPMelrep	-0.069052	0.312255	-0.221	0.8252
WPPanmax	0.005771	0.365767	0.016	0.9874
WPSchpap	-0.090589	0.362240	-0.250	0.8028
WPSetsph	0.012776	0.292024	0.044	0.9651
WPSpopyr	0.249196	0.424551	0.587	0.5578
WPUromos	0.040364	0.306773	0.132	0.8954

Table: Summary of results for linear mixed effects model between the logged number of dead leaves and soil moisture with species as an interaction. Pr (>|t|) column represents the p-value associated with the value in the t value column. (For full species name see table 2.1).

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-0.481445	0.047064	-10.230	< 2e-16 ***
SoilM	0.029853	0.001762	16.942	< 2e-16 ***
Cencil	-0.638536	0.057915	-11.025	< 2e-16 ***
Digeri	-0.641122	0.058162	-11.023	< 2e-16 ***
Erasup	-0.601159	0.057687	-10.421	< 2e-16 ***
Euspas	-0.690358	0.058117	-11.879	< 2e-16 ***
Lousim	-0.222466	0.064973	-3.424	0.000658 ***
Melrep	-0.725820	0.057880	-12.540	< 2e-16 ***
Panmax	-0.970178	0.056846	-17.067	< 2e-16 ***
Schpap	-0.388714	0.057885	-6.715	4.29e-11 ***
Setsph	-0.793852	0.057881	-13.715	< 2e-16 ***
Spopyr	-0.691011	0.058114	-11.891	< 2e-16 ***
Uromos	-0.415135	0.058115		

Table: Summary of results for linear mixed effects model between mean chlorophyll content and soil moisture (SoilM) with species as a random effect. Pr (>|t|) column represents the p-value associated with the value in the t value column. (For full species name see table 1.1).

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	438.1383	8.6830	50.459	< 2e-16 ***
SoilM	-0.8036	0.5933	-1.354	0.175781
Cencil	-7.6147	11.9126	-0.639	0.522764
Digeri	-18.8927	11.5569	-1.635	0.102275
Erasup	50.0010	11.8979	4.202	2.77e-05 ***
Euspas	-16.3077	11.8736	-1.373	0.169782
Hyphir	-18.3126	11.9856	-1.528	0.126718
Lousim	-43.7060	13.0468	-3.350	0.000825 ***
Melrep	-15.7691	11.6721	-1.351	0.176861
Panmax	-32.3013	11.3862	-2.837	0.004607 **
Schpap	30.9996	11.9435	2.596	0.009521 **
Setsph	-17.6266	11.8439	-1.488	0.136861
Spopyr	-11.4513	11.6542	-0.983	0.325941
Uromos	-45.1229	11.7827	-3.830	0.000133 ***
SoilM: Cencil	1.2089	0.8217	1.471	0.141405
SoilM: Digeri	1.8195	0.8222	2.213	0.027022 *
SoilM: Erasup	-2.5269	0.8634	-2.927	0.003469 **
SoilM: Euspas	0.7701	0.8312	0.927	0.354305
SoilM: Hyphir	1.1381	0.8641	1.317	0.187983
SoilM: Lousim	1.1208	0.8718	1.286	0.198768
SoilM: Melrep	0.3158	0.8351	0.378	0.705348
SoilM: Panmax	1.1224	0.8241	1.362	0.173380
SoilM: Schpap	-1.0242	0.8594	-1.192	0.233475

SoilM: Setsph	1.1992	0.8122	1.477	0.139951
SoilM: Spopyr	0.5250	0.8139	0.645	0.518968
SoilM: Uromos	2.5020	0.8193	3.054	0.002292 **

Table of Lmer between fluorometry and soil moisture

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.689243	0.027644	24.933	< 2e-16 ***
SoilM	0.006144	0.002522	2.436	0.015006 *
Cencil	0.048848	0.032174	1.518	0.129238
Digeri	0.042351	0.031222	1.356	0.175233
Erasup	0.044626	0.031645	1.410	0.158764
Euspas	0.086873	0.032484	2.674	0.007599 **
Hyphir	0.041870	0.032053	1.306	0.191740
Lousim	0.035506	0.037804	0.939	0.347822
Melrep	0.033575	0.031914	1.052	0.293012
Panmax	0.018542	0.031446	0.590	0.555543
Schpap	0.067647	0.031903	2.120	0.034194 *
Setsph	0.039392	0.032081	1.228	0.219759
Spopyr	0.105365	0.031767	3.317	0.000941 ***
Uromos	0.066664	0.031788	2.097	0.036208 *
SoilM: Cencil	-0.003660	0.002899	-1.263	0.206966
SoilM: Digeri	-0.002350	0.002894	-0.812	0.417099
SoilM: Erasup	-0.003799	0.002923	-1.300	0.193974
SoilM: Euspas	-0.005812	0.002913	-1.995	0.046247 *
SoilM: Hyphir	-0.002825	0.002930	-0.964	0.335218
SoilM: Lousim	-0.002716	0.003494	-0.777	0.437135
SoilM: Melrep	-0.000868	0.002920	-0.297	0.766296
SoilM: Panmax	-0.002225	0.002921	-0.762	0.446259
SoilM: Schpap	-0.004927	0.002973	-1.657	0.097742.
SoilM: Setsph	-0.002858	0.002893	-0.988	0.323378
SoilM: Spopyr	-0.007279	0.002921	-2.492	0.012837 *
SoilM: Uromos	-0.004452	0.002851	-1.561	0.118747

Table of t test values for chlorophyll content of old vs new leaf

Species	T value	P value	df	CI
Ari jun	-2.24	0.06*	6.26	386.6±472.5
Cen cil	-1.46	0.17	11.41	-39±7.82
Dig eri	-1.75	0.12	7.02	-104±15.47
Era sup	-0.83	0.42	10.87	-116.8±52.56
Eus pas	-1.93	0.08	10.75	358.14±415.14
Hyp hir	-4.22	0.002*	9.51	365.3±441.3
Lou sim	-1.84	0.15	3.5	327±392
Mel rep	-4.25	0.003*	8.39	355.6±417.14
Pan max	-4.35	0.002*	8.49	322.86±424.14
`Sch pap	-2.97	0.02*	7.04	415.83±481
Set sph	-3.19	0.01*	9.21	352.71±436
Spo pyr	-3.68	0.004*	9.39	365.42±450
Uro mos	-0.78	0.46	7.37	363±389.14

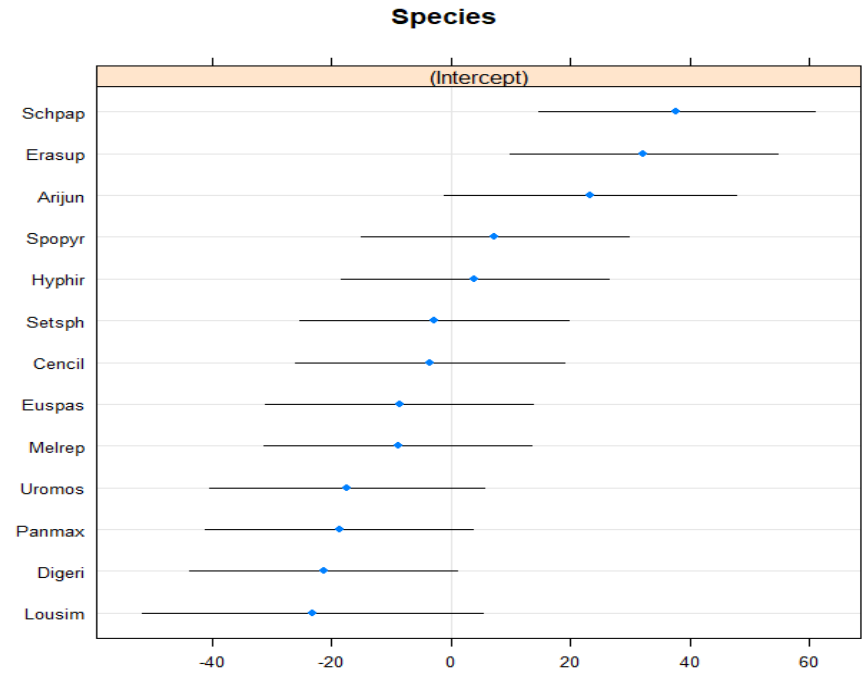


Figure: Random effect extracted from the model, indicating differences between species in the chlorophyll content of grass leaves (For full species name see Table 1.1)

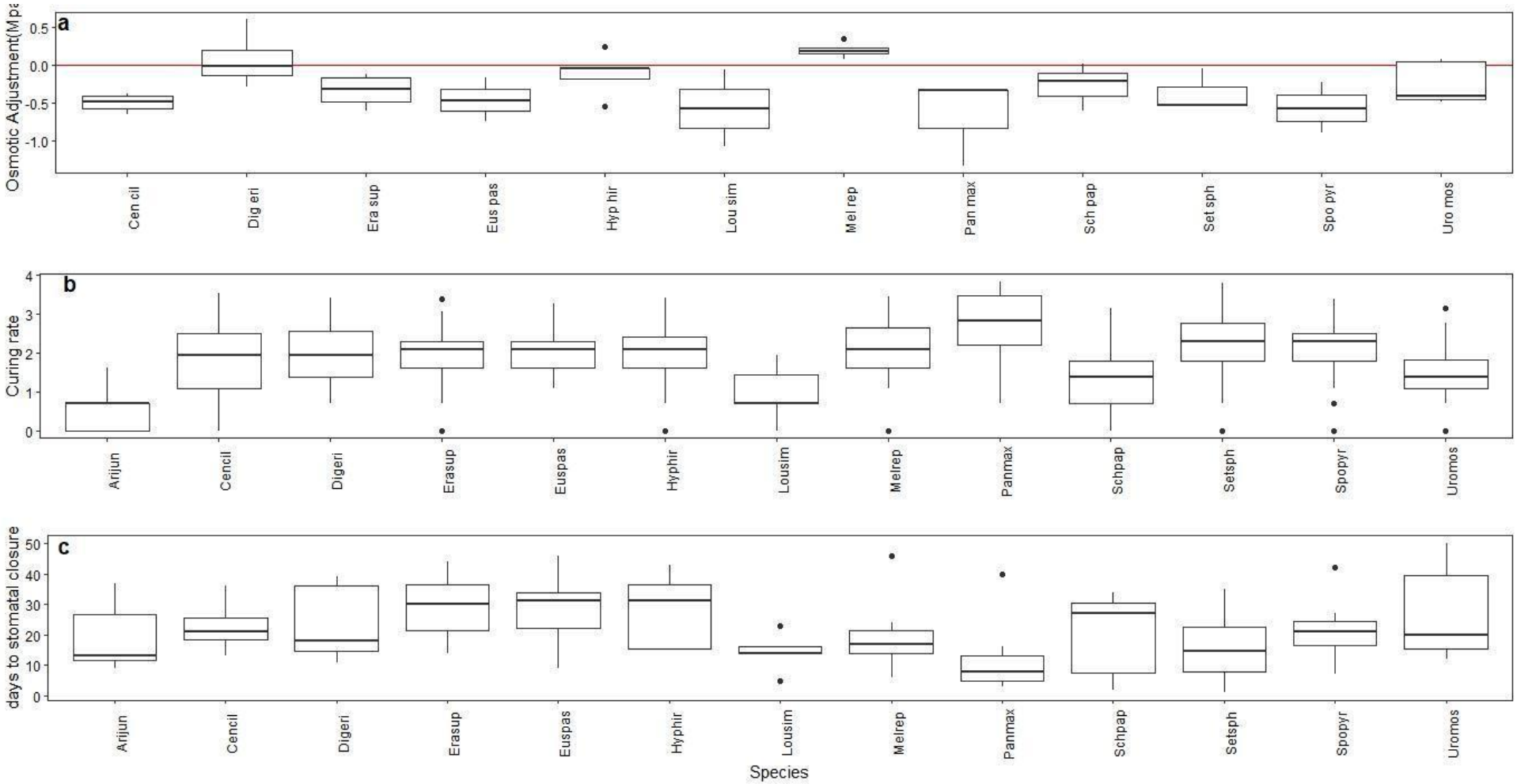


Figure: Boxplot of the osmotic adjustments b) Boxplot of curing rate represented by the curing rate during the drought c) Boxplot of the days it takes grass species to close their stomata of all 13 species. Boxes represent the medians, 25 and 75th confidence intervals and (For full species names represented in the box see Table 2.1)

Table showing the Importance of components in a PCA

	PC1	PC2	PC3	PC4	PC5
Standard deviation	1.437	1.2594	0.8896	0.7466	4.429e-16
Proportion of Variance	0.413	0.3172	0.1583	0.1115	0.000e+00
Cumulative Proportion	0.413	0.7302	0.8885	1.0000	1.000e+00

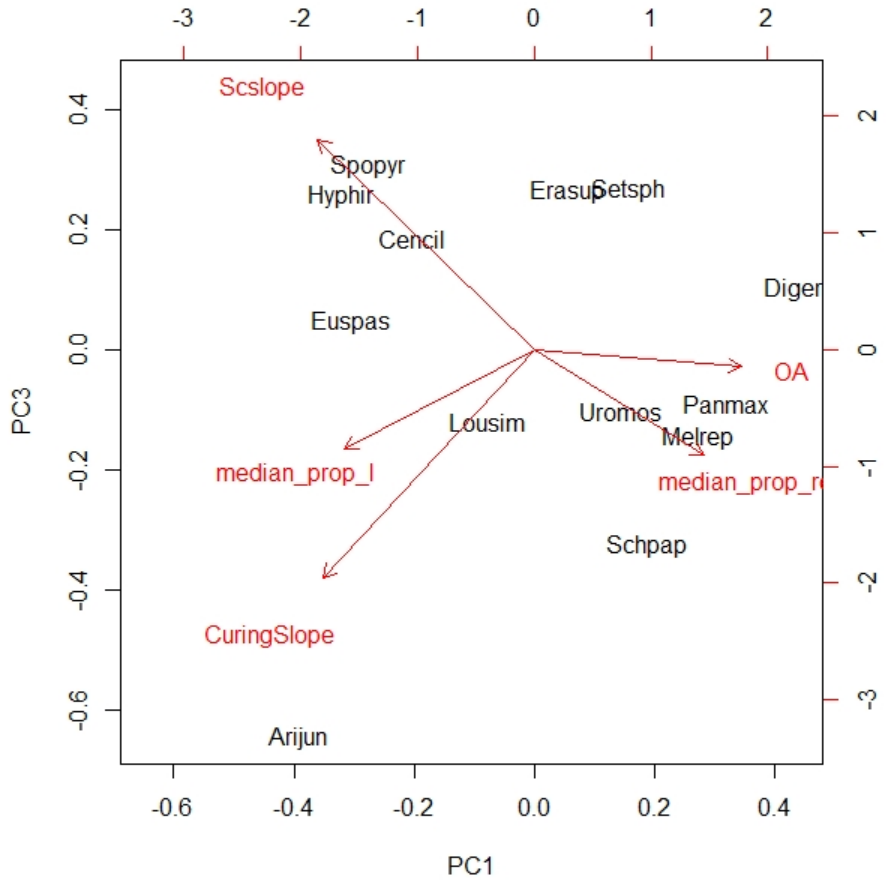


Figure: Biplot showing results of a PCA for drought strategy traits. Drought traits are plotted on the first two PCs of the trait data collected. The relative magnitude and direction of trait correlations with the PCs are shown with red arrows and all 13 recovered species on each day (For full species name see Table 2.1) (For drought strategy traits see legend box and for definitions see table 3.1). Curing slope: high value means faster curing, Scslope: high value means high stomatal sensitivity, Osmotic adjustment: High value means high osmotic adjustment.