

Hydraulic vulnerability of Subtropical Thicket to drought: a remote sensing and physiological perspective.

by

Daniel Buttner

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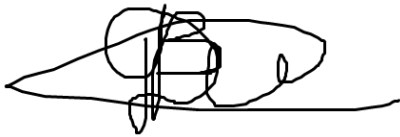
In the Faculty of Science, Botany Department,
Nelson Mandela University

Supervisors: Dr. Alastair Potts, Dr. Robbert Duker, Dr. Robert P. Skelton



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Abstract

Water availability is one of largest constraints on plant survival, growth, and species distribution globally.

The recent escalation in tree mortality coupled with declining precipitation and amplified temperatures has implicated drought as a major cause behind many large-scale dieback events observed across the world. Raising the question, what makes some species more resistant and persist while others dwindle and vanish from the landscape? The observed variability in species drought susceptibility demonstrates the complexity of physiological responses of plants to changes in water availability. Hydraulic dysfunction in plants has been purported to be the key mechanism behind drought-induced mortality provoking interest in hydraulic traits and critical thresholds of xylem physiological function. The capability of species to maintain hydraulic functionality under drought strongly influences the survival and general productivity towards water deficits. Hence, two core objectives, and subsequently aims, of this thesis are firstly to investigate the effect of drought on Subtropical Thicket vegetation health and productivity, and secondly to examine the underpinning physiological mechanisms and functional thresholds relaying species-specific drought vulnerability within this semi-arid biome.

In first data chapter, this thesis offers an assessment of vegetation change under drought and its influence on plant physiological function and productivity across a subsection of Subtropical Thicket distribution. Additionally, this chapter provides a regional scale perspective of drought on Subtropical Thicket flora in the Eastern Cape of South Africa. A severe anomalous dieback event, which coincided with extreme, accumulative drought conditions was observed in 2020. Employing a combination of field-based approaches and remote sensing, this chapter aimed to provide a comprehensive report of the extent and severity of crown defoliation and canopy dieback following this event, additionally describing predisposing and compounding factors. Based on Standardized Precipitation Evapotranspiration and Standardized Precipitation Indices this event began in 2015 and coincided with amplified temperatures, exacerbating evaporative demand. Aerial UAV surveys and field-based investigations were undertaken. Remotely sensed (RS) indices provided an avenue for extensive spatiotemporal investigations to uncover the extent of drought-related impact on vegetation productivity and discuss potential underpinning mechanisms behind drought-induced mortality in Subtropical Thicket. Leveraging long-term time series RS data, Normalized Difference Vegetation Index (NDVI) and Enhanced Vegetation Index (EVI), from Moderate Resolution Imaging Spectroradiometer (MODIS) satellites as a proxy vegetation physiological status in combination with SPI and SPEI describing drought condition and ground-based surveying defining canopy vitality condition this study incidentally addressed the aim of endeavoring to link drought-induced mortality and early warning symptoms observed in the field with RS time series data to describe drought impacts across Subtropical Thicket.

Furthermore, the findings of this chapter demonstrate the spatial and temporal heterogeneity in drought impacts on Subtropical Thicket. The recent drought of 2015 till 2021 is the longest documented period for the past 50 years in which there has been no accumulated net positive water availability, this apparently perpetual state of water deficiency has enacted a high cost for vegetation productivity and growth for the study area, exhibited well by average NDVI values of less than -0.5 for over 70% of the research site. The findings of this chapter report, to the contrary of anecdotal suggestions in the literature, on the intrinsic tolerance of Subtropical Thicket and that

drought has a far more significant role on overall vegetation productivity, growth, and mortality in this region.

The second data chapter assess whole-plant physiological functionality during a drought event in the Eastern Cape, South Africa. This is the first study in Subtropical Thicket to quantify *in situ* hydraulic functional integrity under a natural drought event testing the leading hypothesis describing tree mortality – hydraulic dysfunction. This chapter firstly, investigates hydraulic traits (e.g., turgor loss point) and describe key physiological thresholds (e.g., P_{50}) for maintaining function during drought. Secondly, levels of native embolism were determined under drought conditions and combined with measurements of xylem vulnerability to embolism providing an estimation of degree of hydraulic dysfunction experienced by six dominant woody canopy species in Subtropical Thicket – to quantify interspecific drought tolerance and susceptibility. Subtropical Thicket species exhibited remarkable tolerance towards desiccation (TLP from as low as -3MPa) and hydraulic functional resistance to embolism (P_{50} value as low as -7.89MPa). The observed unexpected drought resilience of subtropical derived lineages highlights the intrinsic evolutionary role aridification had, selecting for greater embolism resistance and community-level conservatism in drought resistance of Subtropical Thicket. The level of native embolism and hydraulic safety margins identified two species (*Schotia latifolia* and *Polyagla myrtifolia*) that were the most vulnerable, and a third (*Pappea capensis*) moderately vulnerable, then the remaining studied species – this could result in community-level adjustments in species composition and structure under future projected climate change scenarios where, increased frequencies of drought events are anticipated. This retrospective assessment of canopy vigor with physiological functional thresholds evokes hydraulic dysfunction as the principal mechanism of observed Subtropical Thicket dieback to drought. This chapter provides crucial *in situ* data for predictive assertions around drought-induced mortality risk in a phylogenetically diverse and climatically unique biome of southern Africa; and is the first to identify critical hydraulic thresholds for tree species within this region.

Keywords: Drought, optical vulnerability technique, hydraulic traits, hydraulic dysfunction, Subtropical Thicket, drought-induced mortality, remote sensing, canopy vitality, xylem functional loss, xylem embolism, drought resistance

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List of abbreviations

DW (g)	Leaf dried weight
EVI	Enhanced Vegetation Index
FW (g)	Leaf fresh weight
HSM (MPa)	Hydraulic safety margin
MAP (mm.yr ⁻¹)	Mean annual precipitation
MODIS	Moderate Imaging Spectroradiometer
NDVI	Normalized Difference Vegetation Index
NSC	Non-structural carbohydrates
OVC	Optical vulnerability curve
PAR	Photosynthetically active radiation
P-V	Pressure-volume curve
P _e (MPa)	Water potential at incipient embolism (synonymous with P ₁₂)
P ₁₂ (MPa)	Water potential causing 12% embolism in xylem conduits
P ₅₀ (MPa)	Water potential causing 50% embolism in xylem conduits
P ₈₈ (MPa)	Water potential causing 88% embolism in xylem conduits
P ₁₂ HSM (MPa)	Hydraulic safety margin of incipient embolism-induced dysfunction
P ₅₀ HSM (MPa)	Hydraulic safety margin conservative threshold embolism-induced dysfunction
P ₈₈ HSM (MPa)	Hydraulic safety margin of critical threshold embolism-induced dysfunction
RWC	Relative water content
SLA (m ² .g ⁻¹)	Specific leaf area
SPEI	Standardized Precipitation Evapotranspiration Index
SPI	Standardized Precipitation Index
TLP (MPa)	Turgor loss point
TLP HSM (MPa)	Hydraulic safety margin for stomatal closure
TW (g)	Leaf turgid weight
Ψ (MPa)	Water potential
Ψ _{md} (MPa)	Minimum leaf water potential

Ψ_{osm} (MPa)	Osmotic potential at full turgor
Ψ_s (MPa)	Soil water potential
Ψ_{TLP} (MPa)	Water potential at turgor loss point
ε (MPa)	Bulk modulus of elasticity

Chapter 1

General introduction

Subtropical Thicket covers approximately 10% of South Africa's land area [extent of planning domain of Subtropical Thicket Ecosystem Plan (Vlok et al., 2003)], hosting a rich diversity under varying abiotic and biotic conditions across its respective distributional range. The recent elevation of this vegetation to biome status, distinguishable from historical classification under Karroid Veld Types (Acocks, 1975), stresses the significance of the Subtropical Thicket as core unit of southern African biodiversity. This biome is characterized by slow growing woody trees (Pierce and Cowling, 1984), having a high standing biomass with subsequently low levels of variation in biomass under successive cycles of drought (Aucamp and Tainton, 1984). Physiognomy of the Subtropical Thicket is consistent with spiny, evergreen trees and shrubs ranging in height of 2-5 meters, often forming dense aggregations of vegetation, creating an impenetrable structure. Phytochorologically, Subtropical Thicket is regarded as an ecotonal or transitional vegetation type – an intermediate between Forest and Savanna accredited to its “blended” species composition, sharing a relatively large portion of species with the Karoo and Afromontane vegetations. This biome offers essential ecosystem services and processes that are expressed on both regional and global levels (Lechmere-Oertel et al., 2005). Services including exceptional carbon sequestration capabilities (relative to available water resources), nutrient cycling, soil stability and maintaining high levels of flora and faunal diversity – a product of environmental complexity experienced by this biome.

It is proposed that three principal axes of environmental variation drive ecophysiological adaptation and niche specialization in the Subtropical Thicket biome. The first being the climatic regime and the episodic appearance of cyclic drought events – the Subtropical Thicket biome is characterized by a spatiotemporally variable bimodal rainfall pattern, with high autumn and spring precipitation declining into summer and winter giving-way to drier periods (Hoare et al., 2006). Secondly, herbivory where higher densities of browsers define the availability of niches and present a considerable evolutionary force of vegetation structure (Everard, 1987). The impact of indigenous herbivores on the evolutionary trajectory of the Subtropical Thicket vegetation is evident under the “predator-prey arms race” paradigm, where spinescent nature of Thicket illustrates a defensive strategy. Additionally, large herbivores directly impact the Subtropical Thicket vegetation via physical damage and opening gaps in dense vegetation stands (Kerley et al., 1995). A recent demographic assessment by van As et al. (2016) of the Thicket succulent *Aloe ferox* Mill. further highlights the impacts of herbivory on species demography and subsequent community dynamics. The third and final axis is fire, although thicket is a relatively non-flammable vegetation, with few fire-adapted species the distributional range of this biome is strongly driven by fire events. AST borders two biomes, namely Savanna and Fynbos, in which fire plays a persuasive role in relegating distributional range of thicket to topographical refugia, such as valley slopes.

Overall, the combined effects of stochastic climate events (i.e., drought, aridification) and herbivory pressure are two most influential forces driving ecological and evolutionary processes in Subtropical Thicket since the Last Glacial Maximum (Palmer, 1990). Consequently, Subtropical Thicket comprises a suite of subtypes, in total 112 different vegetation types (Vlok et al. 2003), distinguishable from one another by climate, vegetation structure, and geography. Arid Thicket

denotes a subtype of the Subtropical Thicket experience hot-dry conditions (i.e., arids) receiving between 200-450 mm mean annual precipitation and found throughout the Eastern Cape Province, growing in frost free areas, often along north facing valley slopes, and dominated by the succulent *Portulacaria afra* (spekboom) – resulting in the characterization of this subtype as Spekboom Thicket (used interchangeably with arid Subtropical Thicket throughout this thesis). This Thicket subtype has fascinated plant ecologists for decades, with questions centering around the persistence of woody canopy trees, remnants of warm-wet forests of the Eocene (Willis and McElwain, 2002; Cowling et al., 2005) and successional processes in these arid vegetation types. The dominance of spekboom in these arid environments has long been attributed to is associated drought tolerance strategies, including succulence (i.e., high water storage capacity) and Crassulacean Acid Metabolism (CAM), a photosynthetic pathway conserving water while maintaining suitable carbon assimilation for basal metabolic survival. The relevance of spekboom to community dynamics and successional processes in Spekboom Thicket are exemplified by its recognition as an ecosystem engineer, directly or indirectly effecting resource availability (e.g., water infiltration, carbon and nutrient cycling) thereby modifying biotic interactions within communities. And while many studies have explored the environmental ameliorating effects of spekboom and inferral of woody canopy tree facilitation [i.e., strong spatial association of woody trees and spekboom, see Adie and Yeaton, (2013)], there are none analyzing the physiological mechanisms underlying these spatial associations.

Structurally Spekboom Thicket describes a patch-like vegetated arrangement, comprising a mosaic of bush clumps with sparse emergence of evergreen, to weakly deciduous, woody canopy tree intertwined with spinescent, multi-stemmed evergreen shrubs within a matrix of spekboom and surrounded by bare ground. The later physiognomic description typifies intact Spekboom Thicket, a distinction between intact and subsequently degraded states is required under intensified pastoralism in these arid environments. The escalation in pastoral activity, specifically goat farming, has had tremendous impacts on the structure, community composition and stability of Spekboom Thicket. Where intensified browsing pressure induces an alternative state transition in Spekboom Thicket, to a “pseudo-savanna” conditional state. This “pseudo-savanna” denotes a step phase transition stage between a yet to be determined stable state from the pre-existing intact thicket state. These savanna-like systems are characterized by sparse emergent remnant woody canopy trees of Subtropical Thicket origin in a matrix of ephemeral shrubs and grasses of karroid origin. This transitional shift under increased goat browsing has major ramifications for Thicket canopy tree persistence where few species remain after spekboom removal and those that do have a higher incidence of mortality (Lechmere-Oertel et al. 2005).

Understanding the physiological strategies and associated trait coordination of plants under changing vegetation structure is central to exploring community processes and stability towards environmental change while simultaneously elucidating too species vulnerability under projected future climate change. In Spekboom Thicket the most persuasive shift is intensified pastoral activity reducing vegetation cover and substantially augmenting structural attributes and biophysical properties (e.g., soil moisture and biogeochemical cycling). The changes in vegetation physiognomy – i.e., spatially dispersed remanent woody canopy trees present have been documented by Hoffman and Cowling (1990) and Evans et al. (1998) and are a response identical to those depicted across an aridity gradient. The emergence of anthropogenic “facilitated” aridity gradients in Spekboom Thicket impose spatially explicit physiological filters defining community turnover and composition at transformed sites. The loss of spekboom, i.e. foundation species in Spekboom Thicket, has considerable ramifications for ecological processes

and community dynamics within these arid ecosystems as evident by fence-line contrasts depicting declining trends in woody canopy tree and shrub recruitment in transformed sites (Lechmere-Oertel et al., 2008; Sigwela et al., 2009). Coupled with the latter decline in seedling recruitment of woody trees in transformed sites is the corresponding escalation in adult tree mortality. This pattern of increased mortality in transformed sites described in anecdotal literature and the recent observed trends of dieback in intact Subtropical Thicket draws into question the underlying mechanism for species loss across the distributional extent of this vegetation.

The incidental trend of increasing tree mortality first documented by Lechmere-Oertel (2003) and later observations made herein describe a case study assessment of canopy dieback, foliage discolouration and whole-tree defoliation impress the significant impacts of episodic disturbance, such as drought, have on vegetation continuity at the arid edge of the Subtropical Thicket biome – Spekboom Thicket. Furthermore, stressing the importance of water availability as a foundational eco-physiological filter in structuring the Subtropical Thicket biome – both in distribution and species composition. This is made no more apparent than in Volk et al. (2003) thicket sub-type classification framework with the most diverging indicator of vegetation categories being climate, specifically rainfall, and by extension water availability. The importance of climate and related perturbations or deviations from historical averages, specifically drought, and its accompanying impacts have largely been overshadowed in Spekboom Thicket by extent of pastoral degradation, which has incidentally, highlighted the demographic instability (i.e., low recruitment success) of woody canopy dominants and increased tree mortality incidence induced by anthropogenic disturbance.

Tree mortality in the arid Spekboom Thicket vegetation represents a major concern for ecosystem functionality, as the loss of entire guilds is to have pronounced effects of functional sustainability of critical ecological processes derived therefrom (e.g., carbon sequestration, nutrient cycling). This stresses the importance of the understanding species-specific vulnerabilities to drought-induced mortality which is essential to predicting the fate of Subtropical Thicket in a future of uncertainty in climatic reliability and anticipated to experience greater magnitudes and frequencies of ecological disturbances, such as more intense and recurrent droughts. The advent and widespread application of trait-based approaches present a promising avenue for predicting the impacts of climatic perturbations, such as drought, have on vegetation dynamics and broader ecological functioning (Violle et al., 2007; Volaire, 2018). These mechanistic traits, whose function is distinctly physiologically defined (Brodribb, 2017), are to be a potentially greater determinant of species relative fitness and persistence under climatic disturbance, facilitating an improved capacity to predicted community dynamics and general vegetation change.

Drought-induced mortality is a complex ecological phenomenon, involving the interaction of plant water balance and carbon dynamics in order to sustain productivity and growth. To this end, two key hypotheses have been proposed to characterize drought-induced tree mortality based on these two physiological processes, deemed (1) hydraulic failure and (2) carbon starvation, respectfully (McDowell et al., 2008). Where hydraulic failure is the result of disruptions (embolized xylem vessel conduits) in water transport pathway of plants, either directly via drought-induced cavitation or indirectly via air-seeding (Venturas et al., 2017); regardless the implications are severe resulting in whole-plant desiccation and impaired function culminating in tree mortality. Carbon starvation describes the depletion of carbohydrate reservoirs and resulting net deficiency in carbon turnover causing whole-plant mortality, the outcome of reduced and/or stringent stomatal closure impeding carbon metabolism and increasing the risk of pest/pathogen

susceptibility (McDowell, 2011). However, while much research differentiates these two pathways as diverging causes behind drought-induced tree mortality – they are not mutually exclusive. Rather tightly interlinked, where hydraulic failure or species demonstrating embolism avoidance response (stringent stomatal control, early closure at the onset of water deficiencies) result in reductions in stomatal conductance and subsequent declines in photosynthetic output causing dependency on stored carbohydrates, however under conditions of zero turnover inducing a deficit state of carbon in the plant. This reduction in available carbon pools impedes new growth of functional xylem tissue or ion-assisted water movement when drought conditions recede (Nardini et al., 2011; Secchi et al., 2020), thereby exacerbating already hydraulically impaired water transport network. While support for both hypotheses have been demonstrated (Anderegg et al., 2012; Allen et al., 2015; Adams et al., 2017), recent studies aiming to identify the driving mechanisms inducing tree mortality have elevated the significance of hydraulic failure. That is, many studies have stressed the relevance of hydraulic impairment, where plants have surpassed species-specific functional thresholds due to embolism resulting in whole-plant desiccation and eventual mortality. These studies have led to the identification critical thresholds of hydraulic function, enabling the characterization of species-specific hydraulic safety margins, delimiting the safe operational range of xylem water potential under drought while still retaining hydraulic conductance (Choat et al., 2018). Hydraulic safety margins are thought to define a plant's degree of conservatism in regulating water and is significantly related to drought tolerance of a species. The findings of Choat et al. (2012) demonstrating that nearly 70% of woody tree species across biomes operate with narrow safety margins, converging close to species-specific critical functional thresholds. This stress the significance of uncovering these critical species-specific functional thresholds and hydraulic traits to conceptualize and understand the drivers of drought-induced vegetation change dynamics.

It is imperative that in understudied ecosystems and biomes, such as Subtropical Thicket, vulnerability to extreme climatic perturbations and stochastic events (e.g., drought and floods) be explored to predict species-specific susceptibility and consequential changes to vegetation dynamics. However, while gaining a mechanistic understanding behind tree mortality processes is critical for predicting species-specific changes, there remains insufficient assessment of the timing and spatial extent of vegetation dieback events in Subtropical Thicket. This elevates the importance and significance of conducting observational studies or surveys recording declining trends and events experienced by vegetation, and understanding the determinants of change, facilitating the development of management strategies to safeguard ecosystem function and services (Anderegg et al., 2013). More importantly identifying points or transition thresholds and the causal factor/s are critical to anticipating mortality impacts at both a landscape-level and at a regional scale (Ogaya et al., 2015). There are few studies that have verified thresholds of change or breaks in vegetation induced by climatic disturbances, even less so in Subtropical Thicket, highlighting a major knowledge gap as to validate anecdotal inferences of causal factors (i.e., such as drought) responsible for changes in vegetation productivity and plant mortality. In this thesis a considerable amount of attention is directed towards investigating the spatial and temporal trends in remotely sensed vegetation indices and determine the effectiveness of the breaks for seasonal trend algorithm in detecting critical transitional events that coincided with known droughts – highlighting the utility and practicality of remote sensed indices as ideal candidate indicators/proxies for determining the occurrence of Subtropical Thicket dieback and whole-plant mortality. Furthermore, this thesis attempts to provide a mechanistic interpretation of these observed trends in vegetation productivity under drought by providing the first

comprehensive report on the hydraulic vulnerability of dominant canopy tree and understory shrub species in Subtropical Thicket.

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Chapter 2

Literature review and rationale

The literature review herein addresses core topics relevant to the intention of this thesis, which explores the role of drought both from a physiological standpoint and as a driver change in Subtropical Thicket. The review is presented as a series of sequential sections each discussing a topic of significance and compiling a synopsis of available literature related to the information being articulated. The core focus of this research is describing the implications drought presents to Subtropical Thicket most significantly at its arid distributional extent – arid thicket subtype. Conventionally, the role of drought as an ecological driver has been largely undervalued in thicket, this may be due to the generality at which drought comparable conditions frequent the distributional extent of this vegetation. This thesis explores the context of drought on the productivity and general condition of the vegetation during such water deficit conditions. While additionally investigating the physiological implications and potential vulnerabilities drought may pose to vegetation persistence and demography.

Drought-induced change and mortality can have profound ecological, biophysical, and biogeochemical ramifications for ecosystems. As such this review attempts to stretch across all potential research themes of relevance to understanding the underlying mechanisms and implications of drought-induced change from a Subtropical Thicket perspective. The research themes reviewed expand on general topics highlighted in the literature stretching across major disciplines to offer a comprehensive and concise account of global type changes in climate, chiefly drought, have on the productivity and physiological resilience and vulnerability thresholds of thicket vegetation. A central theme of this thesis is being the first study to capture core physiological threshold reporting the hydraulic vulnerability of dominant woody canopy species to drought – impressing the significance of providing a mechanistic outlook to ecological processes. Overall, this review stretches across several themes, e.g., physiological, paleo-ecological, and phytosociological, with the main intent of addressing the role of drought in Subtropical Thicket.

2.1. Thicket and drought a thorny issue indeed.

This section presents a conventional review of the literature, discussing a diverse array of topics related to Albany Subtropical Thicket from origin, ecology and transformation. Throughout the literature Thicket species, particularly canopy trees have been portrayed as excellent physiological tolerators, specifically towards drought which has supported their persistence within arid ranges of the sub-escarpment inhospitable for most subtropical tree species. This physiological tolerance of Thicket species, particularly woody canopy trees has intrigued many Thicket ecologists, and more importantly confounded preconceived notions of how species derived from subtropical origins might be capable of tolerating such extremes – chiefly rainfall unpredictability and generally low water availability. And while excellent research within the fields of biogeochemical cycling, phylogenetics, and species distribution modelling has been conducted within this biome, there has been a considerable absence of physiological work and its applicability in an ecological context – “ecophysiology”. The few physiological studies which have been conducted have propelled our understanding of the implications regional scale climate has on the distributional range and performance of these species. The general lack of

ecophysiological work in thicket is in part due to the effort of collecting such data, yet many have presented suppositions as to the possible physiological tolerances and mechanisms of Thicket flora and although hypothetical they provide a basis to which empirical based studies can be founded upon. For example, the early hypothesis that frost is a significant driver for Thicket species distributional fringe was suggested by Cowling et al. (2005), and later quantitatively proven by the work of Duker et al. (2015a,b) using physiological assessments (namely, photosynthetic performance) of freezing tolerance of Thicket species in comparison to frost-hardy Nama-Karoo species – thus it was inferred that frost may dictate the boundary between the two vegetations defining the dispersal range of Thicket to the lower sub-escarpment of the southern Cape, South Africa. The latter work is one of the few published works attempting to describe ecological processes (i.e., distribution) to an environmental character finding a strong link – this emphasizes the significance of physiology to providing a mechanistic and predictive framework upon which ecological patterns can be discovered.

Ecophysiological research is paramount for conceptualizing the fundamental processes effecting change in populations, communities, and ecosystems. Such studies provide us with a mechanistic interpretation and understanding of plant survival, productivity, performance, dominance, and distribution across heterogeneous environments of our planet (Ainsworth et al. 2016). The use of physiological techniques has greatly advanced our knowledge of plant interactions, plant water relations, respiration, photosynthesis, and environmental response strategies across timescales from short-term acclimation to long-term evolutionary adaptation (Lambers et al. 2008). From the perspective of Ainsworth et al. (2016) plant ecophysiology is considered fundamental for understanding (1) plant evolutionary strategies and adaptation; (2) anticipating and predicting future, present, and past vegetation productivity and resource use efficiencies; (3) novel developments for adaptive response for managing ecosystems under climate change and the modification techniques for increasing stress tolerance of breeding crops; and (4) assessing vulnerability of species to global climate change with the end goal of alleviating its impact. The most pressing concern and most relevant, particularly in the context of plant physiology is rapid pace of global change phenomena such as climate. The change in which is expressed most pervasively/substantially at regional to local scales as opposed to global, where synergetic inference and additive processes buffer change that are scaled to these spatial extents.

Plant ecophysiology has largely focused on two main themes (1) mechanisms or responses and (2) recruitment, growth and/ or survival success incorporating both into macroecological theory to describe vegetation patterns and drivers of their change (Harsch and Bader, 2011). Here attention is given to physiology in interpreting Subtropical Thicket vegetation response to global change type disturbance, specifically drought. Historically the drought as an ecological processes and driver of change is underappreciated and frequently overlooked in Subtropical Thicket. To provide context Cowling et al. (2005) stated that “thicket is unusual amongst African ecosystems in that herbivores are solely responsible for defoliation; with drought and fire and gap-producing tree mortality being unimportant.” This statement echoes those of the earlier sentiments of Aucamp et al. (1982) that biomass remains largely stable with little annual fluctuation in cover or biomass irrespective of monthly to decade intensive droughts. This would appear to contradict much of what is expected under drought, most persuasively a reduction in overall vegetation vitality and productivity. Subtropical Thicket has been described as maintaining its high standing woody and succulent biomass (Aucamp et al., 1982; Kerley et al., 1995) irrespective of drought. The reasoning behind the general stability in vegetation condition and biomass has been attributed to

several co-occurring strategies including sclerophylly, belowground storage organs, Crassulacean Acid Metabolism (CAM) photosynthesis, and succulence.

Many of the strategies highlighted and mentioned previously are apparent in arid thicket where succulent life history and lineages dominate the landscape, with scattered appearance of the less evident woody shrubs and canopy trees. These trees and shrubs are conspicuously evergreen sclerophyllous denoting a more costly carbon investment strategy where sclerification of cellular leaf constituents, producing an abundance of fibers and sclereids, are evident giving leaves a rigid and tough appearance (Cowling and Campbell, 1983). This might evoke speculation that the frequency of drought in arid thicket the reoccurrence of sclerophylly in most of the woody tree and shrub species (e.g., *Pappea capensis*, *Gymnosporia buxifolia*) support the long-held hypothesis that drought tolerance is derived from this trait (Poole and Miller, 1975). As such the rigidity and sclerified leaves would be capable of tolerating extremely low water potentials sustaining symplastic turgor driven forces under extend drought (Salleo and Nardini, 2000; Bartlett et al., 2012). Contrary to the water conservation hypothesis (suggested in order to maintain turgor through leaf structural adjustments, i.e., sclerification) is the nutrient deficiency theory positing that sclerophylly represents a consequence of diminished soil nutrient status; where plants have developed metabolic pathways to capture and subsequently conserve resources by extending the lifespan of its component organs (i.e., leaves). Much contention surrounds which of these hypotheses should be given greater credence to the appearance and adaptive role played by sclerophylly. A recent study of *Protea* observed a strong negative response between Carbon-13 (proxy for water use efficiency) and leaf mass per area demonstrating sclerophyllous lessens with greater LMA and correspondingly lowers Carbon-13 (less efficient water use) demonstrating sclerification as a means of resource conservation (Mitchell et al., 2017). At present nutrient deficiency remains one of most convincing hypotheses at characterizing the adaptive appearance of sclerophylly (Salleo and Nardini, 2000). Given that Subtropical Thicket shrubs and trees largely exhibited few if any of these attributes (succulence, belowground storage organs, and CAM photosynthesis) purported in the literature (Vlok et al., 2003; Palmer, 2004; Cowling et al., 2005) ascribing drought resilience of thicket – the question then arises to what other potential traits might ascribe the drought tolerance to this vegetation.

Trait-based approaches are emerging field offering a new perspective to accurately predict changes to vegetation dynamics and trends in response to shifts in environmental variables and climate. As plant functional traits are characteristically correlated well with the environment and climate thus are essential for ascertaining plant performance both under stable conditions but more importantly under abiotic or biotic stresses (Soudzilovskaia et al., 2013). Predicting demographic change, notably mortality, by means of physiological traits has been a central theme of ecology with broader exploration of their linkage with biogeography of species and potential future population responses (Anderegg et al., 2012; Soudzilovskaia et al., 2013). Yet drought-induced mortality predictions have largely been challenging due to uncertainties surrounding the interdependency and coordination of traits delimiting vulnerability risk and modulating loss of function and reductions in performance. Irrespective of this uncertainty (Hartmann et al., 2015), the impairment of plant vascular hydraulic transport is a chief pathway to tree mortality. This hydraulic failure is chiefly driven by the appearance and spread of embolism throughout the xylem network induced by high evaporative demand and low soil moisture, resulting in the impediment of water flow particularly to downstream organs and subsequent loss of function in these tissues. The point at which critical loss in physiological function induced by embolism can be determined by several hydraulic and stomatal traits enabling the identification of thresholds for drought

vulnerability and determination of mechanisms behind observed mortality and changes in vegetation productivity trends.

Plant water relations are crucial to adaptive ecological strategy with vulnerability to vascular dysfunction being strongly collated to vegetation distribution and productivity across ecosystems. By investigating plant water relations and associated traits innately linked to drought tolerance will enable predictions as to species-level sensitivities to drought-induced mortality, or conversely identify species illustrating adaptive response to drought. To this end, this thesis will be the first to quantify key hydraulic traits of six dominant Subtropical Thicket woody species providing insight into drought tolerance and species sensitivities to drought. This thesis will examine the link between these intrinsically drought related physiological traits to explain trends in vegetation productivity through time. However, before any undertaking or discussion regarding the underpinning physiological traits of drought vulnerability can be taken, the detection and description of drought as a fundamental ecological disturbance is needed and future monitoring its effects is of paramount importance.

2.2. Things are beginning to heat up: Drought monitoring and definitions

A lack of consensus of the precise definition of drought has impeded attempts to derive a commonly applicable characterization of this climatic phenomenon. However, the general agreement considers drought as a complex, natural climatic phenomena that has negative implications for flora and fauna within its extent (Paulo and Pereira, 2006). Noteworthy definitions include that of Palmer (1965) description of drought as a “significant derivation from the normal hydrological state/condition of an area” and McKee (1963, pg. 17) drought explanation as a “climatic condition where insufficient moisture of an area is caused by a precipitation deficit over a time period”. While the challenge of defining drought from a theoretical standpoint remains, it's characteristic expression as a deviation from precipitation normality is undeniable. Drought occurs as a deficit of rainfall within an area or region and depending on the duration and severity (expressed as evaporation demand) may influence water availability in ephemeral sources such as soil moisture to more permanent sources such as ground- and stream-water, directly impacting vegetation productivity and other ecosystem processes. The impact of drought on an areas water sources have led to the categorization of varying drought types, namely: (1) meteorological, (2) agricultural, and (3) hydrological subtypes.

Meteorological droughts: Describes an area experiencing general precipitation deficit where rainfall has reduced by more than 25% than average. Again, this is specific to a region due to spatial-temporal climatic variability.

Hydrological droughts: These associated with precipitation deficits which results in declines in surface and groundwater source. Hydrological droughts are an extension of shortages in rainfall as defined by meteorological droughts, however, focused towards assessing changes of the hydrological system, from soil moisture to decreases in streamflow and groundwater levels.

Agricultural droughts: Such a type of drought links several attributes of hydrological and meteorological droughts to a quantifiable outcome, more specifically agricultural impacts, with attention to the implications of increases evapotranspiration (due to increase vapour pressure deficits), and declines in water availability by surface and groundwater, under the broader influential effect of precipitation deficits. These agricultural droughts are concerned with plant

water demand and balance under the established climatic state, coupled with such is biological traits of crop species and soil integrity to sustain growth (i.e. maintaining optimal moisture conditions for development).

The ambiguity around drought and its definition extend to the means by which this climatic phenomenon is quantified with no uniform methodology or singular metric characterizing it, rather several indices have been proposed to monitor and delimit conditions of drought. These indices aggregate, combine variables (precipitation, temperature, soil moisture, etc.) describing the water availability status and deficits a region may/might have experienced by evaluating changes in trends and abundance of water resources over a specific time-period (Zargar et al., 2011). A few widely utilized indices include, the Standardized Precipitation Evapotranspiration Index (SPEI), Standardized Precipitation Index (SPI), Palmer Drought Severity Index (PDSI), and Aridity Anomaly Index (AAI). Two of the most widely applied indices quantifying and monitoring drought are the SPEI and SPI, as such these indices are employed in this thesis as the core proxies for drought characterization.

First formulated and reported by McKee et al. (1993), the Standardized Precipitation Index (SPI) is widely accepted and is advocated under the seminal proceedings of the Lincoln Declaration on Drought Indices to be the standard metric for quantifying meteorological drought (Hayes et al., 2011). SPI is computed as the number of standard deviations of the observed value from the predicted long-term mean, based on the probability function describing distribution of precipitation data (Stagge et al., 2015). Hence, SPI requires only a single input variable, namely rainfall, the simplicity of one parameter derived indexing has propelled the broad application of SPI in monitoring water availability trends at a given location (Cancelliere et al., 2007; Zargar et al., 2011). The intensity scale of SPI, offering both positive and negative values corresponding to wet and dry conditions respectfully, is aptly suitable for rapid interpretative inference and intuitive understanding (Wagan et al., 2015). SPI was created to ascertain the precipitation deficit of an area in addition to being computed across time scales. The temporal scaling based on the probability of precipitation for any time scale enables the determination of how drought impacts water availability of varying sources. For example, short-term anomalies such as 3-, and 6-month SPI describe the impacts of water deficit for those cumulative periods on vegetation and soil moisture, whilst longer periods (e.g., 12-month) describe changes to groundwater reservoirs and more permanent water sources. This flexibility of SPI in conjunction with the capacity to be determined even with missing data is made possible and limited, by the requirement for larger rainfall records of a region (Dalezios et al., 2017). A major constraint of SPI is the inability to capture the effect of thermal fluxes on climatic water balance and use for a region. Such shortcomings limit spatiotemporal comparisons of SPI due to the underlying uncertainty around temperature conditions during these periods.

The Standardized Precipitation Evapotranspiration Index (SPEI) is one such drought index that overcomes the shortfalls of SPI and was developed and first reported by Vicente-Serrano et al. (2010). SPEI builds on the work by McKee et al. (1995) SPI by integrating the influence of temperature. Therefore, enabling SPEI to account for the thermal influence of temperature under drought. By integrating an equation relaying climatic water balance, defined as the difference between potential evapotranspiration and precipitation to the underlying determination of drought severity. This adjustment retains the computation simplicity and statistical practicality of SPI while additionally incorporating a more inclusive quantification of water availability of an area through water balance calculation (Beguería et al., 2014). Similar to SPI, the Standardized Precipitation

Evapotranspiration Index presents an intuitive intensity scale depicting both positive and negative values which correspond to wet and dry conditions. The significance of SPEI as a drought metric is greatly underestimated, its capability for the inclusion of modelling potential evapotranspiration lends itself to a more comprehensive assessment of the physiological costs of drought. Furthermore, the multi-scalarity of SPEI addresses major limitations of other widely applied indices (e.g., PDSI), enabling comparative analyses between varying hydrological systems while additionally discriminating between drought types (Vicente-Serrano et al., 2012). These indices provide crucial information on the meteorological and water availability conditions of an area or region, offering some indication of the predicted physiological strain, they provide little indication of the impact changes in the conditional status may present to vegetation continuity and productivity. Whilst detecting drought is a major priority, particularly from a societal perspective, understanding its implications for vegetation productivity and growth is far more crucial for discernment of the ramifications drought-induced changes and damage presents to species vulnerability and community composition and structure. In forest science, remote sensing is a contemporary and effective tool with high spatial and temporal resolution capturing changes in vegetation performance, physiology, and survival. The combination of drought indices (SPEI and SPI) with remote sensing offers a more comprehensive perspective on mortality and drought impacts by including the monitoring and detection of corresponding change in vegetation performance and physiology that would coincide with changes in SPEI or SPI.

2.3. Monitoring vegetation responses to abiotic disturbances from space

The monitoring of vegetation has conventionally and historically been approached from ground plot-level surveys often limited in their respective spatial and temporal resolution capacity. However, with the advancement of computational technologies and remote sensing platforms, be they aerial or satellite, filling these spatiotemporal gaps are now possible. There are presently several satellite platforms monitoring the planet's surface capturing and relaying data from optical and radar sensors. The purpose of these sensors is to detect and record radiation emitted from the Earth's surface across a range of wavelengths (e.g., visible, Short-Wave Infrared, and Near Infrared). These wavelength ranges provide spectral characterization of the biophysical and biochemical properties (e.g., chlorophyll content, leaf area index) of the planet's surface enabling the monitoring of vegetation, and potentially land use, change (Xie et al., 2008). There is however, one major inherent drawback to using optical derived data attributed to the passive sensor relying on extrinsic radiation sources being refracted off the surface of the earth and captured by the detector (Zhu et al., 2018). Subsequently, limiting data acquisition to daylight hours and cloud free periods when emitted radiation can be capture by the optical sensor (Asner, 2001). This limitation has been resolved by the implementation and deployment of radar sensor, capable of emitting their own electromagnetic radiation and detecting the returning signals (Sinha et al., 2015). While radar remote sensing platforms offer many advantageous over optical, for the purposes of this thesis optical sources are employed due to comparable methodology within the literature and accessibility of data.

Remote sensing platforms such as Moderate Resolution Imagery Spectroradiometer (MODIS), Landsat, and SPOT satellites has developed into an essential and valuable tool for monitoring spatial and temporal changes in vegetation (Reuben et al., 2008). The detection of several wavelength ranges across the electromagnetic spectrum enables the delimitation of numerous vegetation indices in an effort to quantify the magnitude, occurrence, and changes to vegetation

cover and land usage. Vegetation indices have proven essential by successfully quantifying the impacts of desertification (Collado et al., 2002), phenology (Reed et al., 2009), and drought (Tucker and Choudhury, 1987) capturing vegetation responses at both fine and broad spatiotemporal scales. This breadth of scale facilitates real-time monitoring applicability, coupled with historical trends and ever evolving statistical modelling techniques bolsters the significance of remote sensing. Thus, providing a more comprehensive ecological understanding of vegetation dynamics and the implications of disturbance to ecosystem resilience and recovery (Gazol et al., 2018; Harris et al., 2014). Remote sensing technologies are continually advancing both in terms of data capture and computation capabilities incorporating a range of spectral bands – improving detection, accuracy, and identification of potential drivers responsible for vegetation change. Thus, this thesis leverages several such vegetation indices to quantify vegetation change in response to changes in precipitation, focusing on the role of drought.

2.4. Vegetation indices: background and application

The monitoring of vegetation by remote sensing is performed by capturing emitted electromagnetic radiation from vegetation canopies (Rees, 2013). The range of wavelengths observed from plant canopies is influenced by the biophysical properties of the vegetation itself, changing with the functional type, tissue water content, pigment properties, and other intrinsic attributes. The refracted radiation from the vegetation is dictated by the morphological and chemical characteristics of the plant (Knipling, 1970). Such that any factor, abiotic or biotic stressors, may influence these characteristics resulting in corresponding changes to the emissivity peaks of spectral bands deviating from expected of green vegetation. The spectral character of green vegetation is a product of the emission of green wavelength in favour of higher photonically charged red and blue light, with greater energetic radiation, Near Infrared, being emitted as well. Several of the commonly utilized vegetation indices are derived from varying expressions of spectral absorption range (dominantly red visible light: 620-750 nm) of chlorophyll and the scattered/refracted NIR by structural components of the mesophyll (Tucker 1979, Tuck et al., 2014). Of the several wavelength bands captured by remote sensing platforms (e.g., MODIS, Landsat) these two spectra are frequently utilized in quantifying vegetation cover change and dynamics being applied as the index, Normalized Difference Vegetation Index (NDVI), due to the calculation simplicity and suitability for spectral characterization of vegetation condition, health, performance, and productivity. NDVI is one of the most widely and commonly used vegetation indices for monitoring vegetation change and response to abiotic pressures. NDVI has frequently been used as a proxy for vegetation condition, vigor, and productivity – derived from estimated fraction of absorbed photosynthetic radiation – and has additionally been used to derive biophysical properties as well including aboveground plant biomass, leaf area index, and canopy water content and availability (Gamon et al., 1995; Claudio et al., 2006). It is expressed as:

$$NDVI = \frac{NIR - Red}{NIR + Red}$$

Where NIR denotes the Near Infrared band detected by the sensor and Red describes the red spectral band absorbed by chlorophyll. The expression of ratio of refracted wavelengths divided

by the sum total of their intensities, resulting in a NDVI value range of -1 to 1, with values between -1 and 0 describing inorganic surface features (rock, ground) and 0 to 1 denoting varying states of vegetation vigor and health condition. Thus, high index values, approximately 0.6-0.9, denote sites with high vegetation cover, density, and are indicative of high photosynthetic performance and vigor. Whilst lower values, approximately 0.3-0.5, describe sparse, senescent vegetation and overall conditional vigor of standing biomass. The classification of NDVI values to conditional status of the vegetation facilitates the detection and identification of changes in optimal physiological state and proves essential to monitoring the trajectory in productivity and resilience to abiotic factors. For example, Brodrick and Asner (2017) coupled NDVI with health conditional survey of conifer stands to characterize an identifiable NDVI threshold for predicting the probability of deriving the spatial extent of tree mortality, in addition to isolating an appropriate threshold for monitoring future tree death in conifer stands in the US. The usage of NDVI has been widely correlated to net primary productivity, phenological response/change to weather conditions, land use change, vegetation health (Schloss et al., 1999; Lunetta et al., 2006; Hmimina et al., 2013) – demonstrating the ecological significance and applicability this spectral index holds to understanding and interpreting vegetation responses.

The implementation and usage of NDVI for monitoring changes in vegetation cover and condition in semi-arid and arid ecosystems is established from a body of literature (Tucker et al., 1985; Wessels et al., 2004; Brown et al., 2010; Verbesselt et al., 2012; DeVries et al. 2015) illustrating the strong association that exists between precipitation and NDVI. The relationship is strengthened by the significant seasonal and annual fluctuations between rainfall and NDVI, accordingly drought impacts are assessed by the variance in photosynthetic value (i.e., spectral absorptive capacity) of plants. To this end, NDVI is frequently used to monitor and estimate drought impacts and near real-time conditional changes of the vegetation. NDVI capability to accurately monitor drought can only be achieved when coupled with drought metrics, such as SPEI and SPI. These meteorological derived indices such as the aforementioned mentioned Standardized Precipitation Evapotranspiration Index (SPEI) and the Standardized Precipitation Index (SPI) are essential for monitoring and identifying changes in both rainfall trends and general water availability/demand on plants through the integration of modelling potential evapotranspiration exhibiting the physiological implications of water scarcity on ecosystem-level productivity and species-level tolerance thresholds to aridity.

The NDVI is however, sensitive to the geographic heterogeneity and other locational biophysical properties, which many present subtle inconsistencies when employed as a monitoring proxy over a heterogeneous landscape (Burgess et al., 1995). Additionally, atmospheric factors such as water and aerosol content and large areas of bare ground can potentially distort spectral refractive properties influencing detected NDVI value presenting as vegetation change when in actuality there has been little change (Kogan, 1990; Huete and Tucker, 1991). These limitations or once off discrepancy appearances in NDVI has prompted the advancement of spectral vegetation indices and has resulted in the development of several alternatives, including Soil Adjusted Vegetation Index (SAVI), Atmospherically Resistant Vegetation Index (ARVI), and Enhanced Vegetation Index (EVI) which are intended to remedy distortion-induced factors when using NDVI as a sole proxy in vegetation monitoring. The most widely applied index, apart from NDVI, is EVI which is applicable to the same spectral wavelengths capture from MODIS satellite sensors and is calculated as:

$$EVI = 2.5 \times \left(\frac{(NIR - Red)}{(NIR + 6 \times Red - 7 \times Blue + 1)} \right)$$

The modified equation integrates the blue band (0.43-0.45 nm) enabling the calculation of ratio index between NIR and Red band while reducing noise and distortion from atmospheric properties and topographic inconsistencies (Liao et al., 2015).

The Enhanced Vegetation Index differs from NDVI in that it implements the blue-band spectrum into the NDVI equation to overcome the sensitivities of NDVI, namely bare ground, atmospheric distortions and biomass saturation, however, retains responsiveness to variation in vegetation spectral condition (Matsushita et al., 2007). Furthermore, the EVI demonstrates a greater tolerance for the influence topographic variance plays in creating distortion in detect NDVI. Both EVI and NDVI have shown to complement each other well when applied to vegetation change research, improving not only the change detection but demonstrating a stronger accuracy when estimating biophysical conditions of the vegetation (Huete et al., 2002). Remote sensing vegetation indices are a valuable tool for capturing greater spatial and temporal resolution of climate-related impacts and changes on vegetation, however, firstly understanding the ecological processes and historical changes of vegetation is crucial for a comprehensive analysis.

2.5. A tale of a thorny landscape: The origins and ecology of a forgotten biome, Albany Subtropical Thicket

Albany Subtropical Thicket (AST) has proven to be quite a challenge for biogeographers and plant ecologists, attributable to its phytochorological complexity, evolutionary origin, ecological and climatic uniqueness (Cowling et al., 2005). These very complexities may have contributed to the delayed biome recognition of Thicket, with early authors accrediting the vegetation as derived from karroid origins (Bews 1925). It was only until the seminal works of John Acocks (1911-1979) on southern Africa vegetation typology and classification that renewed interest in characterizing and uncovering the fundamental ecological processes of this “forgotten” biome. Acocks, and by extension his research, has made profound contributions to the fields of biogeography (Killick, 1980), pastoral grazing systems (Meadows, 2003), and vegetation dynamics (Hoffman, 2003). All of which have culminated in one of the most noteworthy provisions, still used as a reference text today, published in 1953 entitled *Veld Types of South Africa*. Wherein Acocks describes vegetation patterns and processes, emphasizing the importance of two keystone ecological drivers effecting the former, namely grazing and fire shape vegetation dynamics and plant cover in South Africa (Vlok et al., 2003).

The most significant vegetation type identified, in the authors opinion, is Acocks (1953) Valley Bushveld, a veld type that to this day has frustrated, yet fascinated plant ecologists for the better part of seven decades. With many authors built on the earlier works of Acocks, in an effort to resolve criticisms around the amalgamation of physiognomically and floristically divergent veld types in Acocks classification scheme. It was not until two decades later that Valley Bushveld and disjointed, geographically spread but floristically and structurally synonymous, veld types (e.g., Spekboomveld) were recognized as a collective vegetation unit — under the umbrella of a “thicket biome”, first proposed by Tinley (1975). He described this “thicket biome” as dwarf forest, characterized by closed-canopy with undifferentiated strata (unified structural form), comprising

an impenetrable intertwinement of low-growing trees and shrubs, existing as extensive vegetation continuum or aggerating in clumps as mosaic vegetation across the landscape. This was the first time the AST was acknowledged as a discrete biome, and although the early research of Tinley (1975) did not adequately assess the relevance of this vegetation, both in an ecological and evolutionary context, it paved the way and influenced the later research by Cowling (1984) to formalize the concept of Subtropical Thicket vegetation and ecology in South Africa.

The classification of Subtropical Thicket is a turbulent one, encompassing a number of strong opinions and debates regarding its origin and general ecology, this lack of knowledge has stimulated much interests in this ancient – subtropical-tropical vegetation derived from the Eocene (Cowling et al., 2005). Whilst Cowling (1984) was the first to coin the term “subtropical transitional thicket” integrating this concept into broader systematic vegetation classification providing the benchmark upon which future works have been based. Later authors such as Everard (1987) distinguished thicket four types founded on rainfall and structural attributes introducing the notions of “mesic” and “xeric” thickets [xeric is used interchangeably here and throughout this review as arid]. Although these earlier works have proved invaluable in teasing apart the distinction between thicket types, it was not until the inspiring works of Vlok et al. (2003) providing holistic classification scheme, incorporating ecological, phytosociological, and geographical aspects as founding divisions under a topographical hierarchy. Briefly, Vlok et al. (2003) hierarchical classification framework distinguishes types of thicket based on four features: (1) geography, (2) floristics, (3) structure (i.e. solid or mosaic), and (4) grain. Importantly, the presence of the dominant succulent, *Portulacaria afra* (hereafter spekboom) indicates the relevance as a major contributing factor given its recognition as a discriminating parameter under the current classification framework. Below is Vlok et al., (2003) conceptual illustration of thicket types, classified categorically based on the four latter mentioned criteria respectfully.

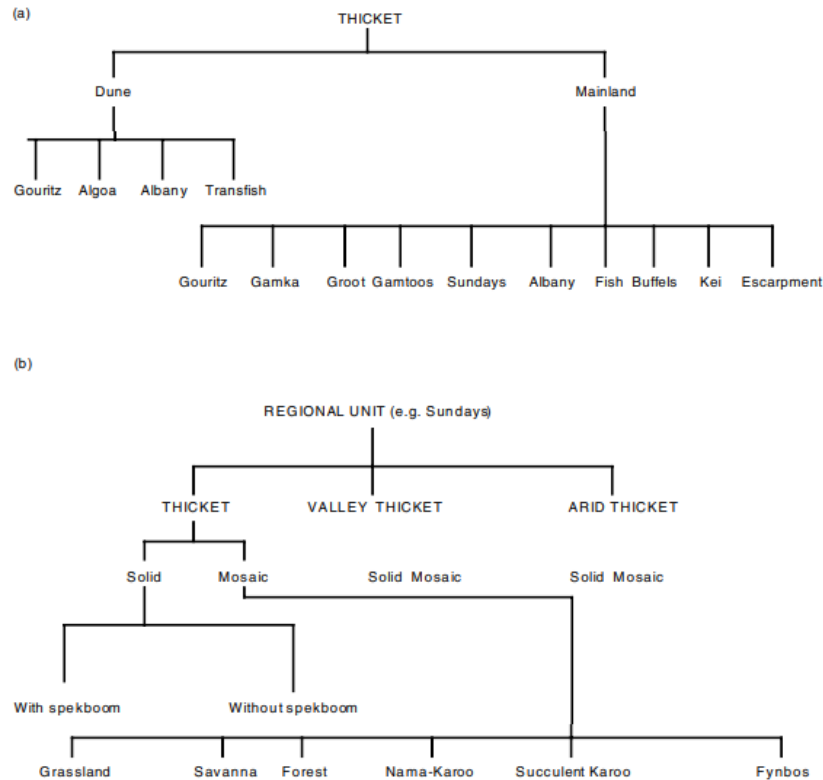


Figure 1: The proposed conceptual classification hierarchy by Vlok et al., (2003) differentiating thicket vegetation into coherent subtypes based on: (1) geography; (2) floristics; (3) structure; (4) grain.

Vlok et al., (2003) classification hierarchy has been the historical benchmark for categorical survey assessments and general landscape management in respect to thicket vegetation for the better part of almost two decades. A brief description of the three dominant subtypes is presented here, including descriptions of the dominant species and generalized ecological properties are provided offering a holistic overview of this ancient vegetation, in addition to outlining the necessary context for this thesis.

These three main subtypes are chiefly differentiated based moisture availability and presented by Vlok et al., (2003) dispersed along a cline of water saturation or accessibility – progressing from dry to humid conditions (arid thicket to valley thicket to mesic thicket) – representative of first order character divergence. While second order character discriminations are parameterized around floristic composition and vegetation structure, with input of other ecological properties (Vlok et al., 2003; Cowling et al., 2005). Briefly, arid thicket is characteristically identified by a general increase in the relative abundance of succulents, most apparent is the appearance of the dominant *Portulacaria afra*, interspersed with woody trees and shrubs (Lechmere-Oertel, 2003). Woody component species include *Pappea capensis*, *Euclea undulata*, *Searsia longispina*, *Grewia robusta*, and *Schotia afra*. A topoclimatic characterization of arid thicket denotes a general elevational distributional occurrence falling between 400 to 1060 meters with a mean modal altitude of 576 meters, while receiving 200 to 450 mm.yr⁻¹ (mean of 296 mm.yr⁻¹) of rain (Vlok et

al., 2003). Arid thicket constitutes the second largest by area of the three mainland types considered as conservation priority under the Subtropical Thicket Ecosystem Planning (STEP) Project with an estimated coverage of 1 504 744 ha – accounting for 32% of the planning area – and comprises of 22 different vegetation types (Vlok et al., 2003; Figure 2). Of these 22 types one stands out from the rest, namely Spekboom-dominated Thicket and Spekboomveld, both in relative size and significance for restoration priority, with an estimated area of 3 149 200 ha ST represents 30% of the total coverage of the Thicket biome.

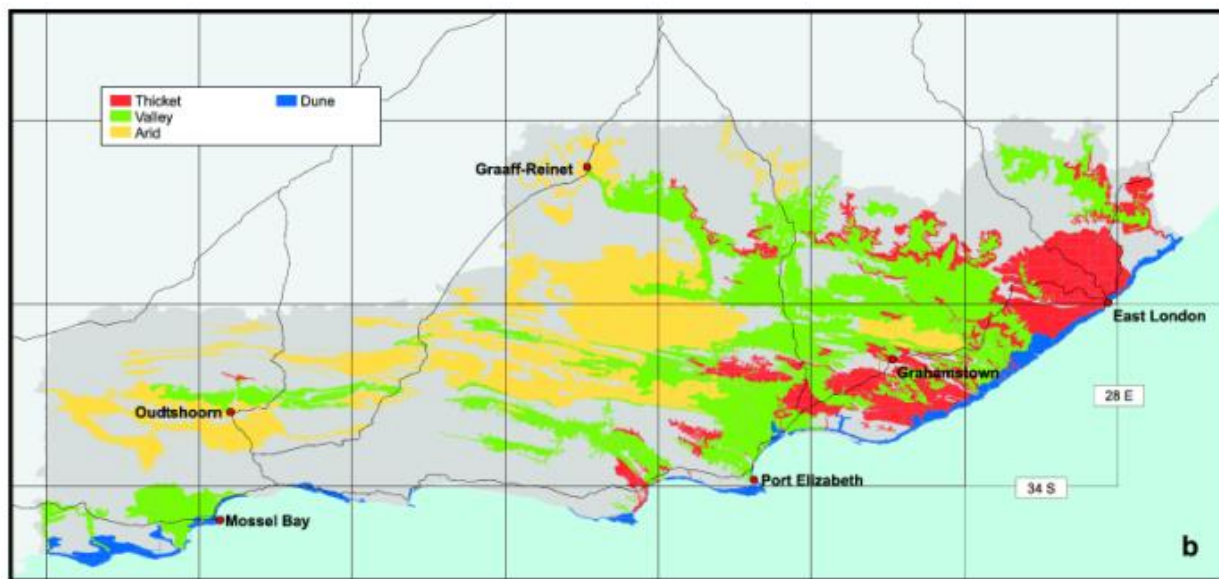


Figure 2: Subtropical Thicket distributional extent, categorized into the four core hierarchical divisions as defined and presented in Vlok et al. (2003).

Valley thicket is categorically the most speciose of the three and is indicative of a biome-specific hotspot in species endemism peaking in sites comprised of a mosaic between fynbos and thicket vegetation (Vlok et al., 2003). Situated throughout the drainage basins and river valleys stretching from the Western Cape well into the eastern limits of the Eastern Cape appearing no further than the Kei river. Valley thicket fittingly embodies the impenetrable nature to which this vegetation has been defined, consisting of dense stratum of woody trees and shrubs, characteristic species include *Azima tetracantha*, *Digitaria eriantha*, *Aloe africana*, *Aloe speciosa*, *Haworthia spp.*, and the conspicuous emergent succulent trees *Euphorbia grandidens* and *Euphorbia tetragona* (Cowling et al., 2005). The rainfall of valley thicket ranges between 400 to 800 mm.yr⁻¹ with a mean of 456 mm.yr⁻¹ and altitudinal distributional average of 380 meters (Vlok et al., 2003).

Mesic thicket in comparison to valley and arid thickets presents a milder climatic envelope, with generally wetter conditions than the latter two, receiving a mean annual rainfall of 586 mm.yr⁻¹. These more humid elements and increased water availability within mesic thicket is an artefact of its distributional occurrence, stretching to eastern vegetation limits past the mapped periphery of the Kei river. Moreover, mesic thicket rarely extends up into the escarpment, often occurring throughout the lowlands and sub-escarpment valleys (given sufficient moisture availability), with a mean modal altitude of 293 meters (Vlok et al., 2003). Mesic thicket strongly mirrors the characteristics of forested ecosystems, although stratification is less apparent, the height of trees often exceeds 3 meters and with minimal canopy gaps conditions beneath are analogous to those

of tropical forests (i.e. high humidity, reduced solar incidence). Species characteristic of mesic thicket include, *Olea europaea* subsp. *africana*, *Searsia pallens*, and *Scutia myrtina* (Vlok et al., 2003).

The work of Jan Vlok and Richard Cowling have been instrumental for “our” (i.e. South African ecologist’s point-of-view) understanding of the ecological principles driving this ancient and floristically complex biome providing innovative ideas as to the dynamics of thicket. Focus here and throughout this thesis is specific to arid thicket, while not species enriched it offers a composite of plants that should demonstrate to be both physiological tolerant and functionally diverse than the latter two thicket types. This subtype of thicket has been admired by many ecologists based on the capability of the vegetation at maintaining phytomass consistency while experiencing substantial physiological stress at both ends of the climatic spectrum, from protracted dry spells and droughts to freezing winter minimum temperatures and prevalent frost events (Hoare et al., 2006), and yet subtropical trees and shrubs have persisted with instances of distributional expansion which is further anticipated to increase under future climate projections (Potts, 2011). While the latter expectation is projected based on climate suitability predicted on existing distribution it doesn’t elucidate to the underlying physiological mechanisms that may explain the persistence of subtropical lineages within the arid ranges of Subtropical Thicket. The disparity between expected physiological range and actualized range of these subtropical trees has been one the fundamental challenges in understanding community ecology of the arid thicket subtype.

The challenge presented by evolutionary-origin-physiological-niche disparity in arid thicket is investigated in this thesis, with specific attention given to the water tolerance thresholds of the dominant canopy trees and shrubs. With further discussion around the possibility of interspecific interactions that may exist between the dominant succulent, *Portulacaria afra* and associated trees and shrubs. The significance of *P. afra* in arid thicket is instrumental, not simply for vegetation type delimitation, but as a key foundational species [synonymous with ecosystem engineer] – being characterized as a keystone species by Lechmere-Oertel (2003) and a miracle plant by Vlok & Euston-Brown (2002). The prevalence (i.e., geospatial frequency) and sheer abundance of this succulent impressed a lengthened discussion of its ecological relevance in arid thicket by Vlok et al. (2003) and thus requires further elaboration here as well. Spekboom (common vernacular for *P. afra*) recently garnered much attention chiefly as a target species for carbon sequestration programmes and in general the global carbon market with intention of offsetting hydrocarbon emissions incentivizing restoration practices — and is the world’s largest active restoration trial with an estimated 10 000 ha of truncheons planted across the greater southern range (Mills et al., 2015). Whilst from a scientific perspective spekboom is admired for its flexibility in photosynthetic expression – characteristically facultative Crassulacean Acid Metabolism (CAM; Ting and Hanscom, 1977; Guralnick and Ting, 1988), alternating between C3 and CAM as a stress-induced response and secondly as abiotic ameliorator providing a more conducive, physiologically relaxed microclimatic sub-canopy (Lechmere-Oertel et al., 2008; Mills & Cowling, 2010; Wilman et al., 2014).

Spekboom is indicative of what might constitute an ecosystem engineer, augmenting proximal microenvironmental conditions, subsequently generating milder microclimates promoting subtropical tree and shrub germination and establishment in dry, arid landscapes which would not be possible without amelioration effects of this prominent succulent. This dependency on amelioration facilitated by spekboom has led many to speculate as to the importance of this

succulent to maintaining biodiversity within the arid thicket. Until recently the role this species might represent to community dynamics beyond ecosystem functional contributions has been poor with a lone publication (see Adie & Yeaton, 2013) exploring its relevance as a facilitator [i.e. nurse plant] in arid thicket with only mentions of such an attribute amongst the literature. Such an underlying dependency on *P. afra* as a facilitator would strongly concur with the proposed theory of niche conservatism stated by Wilman et al., (2014) – suggesting woody canopy species are ecologically fixed with respect to their germination requirements and seedling establishment tendencies mirroring those associated with ancestral origins of the moisture, humid Eocene forests (Willis & McElwain, 2002; Cowling et al., 2005). This hypothesis postulating niche conservatism at the seed and seedling level has further reaching implications than considered by Wilman et al., (2014) potentially extend to other lines of functional ecology driven trade-offs, strategies and/or traits [this will be discussed in-depth below].

2.6. A biome of fire and ice: Albany Subtropical Thicket a tale through time, drivers of distributional change – a physiological perspective.

The AST, and more specifically Arid Thicket has been characteristically ascribed as a “remarkable biome”, chiefly due to its high standing phytomass of the dominant perennial woody canopy trees and shrubs, which depict minor fluctuations in biomass, even under drought conditions (Lechmere-Oertel, 2003). The observation of drought tolerance has remained for a large part a curious caveat of this “rainforest-derived” vegetation for many years. While there is no doubt as to the question of drought resistance strategies and/or tolerance of succulents within arid subtypes of thicket and at the dry range edges of AST (e.g., CAM species) — the persistence of woody canopy species remain elusive and of focus for this thesis. However, there is a marked disparity between the dominant woody complement species occupying arid thicket than those dispersed in mesic and valley counterparts (Vlok et al., 2003; Cowling et al., 2005). These changes in species complement with considerable changes in the dominance of individual species highlight the skewness in distributional evenness and impress the broadscale role of climatic associated macro-evolutionary relationships at play. Pronounced changes in environmental variables can have several consequences for a species range from augmenting climatic niche availability thus heightening the risk of extinction, conversely increasing niche space facilitating distributional expansion; and lastly drive adaptive response to new environmental states. For example, the climatic shift during the middle-to-late Miocene (5-13 Ma) from cold to drier more arid conditions was associated with vegetation changes in Fynbos flora and considerable diversification in Aizoaceae of South Africa (Dupont et al., 2011).

The AST has its origins from subtropical-tropical lineages as far back as the Eocene (Cowling et al., 2005) formed from forest flora established during humid and warm conditions during the early Paleocene. As time progressed and climate became drier and more arid from the mid-to-late Miocene that would have coincided with the contraction of more mesic species and correspondingly the persistence of the more drought tolerant thicket species would have been apparent. The Neogene aridification (~12-5.3Ma) is accredited for the contraction of both Subtropical Thicket and forest from its pre-Eocene western extension being replaced by more xeric vegetations types. (Cowling et al., 2005; Ramdhani et al., 2010; Allsopp et al., 2014). This global scale aridification, coupled with fire disturbances (Bond, 2015), would have resulted in substantial contraction in regional distribution of thicket and forest vegetation, retreating to moist,

mesic and fire-protected valley refugia, and would have certainly resulted in many endemic species extinction.

These shifts in community and associated species level processes would have ever more apparent during the glacial and interglacial cycles of Plio-Pleistocene and its general climatic instability and fluctuations extirpating species (Hamilton, 1983). In southern Africa, the climatic state of this period has been of great interest as to how it would have shaped the paleoenvironment of the time and influence the biogeography of major lineages, but most significantly its role in guiding human evolution effecting early our ancestors (Partridge, 1993; Peter et al., 2004; Marean et al., 2007). Reconstructions and models have played a main role in revealing the past conditions however much of the research use terrestrial proxy records that provide a somewhat representative interpretation of the environment. Most of the research focuses on the most recent changes in climatic and environmental states from the Pleistocene to more recent Holocene epoch.

The mid-to-late Pleistocene (390-12kya) was characterized by a series of interglacial and glacial periods, with coincided with a general global decline in atmospheric temperatures (as much as 5-6°C), with a concurrent shifts in seasonal precipitation regimes (Talma & Vogel, 1992; Chase & Meadows, 2007). Superimposed across these intervals were the appearance of hyper-arid conditions which strongly aligned to precipitation seasonality effecting large areas during these periods (Partridge, 1993). And while initial interest has mainly focused on the effect lower temperatures have had on the vegetation with early indications being that of substantial contraction in distributional range of the AST, chiefly as the increased prevalence of frost events impacting survival of certain species within thicket that are largely frost-intolerant (Duker *et al.*, 2015). Much research has spoken to the ramifications of low temperatures, more specifically the effects of frost, in defining distributional range of the AST (Duker *et al.*, 2015a,b; Duker *et al.*, 2020) – postulating the retreating into thermal refugia during glacial periods (Potts *et al.* 2013) – little work has dealt with the coincidental heterogenous shift in rainfall regimes translating into increased aridity – and by extension the frequency, severity, and duration of drought and possible heatwaves experienced by the AST, particularly at dry range edges (i.e. Arid thicket subtypes, c. Spekboom Thicket and Spekboomveld). Moreover, even less research has explored the physiological implications aridity might have posed to the long-lived perennial canopy species and corresponding changes in water availability might have on thicket distribution and community composition (classification subtype).

Given the role of aridity has played in Subtropical Thicket biogeography in the past and the selective task it currently effects on the current distribution of thicket understanding the physiological limits of core component species will be essential in predicting future changes in this historical diversified vegetation. The influence of aridification has been central to species evolution both as a directional selective pressure, but additionally as a driver for extrinsic ecological change forming distributional barriers and subsequent allopatric speciation due to regional isolation. While there remains much to explore in thicket this thesis considers the role of aridity as a selective force by explaining respective physiological threshold collating to the species survival potential under its current distributional range. This thesis postulates that two primary ecological drivers might have had a considerable role in Subtropical Thicket's evolution and biogeography through time, namely aridity and frost. Where aridity driven by changes in atmospheric water and precipitation deficits acted at a larger widespread, regional scale and frost played a more subtle role due to topographical driven effectors (Duker et al., 2020). Here, the role

of aridity, a largely underappreciated ecological process, is highlighted and proposed as a major factor in driving thicket distribution. Understanding how –physiologically – these species persisted through the climatic fluctuations of the Plio-Pleistocene, and the current environmental conditions of today (and potential response under climate change in the future) has been primarily speculated or theorized in the literature with little to no validation of the physiological properties of the dominant species present in Thicket.

These heterogenous shifts in seasonal precipitation have raised the significance of understanding the water dynamics in this vegetation. Most importantly the arid subtypes/forms (Spekboomveld, Spekboom Thicket), where water represents a major ecological determinant of species-level survival and performance; community-level dynamics and assembly; and ecosystem-level functioning. Water availability is a major ecological driver with far reaching implications for community structure and assembly via its effects on species niche partitioning, coexistence and plant functional trait expression (divergence or convergence) – this thesis will provide much need data on species-specific hydraulic functional strategies that will enrich our understanding of the physiological limitations and tolerance thresholds of major thicket species. This will aid predictive interpretations of the implications increasing aridity and climate change will have on community dynamics, composition, and assembly.

2.7. Forgotten, but not lost: Thicket transformation and future stability/sustainability

The AST is a highly biodiverse biome consisting of over 1553 species of which at least 20% are endemic (Vlok *et al.*, 2003), and has historically supported a rich abundance of indigenous mammalian herbivores across a range of size-classes from the large elephant (*Loxodonta africana*), medium sized Kudu (*Tragelaphus strepsiceros*) to the small common duiker (*Sylvicapra grimmia*). Anecdotal accounts by early settlers and travelers from as far back as 1800s have reported the prevalence of meso- and megaherbivore in thicket. These early observations coupled with the physiognomy and structural attributes of thicket infer the evolutionary significance herbivory has had on this biome as demonstrated by the classic characterization of the “spinescent” nature of thicket. Herbivory is regarded as the most significant disturbance factor in the AST and represents crucial ecological process – recruitment. For example, recruitment of canopy tree ramets is facilitated by top-down browsing pressure from elephants (Stuart-Hill, 1992) and genet recruitment is improved by the dung of black rhinos (La Cock, 1992) — suggesting coevolutionary undertaking between megaherbivores, as disturbance agents, and thicket vegetation. Thickets capacity to retain and maintain phytomass and plant coverage with little to no variation under climatic stressed environments (i.e. ratio of carbon sequestered to water availability) distinguishes it from other disturbance driven ecosystems, such as grasslands and savanna where fire and climate in conjunction with herbivory are factors dictating plant biomass and community continuity.

Disturbance, specifically the regime (frequency and intensity) that the vegetation experiences has a profound effect on the resilience and recovery for plant community. The capability of a community to retain functional integrity while tolerating shifts in biotic and abiotic factors in an environment describe its resilience, while recovery infers the ability of a disturbed ecosystem to return to its pre-disturbance state. Understanding how disturbance impacts an ecosystem or at a more local scale a plant community is essential for assessing the vulnerability of these systems towards anticipated perturbations in disturbance regimes and identify key thresholds of recovery

and resilience. In the context of the AST herbivory by indigenous browsers is regarded as a low disturbance (i.e. reducing biomass but retained patch structure) – consequence of their coevolutionary history with this vegetation (Kerley et al., 1999) – while domestic herbivory via goats seems to result in “run-away” ecological degradation. The cascading degradation of thicket is accumulative response to centuries of pastoral browsing by domestic ungulates which began in the early 1800s with the arrival of European settlers coupled the extirpation of megaherbivores and reductions in population size of other thicket associated browsers such as Kudu and duiker (i.e., hunting). The use of Subtropical Thicket for agro-pastoral activity is predicated on the perceived abundance of high-quality forage and expectation that due to its relative abundance and general stability under variable climatic conditions that it provides the ideal food source.

The abundance of high quality forage was the rationale behind the extensive conversion of thicket to agro-pastoral land culminating in larger than average stocking densities of domestic ungulates ascribed to the supportive capacity of thicket at sustaining megaherbivores. However, there were domestic ungulates that could maximize their use of this forage, with goats being the most successful pastoral browsers capable of exploiting both understory shrubs and taller canopy trees (i.e. targeting lower branches by shifting to bipedal stance with their front limbs propped against the tree bole).

Despite the coevolutionary relationship between thicket and herbivores, the vegetation is incapable of tolerating anthropogenically facilitated goat browsing which has resulted in extensive degradation and ecosystem dysfunction across the AST, particularly in arid subtypes. The extent of degradation is estimated to exceed 42% for mesic thicket types, while more arid subtypes as the later mentioned range between 60-88% (Lloyd et al., 2002). The degradation of arid thickets, specifically Spekboom Thicket is ascribed to two key factors, the first is foraging behavior of goats and the second the life history strategy of the vegetation consisting of long-lived species with slow growth rates. Furthermore, the manner of defoliation by domestic ungulates is localized from branches along the periphery continuing inwards towards the centre of individual or bush clumps preventing vegetative recruitment, in contrast to top-down browsing of indigenous herbivores. The second compounding factor to Spekboom Thicket recovery under pastoral herbivory is the long-life of constituent species, indicative of conservative strategies, conforming to Midgley and Bonds (1993) persistence niche theory. This conservative life history strategy demonstrated by the slow growth rates of the canopy trees, and coupled with the lengthy recovery period of spekboom to defoliation (e.g. 18 months to recover from 50% loss in foliage) increase the vulnerability of this vegetation to disturbance, particularly domestic pastoralism.

The progressive degradation of Spekboom Thicket – specifically the removal of palatable spekboom – in arid landscapes poses a considerable threat for community stability and woody canopy tree continuity. In its intact state Spekboom Thicket is characterized by the dominant succulent *P. afra* interspersed with long-lived, evergreen canopy trees (*Pappea capensis*, *Euclea undulata*) and interwoven with multistemmed spinescent shrubs (e.g. *Azima tetracantha*, *Putterlickia pyracantha*, *Searsia longispina*). The effect of indigenous herbivores can be seen across solid vegetated stands as bare ground patches and pathways throughout the dense vegetation. Furthermore, this high degree of succulence coupled with the lower availability of dry fuel loads is the reason for the absence of fire and its consideration to not being an ecological driver in Spekboom Thicket. Where under extensive browsing Spekboom Thicket is transformed into less productivity state, consisting of an extensive field layer of ephemeral grasses and shrubs of karroid origin. Fabricius (1997) presented a conceptual step-wise model describing the

degradation of semi-arid subtropical thicket of the Great Fish River valley. Briefly, he described degradation based on the loss of biomass in perennial bushclumps, translating into reduced clump size lessening the ameliorating effects of the clump as a refugia from climatic strenuous conditions. The transformation of large, dense vegetation patches coupled with increased proportional bare ground cover described a homogenization of the landscape resulting in the general decline of species diversity and ecosystem functionality under goat browsing.

The impacts of degradation have clear outcomes when considering both phytosociological and ecosystem perspectives in thicket. While much attention has focused on the implications of goats and introduction of other domestic livestock, with even great debate around indigenous game and their impacts, the effects of other disturbances such as heatwaves, drought and pest outbreaks have received far less. Drawing focus to these factors are important given the semi-arid to arid nature of thicket. Historically the propensity for drought is greatest in arid ecosystems where climatic variability is greatest, consequently it is not uncommon in thicket, particularly arid thicket. These periods of characteristic reductions in water availability place significant strain on the productivity of the region, reducing plant growth, nutrient cycling, and eventual mortality of species. The implications of water availability are far-reaching implicating carbon balance, where continued drought conditions would deplete carbon reserves further heightening the risk of mortality. If coupled with the carbon demanding process of herbivory would constitute a great threat to the sustainability of vegetation under highly water deficient conditions. A more alarming outcome is the increased appearance of insect outbreaks during drought conditions, escalating the physiological strain on vegetation significantly influence carbon balance. Regional temperature greatly influences the developmental rate, emergence and survival of insect pest populations. In response species might upregulate defense compounds (e.g., monoterpenes) or resins to deter insects, incurring even greater carbon costs. The impacts of drought has largely been underrepresented within the thicket literature with few comments only being made as to how remarkable the vegetation's capacity to maintain biomass coverage during extended periods of low water availability.

Drought is a severe disturbance, particularly in semi-arid to arid habitats where extended periods of water deficits and atmospheric water demand can be felt for plants. Plants inhabiting arid and semi-arid environments have a tendency to demonstrate a modular architecture consisting of several aboveground clonal stems thereby denoting greater hydraulic redundancy (Schenk et al. 2008). This strategy enables the plant to discard or experience dieback in aboveground tissues while still maintaining a viable reproductive belowground biomass to endure extreme disturbances such as fire or drought (Bond and Midgley, 2003; Zeppel et al., 2015). This avoidance strategy may limit risk of whole-plant dieback and mortality under severe water deficits or drought conditions. This resprouting strategy characterizes much of the thicket woody tree and shrubs supporting the proposed theory of the resprout trait appearance to be a function of disturbance severity as suggested in Zeppel et al., (2015), highlighting the historical climate evolutionary relationship drought or herbivory might have played in thicket.

The co-occurrence of multiple environmental stressors (e.g., drought, herbivory, or insect outbreaks) is predicted to increase in frequency in the near future with major changes in precipitation regimes and water availability trends posing a great threat for thicket and species persistence within these arid environments. Therefore, a mechanistic understanding of drought-related impacts and more importantly mortality is paramount to providing a comprehensive assessment of the compounding effects of drought on thicket degradation.

2.8. Physiological responses to drought: Water regulation processes maintaining plant-water homeostasis

Water is biological imperative at every organizational level of a plant, from cell metabolic activity to physiological maintenance (e.g., as a solvent, transportation of solute, and reaction catalyst – substrate or reactor) to whole plant level (e.g., transportation of macromolecules) (Chaves et al., 2003). The role of this incompressible fluid extends beyond plant physiology to mechanical conservation and structural support of tissues, chiefly via passive pressure gradients (i.e. turgor), with additional carbon investments in structural support features (fibers and lignified cell walls). The functional duality of water within plants infers great demand for this, often, limited resource, which is made ever more apparent considering that nearly all water extracted/absorbed is lost to transpiration via the leaves with less than 1% being assimilated into phytomass (Lambers *et al.*, 2008). This inefficiency at regulating water loss is an inevitable consequence of the structural feature response for photosynthesis — stomata. Undoubtedly stomata have played a foundational role in plant evolution, facilitating the colonization of land by terrestrial plants some 500 million years ago (Berry et al., 2010). Stomatal pores regulate the frequency of gaseous movement between the atmosphere and mesophyll cells of the leaf, thus controlling CO₂ entry into the leaf. These internal airspaces represent the site of gaseous exchange for the plant, expelling O₂ (i.e. accompanied by water) and absorbing CO₂ which dissolves in water at the surface of mesophyll cells. This moist site is where water loss is most proficient driven by atmospheric water vapor deficit – internal airspace is 100% relative humidity while the surrounding ambient conditions are less so generating pressure gradient easing the movement of water outside the leaf (Buckley, 2005).

Stomata thus play a key role in plant response water availability ensuring plant metabolic (i.e. CO₂ uptake) and mechanical support (i.e. turgor pressure) of the plant (Buckley, 2005). The importance of stomatal pores is denoted by their abundance covering up to 70-80 times the leaf surface area indicating the relevance these characteristic structures represent for whole plant metabolic and structural homeostasis (Bertolino et al., 2019). From exerting major effects on plant productivity and growth, to large scale (regional to global) regulation over water and carbon cycling (Henry et al., 2019). The dominance (size and frequency) of stomata is dependent on both environmental conditions that the plants inhabit (acquired adaptation) and the relative position of the leaf in space, i.e., disproportionate solar exposure on the surface of the leaf results in the aggregation of stomata on the adaxial side (Xu & Zou, 2008). The motivity of stomata – i.e., stomatal aperture dynamics – are influenced by shifts in turgor pressure of guard cells and adjacent subsidiary cells, with the net change influencing aperture size and activity (Rodriguez-Dominguez et al., 2016). These changes in turgor pressures effect the stomatal aperture, and subsequent stomatal conductance, which are driven by hydrologically passive or active flux mechanisms (Martin-StPaul et al., 2017). The former invokes shifts in water potentials, while the latter employs changes in osmotic potential (Buckley, 2005), both represent vital mechanisms at regulating plant water relations.

Understanding the role of leaf water relations therefore becomes paramount to conceptualizing the mechanistic processes responsible for stomatal conductance in order for plants to maintain turgidity necessary for sustaining photosynthetic activity and growth. The water status of leaves is quantified by their respective kinetic properties, i.e., water potentials, of two key components controlling stomatal activity: turgor pressure and osmotic potentials (Chaves et al., 2016; Martin-

StPaul et al., 2017). Turgor pressure of a cell, which can be scaled up to leaf, is derived from the rigidity of the cell wall, constructed to resist compression and expansion outside a predetermined tolerance range (i.e., species-specific) resulting in a positive hydrostatic pressure gradient in the cell, prompting water movement/flow inwards — this is the principle driving force of water throughout the plant (Buckley, 2019). When exposed to water deficit conditions the net movement of water is conventionally outward from the cell compelled by the overall water pressure potential demand of surrounding tissues driven negative atmospheric water vapor demands. In these circumstances, water movement out the cell results in declining turgor pressure and decreasing cell volume linearly with relative water content (Lenz et al., 2006).

If water deficiency continues so turgor pressure decreases approaching values near zero (values of zero imply a complete loss in plant water uptake potential, the threshold where plant water potential is equivalent to the environment), until such point as complete turgor is lost. This turgor loss point is frequently referred to as the “wilting point”, accredited to the expression of leaf placcidity at this threshold, denotes the point at which water uptake from the soil is hindered as the sediment matrix potential exceeds that of the plants water potential resulting in net water movement from the plant (Bartlett et al., 2012; Blackman, 2018). In order to prevent such water loss scenarios the plant employs two key strategies to remedy discrepancies in water potentials are osmotic adjustments (i.e. short-term response) and modification of structural attributes, specifically cell wall elasticity (i.e. long-term adaptive response) (Bartlett et al., 2014). As soil dehydrates through evaporation the matrix potential of the sediment declines exceeding water potential of plant cells facilitating passive water transfer to surrounding sediment or the atmosphere at the leaf surface driven by lower water vapor deficits. In response to outward water motivity and potential desiccation risk it posits plants accumulate osmotically active compatible (i.e., enzymatic, and reactively neutral) compounds within the cytoplasm or store in the vacuole — reducing the osmotic potential driven gradient and thus, water potential of the plant (Martorell et al., 2015). Consequently, the adjustment of solute concentrations and accompanying compatible compound (e.g., proline) accumulation within the cells increases plants respective turgor when fully hydrated maintaining cell rigidity. This osmotic adjustment reduces the water potential at turgor loss point enabling plants to sustain a more positive hydrostatic pressure in tissues (Nolan et al., 2017).

An alternative mechanism for the maintenance of water relations within plants corresponds to the cellular structure properties of the species and/or the subsequent modification thereof (Moore et al., 2007). As cells lose water their volume decreases until such point, as turgor loss point, is reached and then the cells become flaccid resulting in the diminishment of most metabolic functionality. The degree to which cells tolerate the decline in volume and thus, the level to which water potential can drop until reaching the turgor loss point is dependent on the capability of cell walls to maintain structural integrity, chiefly via elasticity (Fan et al., 1994). The greater elasticity the improved tolerance towards drought and/or water scarce environments. As cells presenting highly elastic cell walls can accumulate, therefore contain, a higher quantity of water at full turgor (Lenz et al., 2006). This enables cell volume to decrease even further before turgor loss point is reached, thus plants with higher cell wall elasticity have a greater capacity to store water, while additionally retaining mechanical and structural integrity thereby conserving the underlying positive hydrostatic force (turgor) driving water flow into the cell once moisture conditions return (Lenz et al., 2006; Bartlett et al., 2012). It is worth noting that cell wall elasticity is expressed as the elastic modulus of the cell, otherwise referred to the bulk elastic modulus of the plant organ (e.g. leaf).

The maintenance of cell turgor whether by structural modifications (i.e., adaptive selection such as cell wall elasticity) or osmotic adjustment (i.e., reactive adaptation) it is paramount to sustaining hydraulic and stomatal conductance, CO₂ assimilation, and survival under conditions of low water availability, such as drought (Zimmerman, 1978). The significance of cell turgidity as a predictor of whole plant physiological functionality, structural integrity, and performance has elevated the need for a universally applicable metric by which to quantify drought tolerance of plant species. Thus, turgor loss point has recently garnered attention and recognition as a “higher-level” trait quantifying leaf and whole plant drought tolerance (Bartlett *et al.*, 2014), based on pattern that greater negative values of turgor loss point extend the functional range – turgidity retention – of leaf water potentials (Sack *et al.*, 2003; Lenz *et al.*, 2006). The power of this parameter as metric of drought tolerance is demonstrated in Bartlett *et al.* (2012) global meta-analysis of 317 species across varying plant functional types (e.g., woody Mediterranean pines to tropical shrubs) where the correlatory strength of turgor loss point with species habitat water availability is illustrated. A key finding of this comprehensive investigation is that shifts in turgor loss point is driven chiefly by osmotic adjustment, i.e., osmotic potential at full turgor, and not bulk elastic modulus.

Turgor loss point adjustments are regarded as one of several mechanisms employed by plants to improve drought tolerance, hence maintaining physiological functionality under more arid conditions. There are three means by which plants could potentially lower their respective turgor loss point, the first being by solute accumulation (thus decreasing osmotic potential), minimizing symplastic water content of the cell, and lastly modifying cell wall elasticity (Bartlett *et al.*, 2012). Of the aforementioned strategies, osmotic adjustment has most widely been observed in many species enabling the continuance of basal metabolic and physiological function under drought (Merchant *et al.*, 2007). Under water stressed conditions, plants upregulate the synthesis of numerous organic and inorganic solutes ranging from polyamines to soluble sugars such as sucrose all attributing to a reduction in cellular osmotic potential (Silva *et al.*, 2010; De Diego *et al.*, 2013). However, the significance of the other two strategies to maintaining turgor, namely apoplastic water accumulation and cell wall elasticity may present to drought tolerance of plants is the largely unclear (Lenz *et al.*, 2006). Moreover, the latter strategy, of cell wall elasticity modification, requires the upregulation of protein expression and microfibril production to accommodate plasticity of the cell wall presenting a carbon cost (Le Gall *et al.*, 2015). Alternatively, a reduction in elasticity with corresponding boost in cell wall rigidity would preserve hydric status with decreasing water potential (Tyree and Karamanos, 1981). Hence, cell wall elasticity is an ambiguous drought adaptation as either an increase or decrease is indicative of a strategy to drought tolerance (Lenz *et al.*, 2006).

The core mechanism and primary physiological response to dehydration employed by plants is through limiting evaporative loss (Stephenson, 1990; Tombesi *et al.*, 2018). This can be achieved through several means including increasing water acquisition by adjusting root: shoot ratios [a paradoxical approach given drought-induced growth inhibition is widely observed phenomena (Anjum *et al.*, 2011)], a more practical solution that is found across higher plants is reducing stomatal conductance (Zhou *et al.*, 2013). Stomatal regulation represents a rapid defense against desiccation; however, the underlying mechanistic changes in conductance remains largely unclear, involving a complex interaction between both detection of biophysical changes expressed on mesophyll cells and subsequent response trajectories that include hormonal response (Yaaran *et al.*, 2019). The phytohormone Absciscic acid (ABA) is upregulated as the plant dehydrates the core responsibility of ABA is to optimize water use (Sack *et al.*, 2018). Such optimization is achieved through two primary cellular physiological adjustments in leaves

promoting the closure of stomata, and at the roots stimulate water absorbency through morpho-physiological alterations (Hartung et al., 2005; Kuromori et al., 2018). Stomatal aperture changes is considered to be one of the main means by which plants offset water losses, where induced by changes in biophysical properties such as turgor of mesophyll cells or ABA pathways (Sack et al., 2018) – and is a vital physiological pathway for interpreting species drought response and physiological vulnerability to water deficiency.

Understanding the physiological response to drought are pivotal in reasoning the possible strategies plants may employ to avoid and/or tolerate such water deprived conditions. To this end, such a physiological perspective would provide a rationale upon which drivers of mortality and sequence of dehydration thresholds might be defined. Given the global prevalence of drought and observed trends in tree mortality (Allen et al., 2010; Breshears et al., 2005), and general reduction in vegetation productivity it is paramount to identify the physiological thresholds to ensuring species persistence and ecosystem resilience. In order to predict how global change disturbances and climatic anomalous events, such as drought, are to potentially influence forested ecosystems, such as Subtropical Thicket, a greater understanding of the physiological drivers of mortality are needed.

2.9. Drought-induced tree mortality: Physiological mechanisms.

The attention of much ecological research in the recent decade has been devoted to understanding plant drought responses and adaptive strategies providing a hydraulic framework under which to predict mortality risk and plant vulnerability to changes in water availability (Choat et al., 2018). While recent focus has been given to devising an appropriate predictive framework, based on underlying physiological mechanisms, of drought vulnerability its significance was described almost four decades earlier. With the proposed “slow decline” hypothesis by Manion (1981), who attributed tree mortality to initial exposure to a long-term stress predisposing the individual to short-term stressors. Later research has built onto this hypothesis, one prominent addition was citing the plant’s capacity to maintain carbon balance as a fundamental determinant of future mortality risk (O’Grady et al., 2013). Based on these earlier works, McDowell et al. (2008) proposed a hydraulic framework presenting two chief hypotheses describing the underpinning mechanisms of drought-induced mortality. These physiological based hypotheses of McDowell et al. (2008) framework where (1) hydraulic failure and (2) carbon starvation, which indirectly increases the vulnerability and relative impact incurred by biotic factors such as herbivory and disease/pest attacks, impact plant survival.

According to McDowell et al. (2008) carbon starvation hypothesis posits that in order for the tree to survive under water deficit conditions a corresponding downregulation in stomatal conductance is a major strategy preserving plant water relations, however, at the expense of atmospheric gaseous exchange. Consequently, the plant is deprived of CO₂ reducing carbon assimilation necessary to produce the required photosynthates to sustain basal metabolic respiration and regulatory processes such as osmoregulation (O’Brien et al., 2014; Dietze et al., 2014). Although, mortality isn’t immediate as plants may capitalize on stored carbon reserves (i.e., non-structural carbohydrates) over the short-term, if drought stress persists in the long-term plants will face the real prospect of carbon depletion and eventual starvation leading to tree death (Erbilgin et al., 2021). The second hypothesis proposed by McDowell et al. (2008) is potential hydraulic failure, defined by hydraulic dysfunction of xylem conducting tissues (i.e., tracheids and vessel elements)

– either by cavitation or air seeding both resulting in emboli formation – disrupting water transfer between roots and leaves leading to modular or whole-plant desiccation and eventual death. Hydraulic failure occurs in situations where the soil water availability and the evaporation demand culminate in the disruption of hydraulic conductance and ensuing xylem embolism (Mitchell et al., 2013). To avoid such circumstances one strategy plants employ is the regulation of stomatal conductance, reducing the pore aperture, circumventing the development of extreme declines in xylem water potential attributed to increased evapotranspiration and maintaining water transport continuity. This ensures plants avoid potential hydraulic failure, although at a loss of CO₂ uptake and photosynthetic activity (Farquhar & Sharkey, 1982).

Much debate surrounds the dominant physiological mechanism (carbon starvation or hydraulic failure) describing tree mortality trends under drought; and general tolerance to water deficient conditions, i.e., adaptive mechanisms enhancing performance and survival in arid environments. Only recently has it been widely acknowledged that both hydraulic failure and carbon starvation are not mutually exclusive physiological mechanisms for drought-induced mortality, rather they are interdependent and strongly reliant on stomatal stringency, thus both may co-occur under drought conditions (McDowell et al., 2011a, b). For example, Hartmann et al. (2013), using an innovative experimental setup capable of distinguishing the effects of water and carbon dynamics in *Picea abies*, demonstrated that hydraulic failure achieved lethality before carbon starvation occurred, except in scenarios where carbohydrate use is limited. The results presented by Hartmann et al. (2013) not only illustrated the interdependency of these two processes but emphasized its importance to phloem functionality in reducing species drought vulnerability. To put this new interdependency into context, consider the following scenario: during drought stress plants reduce stomatal conductance limiting both water loss and CO₂ uptake, in so doing decreasing photosynthesis while cellular respiration continues. As such accessible carbohydrates (i.e., non-structural) face competing demands for maintaining hydraulic stability (i.e., prevention of turgor loss and refilling embolized xylem conduits retaining water continuity within the plant for limited photosynthetic turnover or photoprotective quenching) and supporting respiration which is extenuated under high temperatures and limited water supply. As drought progresses and stomata remain closed the competing carbohydrate demand between basal respiration and maintenance of hydraulic conductivity continues until such time as either process exceeds a critical threshold concluding in plant mortality. Thus, hydraulic failure and carbon starvation represent the two extremes of the drought-induced mortality spectrum, upon which physiological strategies and hydraulic traits avoiding death may be elucidated to.

Hydraulic dysfunction is accredited as the underlying cause of tree mortality towards drought as made evident by numerous reports across species (Hoffman et al., 2011; Anderegg et al., 2013), documenting a substantial loss in hydraulic conductivity, while carbohydrate availability remained stable. Whereas evidence to suggest the role of carbon starvation as compounding factor in tree mortality is lacking (Sala et al. 2010; Hartmann, 2015; Hartmann et al., 2015), existing research does little to refute the relevance of carbon depletion as a contributing factor (Gessler et al., 2018). O'Brien et al. (2014) observed a strong positive association between survival time and non-structural carbohydrates. Although, their paper might seem to validate the carbon starvation hypothesis put forward by McDowell et al. (2008), the increased survival time or delayed mortality of individuals could just as well be attributed to the retention/maintenance of hydraulic conductivity via osmoregulation facilitated by non-structural carbohydrates. These results confirm the findings of Sevanto et al. (2014), where longer time-to-mortality coincided with reduced concentrations of non-structural carbohydrates at death, suggesting the osmoregulatory

effects of carbohydrates. Sevanto et al. (2014) concluded that carbohydrates play a crucial role in whole plant water balance, not only sustaining metabolism, where impairment of carbon transport or depletion of carbon reserves might result in hydraulic failure. This challenges the idea put forth by McDowell et al. (2008) that assumes carbon starvation and hydraulic failure as independent physiological mechanisms describing drought-induced tree mortality in environments experiencing considerable levels of water deficiency. Instead, carbon starvation is consequential outcome of carbohydrate facilitated turgor maintenance and osmoregulation indirectly prolonging plant survival as opposed to proposed direct mortality delay suggested by the existing hydraulic framework (McDowell et al., 2008).

The ambiguity surrounding the underlying uncertainties or misinterpretations of carbon starvation as a direct mechanism delaying drought-induced mortality, are a consequence of temporal expressions in tree fatality. Where carbon starvation induces mortality over the long-term, given that the plant has appropriate tolerance strategies, while hydraulic failure is expressed over the short-term. This discrepancy in time-to-mortality has often been neglected in research focusing on plant physiological tolerance towards drought and its accompanying response strategies, where carbon starvation is a compounding factor to drought in the long-term as the depletion of non-structural carbohydrate reserves diminishes the plant's capacity to maintain hydraulic conductivity. Hydraulic failure is more apparent attributor of drought-induced tree mortality as water requirement is immediate across all organization levels of a plant, from sustaining respiration to ensuring photoprotection of thylakoid membranes maintaining photosynthesis under stress via nonphotochemical quenching (Muller et al., 2001). Therefore, prioritizing hydraulic failure as an immediate threat of drought-induced tree mortality (Anderegg et al., 2015), thus selective pressure, as opposed to the protracted threat of carbon starvation, although under certain environmental circumstances, i.e., prolonged water scarcity, and efficient hydraulic resilience (thickened xylem vessels) carbon starvation will be key cause of mortality facilitated by impaired phloem transduction.

The significance of hydraulic functionality as a prime candidate for predicting tree mortality can be seen in Anderegg et al. (2016) global meta-analysis where physiological traits such as hydraulic safety margin and xylem water potential strongly correlate to drought-induced fatality. Moreover, a recent multi-species appraisal by Adams et al. (2017) discovered that hydraulic failure was ubiquitous amongst species denoting greater than 60% loss in xylem hydraulic conductivity at mortality with no discernable universal applicable response, exception of gymnosperms and species-specific angiosperms, in non-structural carbohydrate reserves at mortality. This highlights hydraulic functional thresholds as key priorities for providing a mechanistic framework predicting vegetation vulnerability to climate change. Collectively, the works of Anderegg et al. (2016) and Adams et al. (2017) strongly motivate the use of hydraulic traits and related physiological functional thresholds (e.g., P_{50}) as a predictive metrics and a tool for forecasting species-level drought-induced mortality.

2.10. Plant functional traits as proxy for drought vulnerability of Thicket trees.

The capability of plants to maintain whole-plant functionality during environmental strenuous periods, such as drought, are well understood via functional attributes or traits that sustain or improve performance under these stressful conditions (Bartlett et al., 2016; Venturas et al., 2014; Powell et al., 2017). Plant functional traits are defined as any phenological, morphological, or

physiological attribute quantifiable at the individual level which influence plant-level performance via associated impacts on recruitment, growth, and survival – culminating in effects on fitness of a species (Violle et al., 2007). Many of these traits vary systematically across environmental gradients and are regarded as adaptive trait-environment correlations conferring optimal species performance strategies in their respective habitat. The success of functional traits as excellent proxies or predictors in plant ecological, or rationale for evolutionary, deductions is attributed to the presence of trait coordination, permitting the identification of trade-offs between varying phenotypic expressions (i.e., morphological, physiological or phenological) of a species (Violle et al., 2007; Rosas et al., 2021). A seminal example illustrating trait coordination and trade-offs can be conceived under the conceptual Leaf Economic Spectrum (LES) first proposed by Wright et al. (2004) describing leaf structural discrepancies between as species associated with maximizing photosynthetic turnover under varying degrees of optimality – a cost-benefit approach to biological form and function. This cost-benefit paradigm based on functional trait coordination and subsequent trade-offs thereof is represented as a dichotomy between “conservative strategies” (i.e., high structural investment, long life span, conveyed as impeded physiological turnover) to “acquisition strategies” (i.e., maximized physiological activity, low structural investment short life span). These early studies and paradigms to understanding plant-environmental strategies laid the foundation upon which modern interpretations of species physiological tolerances, niche occupancy and community dynamics are based, providing quantitative data points by which correlative distinctions can be made (Ackerly, 2003). The use of functional traits has enabled ecologists to address a number of ecological questions, e.g., determining community assembly processes, and enhancing precision of vegetation modelling systems to integrate trait-based responses to environmental perturbations (Hobbs, 1997). More recent works inspired by the LES have proposed alternative trait spectra, such as the wood economics spectrum suggested by Chave et al. (2009) and the root economics spectrum (Mommer & Weemstra, 2012). The extent to which these principle modular axes converge or coordinate to define the ideal whole-plant economic spectrum which considers inclusivity of all organ levels (roots, leaves, and stems) and resource dynamics remains an open question yet to be clarified in ecology (Reich, 2014). However, plant functional traits have proved to be a valuable tool for ecologists whom are attempting to understand the natural world fundamentally (Reich, 2014).

Despite their wide appeal, there remain concerns around assumptions made when using plant functional traits in examining trait-environment relationships and subsequent inferences regarding plant ecology and evolutionary processes. The first issue surrounds the manner in which functional traits are quantified having been traditionally averaged at the species and community level (i.e., community weighted trait means) — negating the influence of intraspecific variability (Albert et al., 2012; Lepš et al., 2011). This neglect for intraspecific trait variation is quite peculiar considering it has been cited as a principle condition in species coexistence theorem for almost 70 years; as proposed in MacArthur and Levins (1967) seminal paper, “The limiting similarity, convergence and divergence of coexisting species”, in which they submitted a ratio – interspecific variation of niche means: intraspecific niche widths – to ascertain the probability of species coexistence. However, as time progressed community ecology tended towards conceptualizing biodiversity of which niche mean of interspecific variation become a main predictor (Violle et al., 2012). Such focus on niche means, thus interspecific variability, persisted in ecology under the conviction of the mean field theory – the mathematical analysis of the mean behaviour discounting variance of the data (Cam et al., 2002; Klingenberg et al., 2005; Violle et al., 2012). This disregard of variance under the mean field theory has spilled across into several subdisciplines of

community ecology, most significantly functional and trait-based ecologies (Violle et al., 2012). Where its effect can be illustrated by the sentiment of McGill et al. (2006) that “for traits to be useful in community ecology, they should differ more between than within species” — concerning is this sentiment as it underpins a key assumption in functional trait ecology (Albert et al., 2011). McGill et al. (2006) sentiment is further strengthened by highly cited meta-analyses using trait data, acquired from global databases such as TRY, averaged at species and community levels to substantiate ecological theories and concepts. However, recent reports and studies have come to recognize the importance of interspecific trait variability (ITV) to community ecology, and its influence on species coexistence, functional diversity and plant trait coordination at varying scales (Albert et al., 2010; Jung et al., 2010; Laforest-Lapointe et al., 2014) – most apparently when descending from global to regional or local levels and expressed at whole-plant as opposed to modular trait comparisons (Siefert *et al.* 2015).

The acknowledgement of phenotypic plasticity (i.e., the ecological process) and its expression, ITV, contribution to community ecology is still in its infancy, however, has thus far profoundly influenced our interpretations of ecology and plant evolution in the natural world. This general agreement amongst the relevance and subsequent importance of accommodating continuous plant trait distributions can be demonstrated by newly developed modelling approaches that attempt to adequately capture ITV to enhance the low predictive power of contemporary dynamic vegetation models which use amalgamated properties such as Plant Functional Types (PFT) for grouping vectors in model rendering and analysis (Moran et al. 2016). Thus, the integration of ITV will prove invaluable providing a fundamental understanding of reactive environmental trait consideration; bolstering inferences and cognition of the role ecophysiological responses represent to plant function and effecting change in community dynamics and species distribution to environmental conditions.

The second fundamental assumption under the plant functional trait framework (Violle et al. 2007) is the supposition that functionality of traits is dependent on their relative effect on an individual plant's performance as an expression of its respective fitness. While many studies have made inferences relating traits to demographic changes there is yet a consistent trend that emerges illustrating traits that present the largest demographic impact on populations (Chalmandrier et al., 2021). Those studies which have attempted to investigate this fundamental premise have rarely isolated a strong trait predictor accounting for a large proportion of the total variance in growth or mortality (Yang et al., 2018). Most studies have employed common garden experiments in an effort to fill this foundational gap in functional trait research, although to their best intentions the conditions experienced in controlled glasshouse cannot be applied to those in the field. The lack of experimental analogs in conjunction with the weak prognostic capacity of traits measured by the few *in situ* studies has led many to question as to whether or existing approaches or target traits are the best appropriate to quantify ecological responses or provide sound conclusion to draw upon.

Currently, the most popular used trait hierarchical classification scheme has been the “hard” and “soft” dichotomy, which parallel the “effect and response” characterization of traits (Violle et al., 2007). Many studies focus on or employ soft traits as proxies for plant ecological strategy or environmental response, attributed to the relative easy and simplicity of acquiring such traits (Laube et al., 2013). Contrary to the latter, hard traits are those which describe a precise plant function – these are usually physiological features and processes – which are often laborious and expensive to quantify yet they directly relate to plants mechanistic response or ecological

strategies to environmental conditions (Violle et al. 2007). Although this dichotomy has bettered our understanding of trait ecology there remains a vague distinction of functionality. A recent commentary by Brodribb (2017) delivers (in this authors opinion) a more robust description disentangling the ambiguity of functionality by classifying traits as either “mechanistic” – features that are clearly associated/defined to physiological process describing function – and functional traits that denote syndromes of trade-offs between various physiological processes or consequences of divergent coordination amongst whole-plant traits. Where many of these “soft” traits are rather secondary functional correlative indicators – demonstrating general adaptive response (i.e., may display similar outcomes under different scenarios) instead of direct trait-environment adaption to a particular environmental stress (Poorter et al., 2009). For example, leaf mass per area (LMA) is frequently cited as an adaptive trait in plant environmental response and resource dynamics in many plant ecological research articles (Reich et al., 1999; Poorter et al., 2009). However, the underlying mechanism of LMA variation, inferring species adaptive trait strategy, across environmental gradients is challenging to identify due to a lack of specificity, where high LMA could indicate a response to aridity or alternatively as a protective tactic for long-lived perennial species in nutrient impoverished habitats (Niinemets, 2001; Turner, 1994). In contrast many mechanistic traits illustrated strong predictive power of demographic responses to environmental perturbations illustrating strength of association between trait expression and fitness of a population (Volaire, 2018). Its therefore, imperative that traits indicating direct adaptive trait-environmental responses to stress – mechanistic traits – be of focus as they will provide fundamental insight into conceptualizing plant ecological strategies and increase predictive certainty relating to the impacts of environmental change on community dynamics and ecosystem function. Particularly, those traits which are accepted as having a mechanistic association with a specific environmental stressor, such as drought, provision a better functional conception of species responses to their environment and the implications this represents for community assembly and resilience.

The recent increase in scientific publications exploring tree mortality and associated physiological drivers behind the escalated trend in the loss of many climax species (i.e., vital components for maintaining ecosystem functionality) impresses upon the significance this study. Currently, the prime, and most abundantly cited, example exploring the divergence between hydraulic strategies directly linked to drought-induced mortality patterns is the piñon-juniper woodlands of the southwestern United States. In this system widespread mortality of the isohydric *Pinus edulis* following extended periods of drought, while the anisohydric *Juniperus monosperma* is capable of tolerating these protected water deficit conditions (West et al., 2008; Plaut et al., 2012). While this example has presented a fundamental ecological example for drought theory there remains urgency in extending our understanding of hydraulic strategies to systems outside continental United States and the Northern hemisphere in general. More importantly, attention should be diverted to those systems demonstrating great ecological and species diversity anticipate hosting communities demonstrating an array of functional attributes and growth forms capturing a range of hydraulic strategies to resource constriction events such as drought. Moreover, the overwhelming majority of reports throughout the literature have chiefly focused on the tree growth form (Cailleret et al., 2017), yet within functionally diverse ecosystems with its plethora of plant functional types and traits, few studies have explored the responses these species have towards drought, despite their respective roles in retaining ecosystem functionality and resilience in the face of environmental heterogeneity and under climatic perturbations, such as drought.

2.11. Thesis objectives and intentions

Globally, the prevalence of droughts has escalated resulting in an increase the documented rates of forest dieback and generally higher mortality rates during the dry periods/seasons than expected or which has been predicted under global change models (Allan et al., 2015), and in some instances have present as delayed lethal impacts on community structure. Understanding how species are predicted to respond and more importantly their relative sensitivity has fundamental implications for species persistence and geographic distribution under climate change with associated increasing temperatures and shifting rainfall regimes. The significance of predicting drought response strategies and species mortality (i.e., hydraulic vulnerability) is an urgent challenge facing plant physiologists and conservationists given the unprecedented climatic flux we find ourselves in. Among the number of environmental factors (e.g., temperature, nutrients) limiting plant growth, performance and survival none are more persuasive than water availability - regarded as the chief limiting variable of plant, community and ecosystem-level productivity (Boyer, 1982). Water availability acts as an ecological filter within ecosystems which experience drought, selecting species capable of tolerating or surviving periods of water deficit conditions which is dependent on associated hydraulic and plant functional traits. Thus, given the circumstances of predicted climate change – frequency and magnitude of drought – it is essential to identify plant traits that dictate functional responses to increasing aridity inferring adaptive strategies to climate and corresponding changes thereof.

The detection and monitoring of vegetation change, particularly identifying the trends and magnitudes, at high spatial and temporal resolutions in response to local drivers, such as climatic variability, provides benchmarks against which future changes can be assessed. An accurate characterization of the extent to which vegetation change has occurred in Subtropical Thicket through time has remained obscured by the preconceived presumptions of its general resilience to disturbances, particularly climatic variability. These early notions are limiting to understanding the role of climate to vegetation change in thicket, but other challenges remain too. Subtropical thicket covers a large, bio-climatically diverse range denoting rapid changes in precipitation across a topographically variable landscape, additionally long-term data and observational records are lacking thereby hindering the reconstruction of vegetation change and response to disturbance through time. The literature remains scarce in research assessing the response of thicket to disturbance, more so the use of break detection and satellite imagery techniques to monitor and evaluate patterns and trends in vegetation change. Remote sensing techniques and analyses are becoming increasingly more important to vegetation change monitoring and disturbance detection, particularly in semi-arid and arid environments filling data gaps both from wide coverage in spatial extent and far-reaching temporal scales. The use of vegetation indices, derived from remotely sensed spectral platforms, has proved to be a valuable proxy of vegetation productivity (Zhang et al., 2016; Gazol et al., 2018) and mortality (Byer et al., 2017) being employed to study climate vegetation feedbacks, observing trends in growth and phenology (Zhang et al., 2003; de Jong et al., 2011).

Remote sensing in conjunction with plant hydraulic physiological traits were made use of in this study to evaluate trends in Subtropical Thicket response to water availability and provide a mechanistic interpretation of recent trends of increased mortality and canopy dieback. The detection and trajectory of vegetation change is paramount for understanding the mechanism of change and identifying its associated causes. It is proposed herein that understanding of physiological processes of vegetation response to recent drought (2020) and its associated

climatic state responsible for change would enable predictive assertions as to the future character response of thicket to global type change settings. With future projections indicating considerable changes both in temperature extremes and declines in precipitation trends most severely in semi-arid and arid environment could result in significant decreases in the distributional extent of Subtropical Thicket in the future.

This study has the two principal objectives, firstly to determine whether drought acts as a major driver of vegetation productivity and the implications thereof in Subtropical Thicket. To this end, highlighting and emphasizing the role of drought disturbance to ecosystem resilience and as an ecological driver worthy of consideration in future research regarding thicket. Secondly, this study aimed to investigate the hydraulic vulnerability of dominant woody species in Subtropical Thicket from *in situ* measurements of water stress to hydraulic trait thresholds retrospectively following a dieback event.

This thesis combines remote sensing and plant hydraulic trait physiology to provide an account of the effects a multi-year drought (2015-2021) has had on the canopy vigor of dominant arid thicket tree and shrub species; and present a mechanistic account of the ecological significance of drought disturbance represents to the vulnerability of Subtropical Thicket. To this end, this study addresses the following questions:

- When and how has vegetation changed under drought in Subtropical Thicket over the past two decades?
- How has the trajectory of change in vegetation productivity responded to drought disturbance through time?
- Do hydraulic traits provide a useful indication of drought tolerance in thicket species?
- Do dominant woody canopy Subtropical Thicket tree and understory shrub species exhibit converging vulnerability to loss in hydraulic function to drought or are there divergent functional thresholds across species indicating species-specific drought resistance?
- Is hydraulic impairment responsible for canopy dieback of *in situ* Subtropical Thicket species?

This study is unique in that it not only challenges preconceived notions of the role drought plays in Subtropical Thicket and more importantly highlighting its role as a driver of defoliation, and by extension vegetation change but that it is the first to document the hydraulic vulnerability of foundation species within thicket. Furthermore, assessing the impacts of drought, chiefly loss in plant function, under field conditions is often dependent the capacity to rapidly detect and mobilize in response to the events of extreme drought. This is further compounded by the heterogeneity of population mortality and identification individuals being impacted by drought. However, this study was fortunate to have been undertaken during a severe drought inflicting the Eastern Cape enabling assessment of water strain on thicket species *in situ* and comparisons with associated threshold of hydraulic impairment and loss of function. To this end this study impresses the significance and role of drought as a major ecological driver of productivity in Subtropical Thicket and combines these findings with a mechanistic interpretation of the underlying physiological implications water deficits impose on the hydraulic integrity of core thicket species.

2.12. Thesis outline

The core intent of this thesis was to explore the role of drought on Subtropical Thicket, both from the perspective of vegetation change and physiologically – the impacts of water availability on the hydraulic functionality and whole plant survival under drought conditions. This thesis consisted of a total of five chapters: an introductory section (Chapter 1); a comprehensive accounting of the literature as a review thereof (Chapter 2); two data chapters (3 and 4); and lastly a concluding synthesis and synopsis of the study (Chapter 5). Both data chapters were presented in scientific publication format, which accounts for possible content replication throughout the thesis. Each of the corresponding data chapters adopt the reporting style of the journal *Functional Plant Biology* and for uniformity was applied throughout this thesis.

The thesis was structured as follows:

- Chapter 1, contextualizes the study, providing background and brief introductory remarks as to the intentions and purpose of this study. This section outlines core research problem and general the objectives of the study.
- Chapter 2 constitutes the literature review, this section discusses briefly the core literature introducing relevant topics and providing a general background as to existing knowledge pertinent to the study. Here discussions relating to the ecology, biogeography, and phytosociology of Subtropical Thicket are presented. Moreover, information relating ecophysiological process and the drought-related drivers of loss in whole-plant function and mortality are compiled briefly to offer a comprehensive account of the literature.
- Chapter 3 is the first of the two data chapters. Its core intention was to investigate role of drought on vegetation change in Subtropical Thicket, specifically arid thicket subtype, using remotely sensed data in conjunction with meteorological data in characterizing the effect-response pathway of water availability as a driver of ecological change (i.e., defoliation) in thicket. This chapter utilizes climatic data to characterize drought conditional status of the Eastern Cape, by way of multi-scalar indices such as Standardized Precipitation Index (SPI) and Standardized Evapotranspiration Index (SPEI). Furthermore, this chapter explores interannual trends and detection of drought disturbance changes using vegetation health proxies, NDVI and VCI, over the past two decades. Change monitoring methods are applied on MODIS time series to isolate drought-related changes. Moreover, this chapter provides a firsthand account of widespread defoliation and dieback in dominant evergreen woody canopy tree and shrub thicket species during the existing protracted decadal drought being experienced presently in the Eastern Cape. Accordingly, visual assessment were conducted of canopy vitality and health conditional status of effect species the purpose of which was to provide baseline data and established technique for the future monitoring of these individuals in the long-term. Additionally, this study established two permanent plots in pristine arid thicket for future research relating to the role water availability on vegetation change at the arid distributional extent of Subtropical Thicket.
- Chapter 4 investigates the variation of hydraulic traits across dominant thicket woody canopy tree and shrub species and identifies physiological thresholds that may offer a mechanistic account of observed trends in mortality and canopy dieback under present drought conditions in the Eastern Cape. This chapter undertakes a retrospective assessment of canopy dieback and predicted loss of function to embolism as drivers for historical trends in vegetation change. This retrospective assessment of hydraulic traits

and associated physiological thresholds will explore the significance of hydraulic impairment as the primary mechanisms influencing canopy dieback and defoliation in arid Subtropical Thicket.

- Chapter 5 provides a comprehensive synthesis of the key findings from this thesis. Additionally, this chapter draws conclusions from each of the thesis chapters stressing the broader valuable and applicability of the findings. Lastly, Chapter 5 discusses future research avenues and priorities based on the findings presented in this thesis.

2.13. Research publications and conference contributions

At the timing of submission this thesis made several contributions to scientific audience. These contributions include the submission of an article on the 24 November 2021, passing editor desk decision and is currently under review in *Journal of Arid Environments*. The article titled: “A case of heatstroke: Widespread canopy dieback, defoliation, and historical drought impacts in Subtropical Thicket in southern Africa – a drought report” and includes contributing authors: Daniel H. Buttner, Robbert Duker, Robert P. Skelton, Alastair Potts. This article addresses the first component and data Chapter 3 of this research, providing for the first-time a documented account of canopy dieback and crown defoliation in evergreen, sclerophyllous arid thicket of the Eastern Cape in South Africa. Whilst Chapter 4 remains to be submitted with continual edits being made this chapter, with the core focus on the physiological underpinnings of drought in arid thicket, providing a mechanistic interpretation of the earlier descriptive findings in Chapter 3. Although Chapter 4 is yet to be submitted at the timing of thesis submission, the findings and interpretations of the data were presented at the annual Thicket Forum in July 2021 in this year being first online conference. This forum provided engagement with researchers from across the country as well as local landowners to present and articulate a comprehensive account of the vulnerability of arid thicket and the impacts of drought threats to community composition and structure of this biome stressing the importance of physiological research as a priority.

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Chapter 3

A case of heatstroke: Widespread canopy dieback, defoliation, and historical drought impacts in Subtropical Thicket in southern Africa– a drought report

3.1. Abstract

Extreme drought occurrences and escalated aridity have led to widespread drought-induced mortality and dieback events in many forested ecosystems across the globe. However, there are scarce accounts of such drought triggered canopy dieback, mortality events outside the Northern Hemisphere – stressing a fundamental gap in understanding as to the vulnerability of other wooded ecosystems globally. Particularly, South African tree dominated biomes which have rarely document historical drought-related impacts. This study reports on the first case of drought-induced canopy dieback, crown defoliation, and mortality event in Subtropical Thicket which has historically been considered strongly drought tolerant seldom succumbing to water deficits. A severe anomalous dieback event, coincided with extreme, accumulative drought conditions was observed in 2020. Employing a combination of field-based approaches and remote sensing, this study aimed to provide a comprehensive report of the extent and severity of crown defoliation and canopy dieback following this event, additionally describing predisposing and compounding factors. Based on Standardized Precipitation Evapotranspiration (SPEI) and Standardized Precipitation Indices (SPI) this event began in 2015 and coincided with amplified temperatures exacerbating evaporative demand. Aerial UAV surveys and field-based investigations were undertaken. Remotely sensed (RS) indices provided an avenue for extensive spatiotemporal investigations to uncover the underpinning mechanisms behind drought-induced mortality and changes in water availability. Leveraging long-term time series RS data, Normalized Difference Vegetation Index and Enhanced Vegetation Index, from Moderate Resolution Imaging Spectroradiometer satellites as a proxy of vegetation physiological status in combination with SPI and SPEI describing drought condition and ground-based surveying defining canopy vitality condition this study attempted to address the core aim of endeavoring to link drought-induced mortality and early warning symptoms observed in the field with RS time series data to describe drought impacts across Subtropical Thicket. The spatial extent of drought varied considerably between sequential drought years between the study period (2001-2021), however, a consistent spatial pattern was observed with western interior of the region suffering most severely. Widespread crown defoliation, canopy dieback, and whole-plant mortality was observed throughout the study site conveying the landscape-level structural impacts drought presents to Subtropical Thicket. Moreover, the evergreen leaf habit of this vegetation potentially contributed to exacerbation in drought impacts, with species such as *Putterlickia pyracantha*, *Pappea capensis*, and *Polygala myrtifolia* suffering extensive dieback, defoliation, and in severe cases mortality. Given recent projected climate models stressing the probability of elevated frequency and severity in drought events, the potential for Subtropical Thicket biome to exceed an ecological tipping point and vegetation transitional outcomes is of research priority.

Keywords: Remote sensing, Subtropical Thicket, drought vulnerability, defoliation, canopy dieback, NDVI, EVI, MODIS, SPEI, SPI, drought resistance

3.2. Introduction

The prevalence and severity of catastrophic climatic anomalous events, such as droughts, have been detected across the globe (Breshears et al., 2005; Breshears et al., 2013; Allen et al., 2015), affecting plant demographic performance, species distribution, community composition, structure, and functioning (Mathys et al., 2016). The escalation in drought frequency has garnered much interest, particularly regarding the mechanistic processes underlying species-specific tolerances and broader demographic implications drought-induced mortality presents to plant communities. Severe drought conditions have resulted in several prominent tree mortality events globally (Allen et al., 2010), stressing the need to understand the climatic thresholds and plant-level responses required to trigger such severe drought-induced fatality episodes. Understanding plant drought responses, particularly at ecotones, is a primary focus of global change biology. These transitional zones or edge communities are often inhabited species operating at their physiological limit suggesting that these communities denote greater environmental and disturbance responsiveness than spatially shielded communities.

Arid Subtropical Thicket constitutes one such spatially transitional community, denoting unique phytosociological and physiognomic characteristic flora (many endemic succulents: *Mesembryanthemum* and woody trees), this ecotype represents the arid subtype of the greater Subtropical Thicket biome. The Subtropical Thicket biome is a recently recognized biome of South Africa differentiated from forest by (1) vegetation height limited to 3 meters, and (2) comprising no more than two strata in contrast to three or more in forests. The novelty of this biome is evident by the underlying typology indicative of its ancient origins and evolution of this vegetation (Cowling, 1983). Interestingly, however, Subtropical Thicket demonstrates strong correlation with climate illustrating mesic flora in the east to more arid species assemblages to the west. The absence of a pronounced seasonal precipitation regime is a major correlate of Subtropical Thicket distribution. So much so that changes in proportional contributions in winter rainfall dominant west reaches result in the replacement of thicket species with more arid Nama-Karoo shrublands and nearer the coast Fynbos which displace thicket species due to increased fire frequency. In contrast, progressing eastwards results in higher summer rainfall and most significant amount of precipitation, often in excess of 1000 mm, causing the displacement of thicket species with Afromontane Forest, shadowing thicket hindering establishment (Vlok and Euston-Brown, 2002). The distribution of Subtropical Thicket occurs within a confined zone, where the annual rainfall quantity is not the sole limiting factor and includes seasonality as an important driver (Cowling et al., 2005; Vlok et al., 2003). The latter suggests that variability in annual precipitation is potentially a major driver for change both to community composition and structure. To this end, rainfall is known to be highly erratic and unpredictable across Subtropical Thicket, particularly within edge communities (such as arid thicket) where precipitation denotes a coefficient of variation as high as 40% (Hoare et al., 2006; Vlok and Euston-Brown, 2002). Moreover, in any given year there is a 25% chance of not receiving 80% of mean rainfall (Aucamp and Tainton, 198). Coupled with the uncertainty around rainfall are extreme temperatures (instances of 50°C and excess) faced by arid thicket.

The erratic climate and unreliable precipitation regime of arid thicket results in frequent drought occurrences within this region. Whilst there is not a major concern for the dominant succulent species there is alarm for the less abundant woody component trees and shrubs. This concern is driven by the observation from a meta-analysis (Choat et al., 2012) describing that 70% of woody species are operating at the physiological limits with regard to water stress highlighting the risk

and potential vulnerability to anomalous climatic conditions might pose to this crucial functional guild in arid thicket. The underlying mechanisms of drought-induced tree mortality can present as direct or indirect (Choat et al., 2018). Hydraulic failure due to water stress describes a process where the critical water potential thresholds are surpassed causing xylem embolism dysfunction impeding water transport limiting photosynthetic capacity (Brodribb et al., 2003; Brodribb, 2009), impacting growth and phenology (Cardoso et al., 2020). In response to moisture deficits trees and shrubs mitigate water loss, and indirectly avoid exceeding plant functional thresholds, through stringent stomatal control. However, in so doing plants risk depleting vital carbohydrate reserves used in respiration heightening the vulnerability to pathogens and pest attacks (Hartmann et al., 2013; Hartmann, 2015; Sevanto et al., 2014). While understanding the processes that underline drought-induced mortality is essential for predicting vegetation dynamics in the future – a more important focus on the detection of, and description of climatic correlates with drought-related symptoms of plant vitality are paramount for interpreting the implications of water stress for plant communities and management responses.

Regular monitoring and detection of the timing, locality, and severity of declining canopy vitality (via observed symptoms: foliage discolouration, crown dieback) and mortality across varying spatial scales is crucial for land managers and government agencies responsiveness to environmental disturbances and for anticipating future vulnerability risks of biomes to water stress. First visual symptoms of canopy dieback in water stressed vegetation is crown defoliation and foliage discolouration (i.e., brownness) due to diminished water availability resulting in branch and leaf death (Manion, 1981). Conventional monitoring practices based on plots-level characterizations of canopy vigor and mortality trends have great predictive utility, however due to the spatial limitation (i.e., ground-based surveys) the scalability across landscapes or regions are hindered. The success of ground-based plot surveys is attributed to the quantification of early warning symptoms of mortality in productivity metrics. Remote sensing transcends the spatial limitations of the latter, capable of observing changes in vegetation dynamics, such as productivity, at the individual plot-level to broader spatial scales such as landscapes. For this reason, remote sensing offers an approach that bridges the gap between restricted ground-based plot-level observations and more coarse-scale detections of declining canopy vigor and mortality. Remote sensed vegetation monitoring widely employs varying spectral indices each of which describe crucial physiological related performance and general canopy vigor (Jiao et al., 2020). To the extent that they document plant productivity dynamics portend tree mortality, these indices have demonstrated value in detecting early warning symptoms of vegetation condition (Anderegg et al., 2019; Rogers et al., 2018). The most common early warning symptom of drought-related mortality is “wilting”, defined here as leaf discolouration and defoliation. Whilst early wilting occurs both in deciduous and evergreen species, in the former it's often a strategy to avoid physiological water stress, though in evergreen trees it's more strongly an indicator of mortality (Brun et al., 2020). Hence, early wilting, which is easily detectable using remote sensing (expressed as productivity), provides a great opportunity for monitoring vegetation vigor and the threat of drought-induced mortality based on leaf habit.

In contrast to the evident escalation in the global trend of drought-induced tree mortality, there have been comparatively fewer reports of analogous events in South Africa. The absence of such literature coupled with drought appearances sparked renewed interest in drought-related impacts in South Africa ecosystems and led to the formulation of a Special Issue in African Journal of Range and Forest Science. Whilst the scientific reports in the latter have contributed much understanding and characterization of drought impacts to productivity and tree mortality, the

general scope of the literature on drought-induced tree mortality has largely been limited to the savanna (Swemmer, 2020) and fynbos biomes (West et al., 2012) with no accounts of drought-related die-off events in other tree dominated biomes of southern Africa. This poses an important question, are extensive drought-induced tree mortality occurrences rare amongst other South Africa trees? Or do they merely go unreported? Despite detailed accounts on the implications and outcomes of drought events on agriculture, hydrological resources, and socio-economic stability (Baudoin et al., 2017; Vetter et al., 2020), there remains a marked gap in quantifying drought related effects for South African woody tree and shrub species. More importantly, the detection of early warning signals of advancing incipient mortality events are paramount for anticipating and modeling potential changes in community dynamics, in addition to elucidating to species-specific vulnerabilities to climatic anomalies, such as drought.

In South Africa, the cases of severe tree decline, and drought-induced mortality have rarely been documented in major tree functional type biomes – to this end, this report is the first to document widespread defoliation and die-off within a woody tree biome, the Subtropical Thicket, affecting contemporaneously large number of coexisting species. Given that the Subtropical Thicket biome constitutes chiefly evergreen species, which are physiologically more tolerable to water stress, however, are at greater risk of experiencing drought-induced mortality under extreme drought conditions, the appearance of these early warning symptoms elevate concern for the survival of species and community compositional shifts due to this anomalous climatic perturbation. Leveraging the occurrence of a climate change-type drought – drought conditions exacerbated by high temperatures (Anderegg et al., 2019) – of multi-year origin (commenced 2015 to present) in the southern Cape South Africa which drove widespread defoliation and dieback, particularly in the canopy dominant species Jacket Plum (*Pappea capensis*) in the region, this study strived to comprehensively analyze the (1) patterns; (2) drivers; and (3) impacts of widespread drought-induced symptoms of mortality in Subtropical Thicket during the height of the drought in 2020. Employing a combination of field-based approaches and remote sensing, this study attempts to characterize the severity of canopy dieback following this anomalous drought event, in addition to stressing the importance of predisposing and compounding factors. Deliberately, this study did not intend to enter into addressing the question of causal physiology behind defoliation and canopy dieback which would doubtfully be revealed by observational studies alone. However, such query is marginally considered in this report on drought and arid thicket tree species response to unravel the underlying physiological processes affecting these observations. In the present study, original information regarding the case of canopy dieback and tree mortality in south-eastern South Africa contributing to a more comprehensive understanding of the implications and outcomes of recurrent drought in arid thicket of this climatic unique region.

3.3. Methods and materials

3.3.1. Study site and case description

An auspicious opportunity to investigate and report on the drought vulnerability of arid thicket presented by an extreme multi-year drought in southern Cape of South Africa. This multi-year drought period commencing in 2015 until present demonstrated minimal impact in the first three years on woody perennial, evergreen trees, however, significant declines in productivity of grasslands with minimal mortality were described (Archer et al., 2022). It was not until late 2020 that early warning symptoms of mortality, crown defoliation and canopy dieback, were detected in evergreen perennial arid thicket tree species. Crown dieback and collapse was most prominent

in the canopy dominant *Pappea capensis* and the understory shrub *Polygala myrtifolia* in this region. In contrast to the canopies of subdominant species, *Euclea undulata* and *Boscia oleoides* illustrating less severe symptoms and in many instances only partial crown defoliation. However, more information is required regarding the factors contributing to these observed trends. Hence, the core intent of this study was to describe and quantify the response patterns-symptomology of the major canopy and understory arid thicket species, and climatic characteristics, following the height of multi-year drought and observed dieback event.

This study was undertaken at Kabouga Private Nature Reserve nestled in the Zuurberg mountain range of the Eastern Cape (S 33°15'44" E 25°22'55") situated approximately 90 km northwest of the major metropole, Port Elizabeth. The site constitutes largely arid thicket subtype of Subtropical Thicket, representing the arid edge of this biome. The site has a mean annual rainfall of 340 mm with a high degree of variability. The geology of the site is predominantly karoo lithologies, namely of the Dwyka and Ecca transition, resulting in deep and well stratified sediments (Norman and Whitfield, 2006). The Dwyka tillite characterize the area constituting the fine grain shales, while transitional geologies with Ecca group containing higher content of coarse sandstone derived sediment (Baiyegunhi et al., 2017). The heteroscedasticity in geology across the Subtropical Thicket biome suggest that edaphic properties are not an influencing component for this vegetation. The seemingly absent reports of plant water stress in Subtropical Thicket under the multi-year drought (2015 to present) might suggest that this unique southern cape biome demonstrates a high tolerance towards precipitation uncertainty and drought in general. The capability of woody perennial tree species to persist in arid thicket, characterized by unpredictable and low rainfall coupled with extreme temperatures experiencing frequent drought (Hoare et al., 2006), elucidate to its resilience of this vegetation in the face climatic variability and disturbance. These species use several morphological, phenological, and physiological strategies to survive within this climatic variable biome. To the extent that post-disturbance, chiefly drought, resprouting is a commonly observed response in several tree species within arid thicket, assisting the persistence of these perennial plant species. Whilst this response strategy of resprouting might account for resistance in arid thicket to water stress, the assumption first reported by Zeppel et al. (2015), that systems dominated by these species denoting this response will experience minor drought-induced effects, such as crown defoliation and mortality, is challenged by the observations of the recent drought in the southern cape.

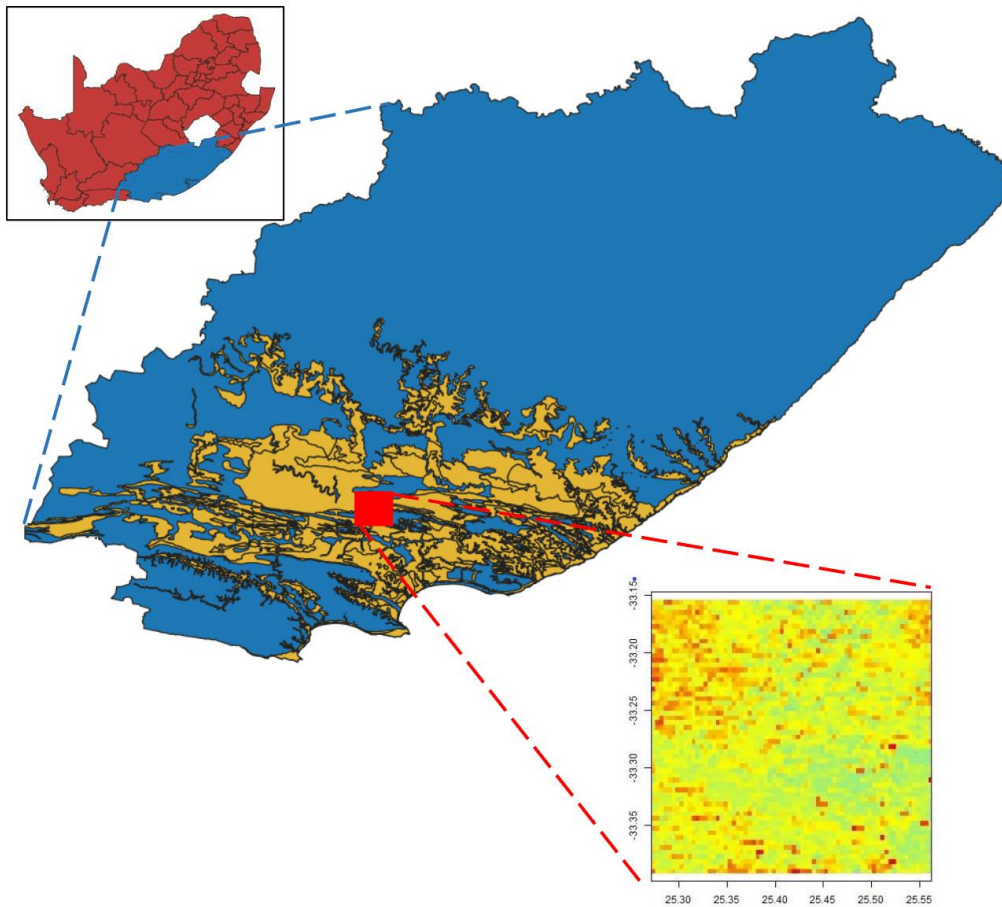


Figure 1: The location of study area within the Eastern Cape Province of South Africa. The orange shape [adopted from Dayaram et al. (2017)] extents delineate the distributional range of Subtropical Thicket biome. [Colour image on web; black and white for print]

3.3.2. Canopy condition and health assessment

A preliminary visual field assessments was carried out in late-September to mid-October 2020 across 100 observation locations across the study site varying in observed decline in canopy vigor and productivity metrics (i.e., defoliation, branch dieback, foliage discolouration) on thicket stands – providing firsthand account of drought-induced impacts. These initial observations resulted in the establishment of three permanent plots (Figure S6), ranging from 1-to-2 ha depending on vegetation structure, situated at the study site in unmanaged thicket stands. At each location dominant canopy woody tree species were selected, followed by an extensive plot-level summary describing the canopy condition and vitality characteristics. The descriptions noted at each location include qualitative analysis of the species present, general architectural and structural attributes, and drought-induced symptoms: foliage discolouration, branch desiccation and dieback, defoliation, presence of pest attacks. Fifty dominant *Pappea capensis* trees, and at least a minimum of 10 trees for subdominant species *Euclea undulata*, *Searsia longispina*, *Ptaeroxylon obliquum*, *Brachyleana ilicifolia*, *Gymnosporia buxifolia*, *Putterlickia pyracantha*, *Schotia latifolia*,

and *Polygala myrtifolia* were randomly selected across study site. For each tree a canopy health score was derived following modified version of the scoring system devised by Stone et al. (2008), which was applied in a eucalypt forest describing the effect of bell miner (*Manorina melanophrys*) on the individual tree vitality and noting the demographic consequences thereof.

This modified scoring system (see Table S2) was based on several categorical descriptors of canopy vitality condition and followed a similar value ranking approach as Souter et al. (2010) TLM visual technique on canopy condition, which involved numerically scoring trees on a scale of 1 to 6 for canopy architectural attributes (crown extent and crown density); from 1-4 for foliage and growth characteristics (leaf dieback, leaf damage, leaf colouration [denoting tones outside the green spectra of the Munsell Plant Tissue Colour Chart], epicormic and/or basal resprouting); from 1-5 denoting the degree of vulnerability via conditional status of protective features (bark condition) to abiotic perturbations. Moreover, Souter et al. (2010) included an assessment of pest-induced damage investigating the role of drought strain on enhanced vulnerability to insect attacks or fungal infestations, to this end this study embraced this attribute under the modified scoring system of Stone et al. (2008), scaling from 1 to 4. A final canopy vitality condition score was obtained by summing each of the component attribute scores, further details on scoring outline are reported in Supplementary Tables S1, on each feature scored. The maximum score of 37 represents a healthy, vigorous canopy tree, while conversely a score of 8 describes a defoliated, pest-infested, damaged canopy tree. To provide standardized comparative analysis of the findings relaying canopy vigor this study presented the corresponding percentile estimations of drought-related symptoms which centered around the severity classes of the adapted scoring system (expectation under ICP Forest guidelines).

Site-specific features were noted such as aspect, sediment type, slope, and each location delineated and geo-referenced using differential GPS (GPSMAP 72, Garmin®, USA). In addition, images at each point location were captured of selected canopy trees from multiple angles, for canopy vitality reference and symptomology at later stages of the study, using a digital camera (TG-5, Olympus, OM Digital Solutions, Tokyo, Japan).

In response to these early ground-based field observations characterizing and reporting canopy damage amongst co-existing woody canopy species in arid thicket, an aerial survey of the area was conducted in late May 2020 and once more during September-October 2020 using a DJI Mavic 2 Pro. As this presents the first use case of aerial monitoring for Subtropical Thicket, a dual flight plan approach was undertaken in this study capturing both broader landscape-level photography of vegetation canopy and more localized focus flights for crown symptomology.

The purpose of these drone flights and ground-based surveys were to provide baseline data documenting vegetation drought response, community and species demographic impacts, with the intention of repeated monitoring aspirations in the future following varying phases of mortality or recovery in the long-term. Three newly established, and first-ever, Subtropical Thicket plots were found describing changes in canopy vigor. These plots are situated on private research land enabling accessibility and making continued monitoring possible. Plots were selected along the slopes of lowland in the Zuurberg mountains, where arid thicket dominants. These arid thickets are home to many woody canopy trees and midstory shrubs that have frequently been purported as drought tolerant (Lechmere-Oertel, 2003; Lechmere-Oertel et al., 2005). This speculation is driven by the underlying inference that occurrence defines tolerance and due to the presence of this woody species they must be tolerant to drought (Lechmere-Oertel et al., 2008). This study discusses this inference in the greater context of vegetation change dynamics under global

climate change, with focus on the historical perspective of meteorological drought and Subtropical Thicket response.

3.3.3. Meteorological data and drought metrics

Precipitation, both seasonal and annual distributions, is a major determining ecological parameter influencing growth and productivity of vegetation in arid and semi-arid ecosystems, the dynamics in which alter the properties and structure of these systems. The influence of rainfall is apparent in Subtropical Thicket vegetation and is described as a defining feature determining the community composition and functional guild distributed within an area based on annual trends in precipitation across its range.

To calculate the selected drought indices, Standardized Precipitation Index (SPI) and Standardized Potential Evapotranspiration Index (SPEI), monthly meteorological data was obtained from 10 automatic weather stations situated in the Eastern Cape Province of South Africa (see Figure S11). The data comprised daily and monthly precipitation, daily and monthly average minimum, maximum and average temperature records for the following available stations in the Eastern Cape (i.e., study area).

3.3.3.1. SPI and SPEI calculations

SPI was conceived and proposed by McKee et al. (1993) to capture precipitation deficits purely based on the amount of rainfall an area receives. SPI is defined as a monthly indicator contrasting accumulated precipitation during a specified period with long-term rainfall distribution trend for a historical timeframe. As such the SPI is regarded by the World Meteorological Organization as the global standard for detecting and defining drought. The strength of SPI as a drought metric is its temporal flexibility capable of being calculated across a variety of timescales from 1 month to 24 months. However, it should be noted that the length of time upon which SPI values are calculated have varying implication for interpretation of water deficit conditions, for example short-term (i.e. 1 month) timescales can strongly align to dynamics in near-surface soil moisture status affecting productivity of plants (Caloiero et al., 2018); whilst length intervals, such as 12- or 24-months SPI, are tied to more permanent water sources such as groundwater having minimal direct effect on physiological plant function under drought. The temporal flexibility of SPI makes this metric an ideal proxy for differentiating drought effects on plant physiology to long-term ecosystem water availability (Caloiero et al., 2018). Accordingly, McKee et al. (1993) defined a drought to ensue when SPI values reach -1 and is concluded when values return to and exceed 0 (see Table S1).

In conjunction with SPI a second commonly employed drought metric, and applied here, is the Standardized Potential Evapotranspiration Index (SPEI) first proposed by Vicente-Serrano et al. (2010) and is based on the climatic water balance principle. SPEI is derived from probability distribution of the difference between precipitation and potential evapotranspiration, which are used as input parameters to calculate a standardized value, describing the conditions (i.e. dry or wet) of an area over varying timescales.

Briefly, the three core steps in calculating SPEI are mentioned, the first being the calculation of potential evapotranspiration is undertaken by means of the Thornthwaite method, inclusive of temperature. Secondly, due to drought impacts being cumulative, water balance accumulated is

derived from the subtraction of precipitation from potential evapotranspiration across varying timescales (1-48 months). Lastly, SPEI is calculated from the normalized water balance series via log-logistic probability distribution to derive standardized units from values that are comparable spatially and temporally. For detailed description of SPEI calculation procedure see Vinente-Serrano et al. (2010). Both SPI and SPEI have been applied widely in meteorological studies around the globe to describe drought severity and duration, a major advantage of these to indices is their temporal discretion being calculated on varying timescales. They are spatially coherent, enabling comparisons of various locations under different climates. Most importantly, SPI and SPEI probabilistic nature strengthens its historical applicability to contextualize climatic patterns facilitating predictive utility. Accordingly, both indices were used to categorize and describe the drought conditional status of the region being delimited at particular thresholds of severity (Table 1). Moreover, each index (SPEI and SPI) was calculated at short-, 1 and 3-months and long-term, 6 and 12-month, intervals, respectively.

3.3.4. Remote sensing data

3.3.4.1. Data preparation

Normalized Difference Vegetation Index (NDVI) data obtained from Moderate Resolution Imaging Spectroradiometer (MODIS) platform (designation MOD13Q1 satellite) and prepared for analysis. The utility of this index is strengthened by its wide acceptance and application as a proxy for photosynthetic performance and by extension gross primary productivity of vegetation makes it the ideal candidate in vegetation disturbance monitoring providing spatial and temporal characterizations, spectrally, of foliage greenness. NDVI is derived from two regions of the electromagnetic spectrum, corresponding to the nonlinear product of the Near-infrared (NIR; at 620-670 nm) and Red (at 840-870 nm) spectral bands defined in Eq. 1:

$$NDVI = \frac{NIR - R}{NIR + R}$$

where NIR denotes near-infrared band (620 – 670 nm), sensitive to leaf mesophyll structures, and R is the red spectral band (840 – 870 nm).

In addition to NDVI this study included analyses of EVI, an more recently employed vegetation index, that overcomes the shortcomings (i.e., biophysical factors, topography) that introduce noise in NDVI for the detection of vegetation change. The Enhanced Vegetation Index (EVI) differs from NDVI in that it integrates the blue band spectrum into its computation analysis, using the following Eq. 2:

$$EVI = 2.5 \times \left(\frac{(NIR - Red)}{(NIR + 6 \times Red - 7 \times Blue + 1)} \right)$$

MODIS NDVI data was obtained for the time-period of start date 2000-01-01 and end date 2021 (latest accessible data) of 16-day composite images at 250-meter spatial resolution from MOD13Q1, specifically version 6 where the 16-day composites are comprised of two 8-day

composites, of NASA's Terra and Aqua satellite platform (Land Processes Distributed Active Archive Centre; LP-DAAC; <https://lpdaac.usgs.gov/>).

While NDVI and EVI analyses above will provide an absolute quantification of relative vegetation condition, they, however, do not offer a relative measurement against a defined reference conditional status, such as the historical average or worst conditional stage of the past year. To this end, Kogan (1990) proposed the Vegetation Condition Index (VCI) a metric that conceptualizes the optimal conditions, i.e., maximum vegetation productivity (or NDVI) describing the upper (favourable climatic state) against the worst, i.e., minimum vegetation productivity (or NDVI) denoting the lowest (unfavourable climatic state) historical limits. The VCI enables the identification of the relative pixel carrying threshold facilitating the quantification of changes in vegetation productivity during suboptimal climatic conditions, such as drought (Kogan, 2002). This relative measure of conditional status of vegetation against a reference status is essential for interpreting, quantifying, identifying, and understanding changes in vegetation condition (Kogan, 1995).

The VCI was derived from NDVI inputs, providing a pixel-based assessment denoting mean-annual variance in vegetation condition as described in Kogan (1990) using the following Eq. 3:

$$VCI = \left(\frac{NDVI_i - NDVI_{min}}{NDVI_{max} - NDVI_{min}} \right) \times 100$$

where $NDVI_i$ denotes the value of pixels for length i , in this study a 16-day NDVI interval, $NDVI_{min}$ and $NDVI_{max}$ describe the long-term minimum and maximum multiyear NDVI, respectfully, estimated for each pixel the over the study period, 2001-2021. This study will prioritize the “normal condition” measure of vegetation state characterized as the historical average when addressing VCI comparative analyses. The VCI contains real-time and historical data relating to NDVI values, where VCI is defined on a percentage scale from 0 to 100, facilitating intuitive interpretations of severity of change in vegetation condition. Accordingly, VCI value severity characterization in this study follows that reported by the United Nations Platform for Space-based Information for Disaster and Management and Emergency Response (UN-SPIDER), where VCI of > 40 indicate the drought effects of vegetation. Moreover, Kogan (1995) suggested a VCI value threshold of 35 and below as an indicator for drought conditions, which has subsequently been adopted.

A major concern when analyzing remotely sensed data is the influence of sources of noise, such as cloud cover, shadow and snow in the data, distorting the reliability of pixel change determination analysis. Hence, it remains imperative that appropriate filtering pre-processes be applied beforehand. To ensure NDVI data robustness, i.e., preferential selection of high-quality data, it was first filtered using a pixel reliability mask, a file layer derived from the extracted MOD13Q1 file using the MODISTools package in R (Tuck et al., 2014). The pixel reliability layer designates a descriptor for each time stage an image is captured describing the pixel quality based on a standardized value scale, where -1 is no data, 0 is good data, 1 is marginal (still usable), 2 is snow or ice, and 3 is cloud cover and shadow. In this study NDVI values with a reliability score of 0 and/or 1 were only considered for each pixel. This ensured that only high-quality data was used to interpolate NDVI. In addition, Savitzky-Golay filter was applied (Chen et al., 2004) during pre-processing.

3.3.5. Water availability and vegetation trend analysis

To explore the intrinsic role of water availability on vegetation productivity and monitor possible declines indicative of mortality and general reduction in biomass, this study included spatiotemporal analysis of the calculated NDVI anomaly and water availability using cumulative precipitation. This study took a similar approach to monitoring trends as that described in Jiao et al. (2021) utilizing NDVI anomaly to remove the potential influence of seasonality, calculating “z-score” equivalent value using the Eq. 4:

$$NDVI_{k,i} = \left(\frac{NDVI_{k,i} - NDVI_i}{\sigma} \right)$$

where $NDVI_{k,i}$ denotes the anomaly value (i.e., z-score) for the month k in the year i . And where the $NDVI_i$ describes the mean NDVI for year k over the entire study period 2001-2021, lastly σ denotes the standard deviation for the NDVI averaged for year k over the same period. NDVI anomaly scores were contrasted with interannual precipitation to investigate the response of the vegetation to drying. Moreover, the NDVI anomaly (z-score) offers a more universal metric by which to quantify vegetation vitality, both spatially and temporally (Byer and Jin, 2017).

3.4. Results

The results described here, firstly characterize the climatic state facing the Eastern Cape, and the study site over the past 50 years demonstrating the temporal trends of drought from a local (site) to a larger regional level of influence for vegetation dynamicity and vigor. Secondly, this study reports on the spatiotemporal trends of vegetation change, contrasting the degree of “greenness” and overall productivity between drought years and the historical average. Moreover, this study explored the temporal alignment between NDVI anomaly, derivations in productivity indicator of mortality, and drought conditions with focus on the associative trend water availability to broadly discuss potential causes and interpretations of the findings.

3.4.1. Interannual variability of water availability and NDVI anomaly from 2001 to 2021

Across two decades of concomitant precipitation and NDVI accessible data years, both cumulative precipitation and yearly NDVI anomaly illustrated two discernable shifts to declining productivity and incumbent mortality for the study area. First in 2009 to 2010, preceded by positive regrowth and positive productivity trajectory in the following highest record rainfall year (2011) (Figure 2). However, since 2015 the study area and region in general has and remains under a multi-year drought state with yearly cumulative precipitation rarely exceeding 300 mm.yr⁻¹ resulting in considerable strain on productivity with early indications of incipient mortality observed as of 2020. This declining trend in productivity is evident by the yearly NDVI anomaly, z-score, continuing to be negative with no clue of an upward trajectory under present rainfall conditions. The extremely low NDVI anomaly values, and coincidentally low precipitation values, of 2008-

2010 and 2015-2021, suggest that the severe lack of water availability and drought occurred across these years incurring considerable physiological and productivity strain for this region. Distinctively, the impact of 2015-present drought has had great ramifications for Subtropical Thicket, given both the nonlinear accumulative decline trend in contrast to the rapid shift type response observed in 2009-2010 which did not coincide with any observed plant water stress symptoms such as defoliation or dieback (pers. Comm. with the landowner).

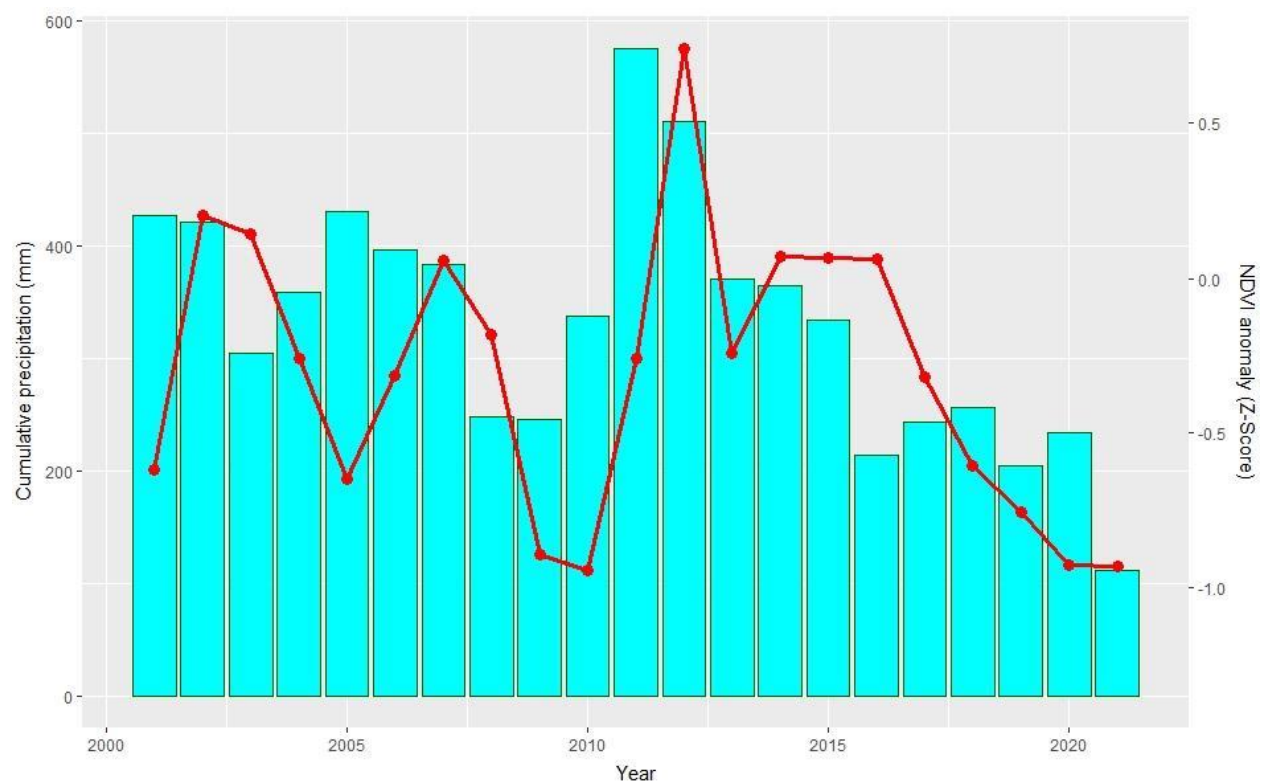


Figure 2: Interannual variation of cumulative precipitation (proxy of water availability) and yearly NDVI anomaly (Z-Score) at the study site for 2001 to 2021. [Colour image on web; black and white for print]

3.4.2. Canopy vitality indicators and health status under drought

This study explored the strength of correlation between observed variables with one another (Figure 3) to examine the responses between each parameter. The strongest correlation ($p < 0.05$, $r = -0.85$) demonstrated was between the percentage of crown density and defoliation, denoting an inverse association between these two parameters. There was a significantly positive correlation ($p < 0.05$) between percentage leaf discolouration and dead leaf proportion of the canopy, indicating the capacity of species in arid Subtropical Thicket to retain high abundances of senesced leaves within the canopy.

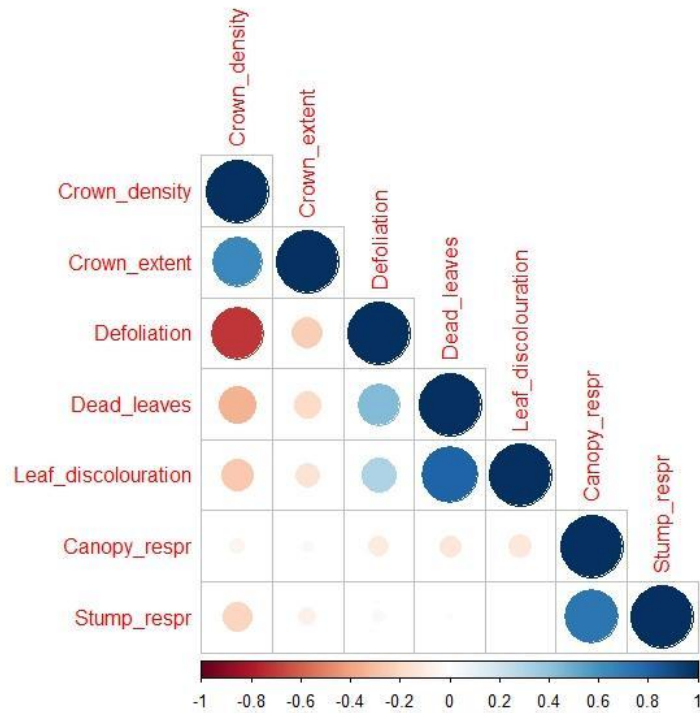


Figure 3: A non-parametric, Shearman rank correlation matrix of observed canopy parameters in the newly established plots characterizing the species collectively. The colour bar indicates the corresponding r values for each independent association, while the magnitude of the correlation is denoted by the circle area extent. [Colour image on web; black and white for print]

While the crown extent and density was largely similar across species, with minor variations between a select few (see Table 1), considerable differences were observed in the degree of defoliation, dead leaf proportion, and severity of leaf discolouration under drought. Of the nine species *P. pyracantha* was most severely impacted by the drought exhibiting greater than 90% dead leaf proportion and leaf discolouration (Table 1, and Figure 4C) in comparison to the other species. The impact of drought on arid Subtropical Thicket had a dramatic effect on vegetation vigor at landscape level (Figure 4A) with many canopy trees denoting substantial canopy dieback (Figure 4B), such as the dominant *Pappea capensis*.

Table 1: Drought-induced canopy defoliation and damage variables described the for the dominant species in arid Subtropical Thicket in the study plots of the Eastern Cape, South Africa.

Canopy metric	Species								
	Br	Eu	Gb	Pc	Pm	Po	Pp	Sc	Sl
Crown extent (%)	67.5 ± 4.36	75.36 ± 6.2	78.8 ± 4.16	66.43 ± 3.02	70.1 ± 5.13	77.9 ± 2.59	75.1 ± 4.12	70.2 ± 4.97	82.1 ± 3.9
Crown density (%)	46.9 ± 6.15	45.18 ± 7.49	51 ± 5.72	49.94 ± 3.92	57.2 ± 5.77	31.6 ± 5.51	49.2 ± 6.95	56.7 ± 6.09	46.9 ± 8.91

Defoliation (%)	27.6 ± 5.38	32.63 ± 7.03	43.3 ± 4.82	26.09 ± 2.97	26.7 ± 4.21	47.4 ± 7.28	36.3 ± 4.67	30.9 ± 5.09	41.9 ± 8.39
Dead leaves (%)	23.4 ± 4.43	10.9 ± 2.76	18.4 ± 3.18	25.86 ± 4.32	9.1 ± 1.91	11.3 ± 2.41	90.3 ± 1.81	9.2 ± 1.72	24.5 ± 9.12
Leaf discoloration (%)	35.9 ± 4.35	19.81 ± 7.53	30 ± 3.94	38.47 ± 4.93	20.6 ± 4.38	34.6 ± 4.76	94.6 ± 1.91	11.6 ± 2.33	51.4 ± 11.61
Canopy resprouting (%)	0 ± 0	27.28 ± 9.36	4.5 ± 4.37	22.25 ± 10.5	0 ± 0	18.2 ± 5.24	0 ± 0	0 ± 0	8 ± 3.49
Stump resprouting (%)	0 ± 0	11.54 ± 4.13	6.8 ± 3.79	14.56 ± 5.91	0 ± 0	15.5 ± 3.68	0 ± 0	0 ± 0	7.7 ± 2.49

Note, Br – *Brachylaena ilicifolia* (n = 10), Eu – *Euclea undulata* (n = 11), Gb – *Gymnosporia buxifolia* (n = 10), Pc – *Pappea capensis* (n = 51), Pm – *Polygala myrtifolia* (n = 10), Po – *Ptaeroxylon obliquum* (n = 10), Pp – *Putterlickia pyracantha* (n = 10), Sc – *Schotia latifolia* (n = 10), Sl – *Searsia longispina* (n = 10).

The impact of droughts in arid Subtropical Thicket are often overshadowed by the superior persistence of succulents, particularly the dominant *Portulacaria afra*, to maintain chlorophyll and general physiological functionality, giving a widespread appearance of minimal/little effect of water deficiencies (Figure 4A, B). However, the current drought in this region has had substantial influence on this vegetation type, with widespread defoliation of understory shrubs (e.g., *P. pyracantha*, Figure 4C) and woody canopy species, such as the regional dominant, *P. capensis*, (Figure 4B). The impact of crown defoliation and observed landscape-level canopy dieback is starkly evident when viewing aerial UAV footage of one of the first established long-term monitoring plots for this site in this report (Figure 4A). All woody component species considered in this study (*P. capensis*, *G. buxifolia*, *P. myrtifolia*, *P. obliquum*, *E. undulata*, *B. ilicifolia*, *S. longispina*, *P. pyracantha*, and *S. latifolia*) were affected by drought. Many species experienced considerable dieback of foliage followed by branch senescence of many canopy species, such as *P. capensis* and *P. obliquum*. The presence of fungal and/or pest damage was apparent, particularly within *P. capensis*, where much of the hardwood had been consumed and several portions of the bole had been subject to bark removal and signs of borer beetle attack (Figure S5). A secondary survey was undertaken six months later following the wet season, however, little change in canopy vitality and general condition of the arid Subtropical Thicket vegetation was observed – stressing the significance of drought to this vegetation influencing productivity turnover and persistence of woody canopy trees and understory shrubs.

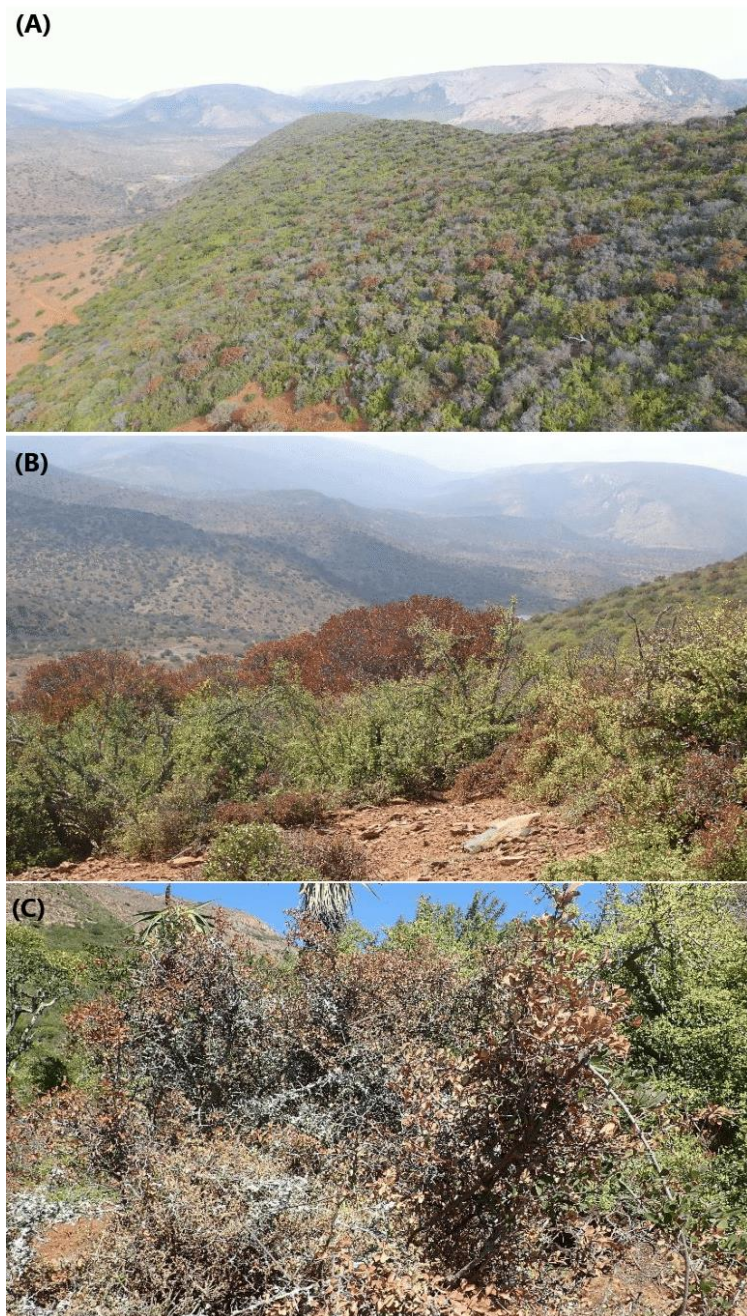


Figure 4: Drought-induced impacts on arid Subtropical Thicket in the Eastern Cape, South Africa of 2020. (A) Landscape-level impacts of the drought event on canopy woody tree species, as observed by complete foliage discolouration of canopy trees *Pappea capensis* (B) and understorey woody shrubs, such as *Putterlickia pyracantha* (C). [Colour image on web; black and white for print]

3.4.3. Inter-annual analysis of drought metrics, SPEI and SPI

Oscillating wet and dry periods which is made most evident from the 12-month Standardized Precipitation Evapotranspiration Index (SPEI) and the Standardized Precipitation Index (SPI) (Figure 5G, H) for Somerset East weather station, i.e., the closest proximate climatological data record to the study site. Temporal variation of water availability at differing time scales (1-,3-,6-, and 12-month) for Somerset East were derived for this study (Figure 5A-H) using SPI and the multi-scalar drought indicator SPEI. Generally, both SPEI and SPI exhibit similar trends and patterns of variability across time scales but differ in magnitude and durational extent of water deficit states for the study period. Additionally, the frequency of dry, arid conditions (red colour, below zero values for SPI and SPEI) occur more often for shorter rather than long time scales, attributed to the requirement of less than or of a month deficiency in precipitation to correspond to a dry conditional state in contrast to long-term accumulated shortages in rainfall to result in change for protracted timescales. The sensitivity of SPEI and SPI at varying timescales was temporally dependent, with short-term denoting more frequent alternation between dry and wet and more evident fluctuation of values from negative to positive. Whereas, long-term timescale demonstrate slower changes from dry-wet conditional status (compare Figure 5A with E).

The values of SPI range from 1.98 to -2.31 and from 2.15 to -2.47 for SPEI across timescales. The most evident temporal pattern observed is the prolonged period of water deficiency commencing from 2015 to present (07-2021) reaching a most severe peak of negative -2.47 for SPEI 3-month interval in 2020 similarly low SPI -1.86 in the same year. The magnitude of SPEI was greater than detected by SPI, this escalation in severity of water deficiency impact can be rationalized by the compounding effect of evapotranspiration (i.e., influence of temperature) on water demand. The converse can be seen for 2017 where SPI is the most negative at timescale of 3-months with a value of -2.31 compared to a more positive SPEI 3-month of -1.11 (Figure 5D), highlighting the influence of temperature on water demand and by extension drought impact for the region of study.

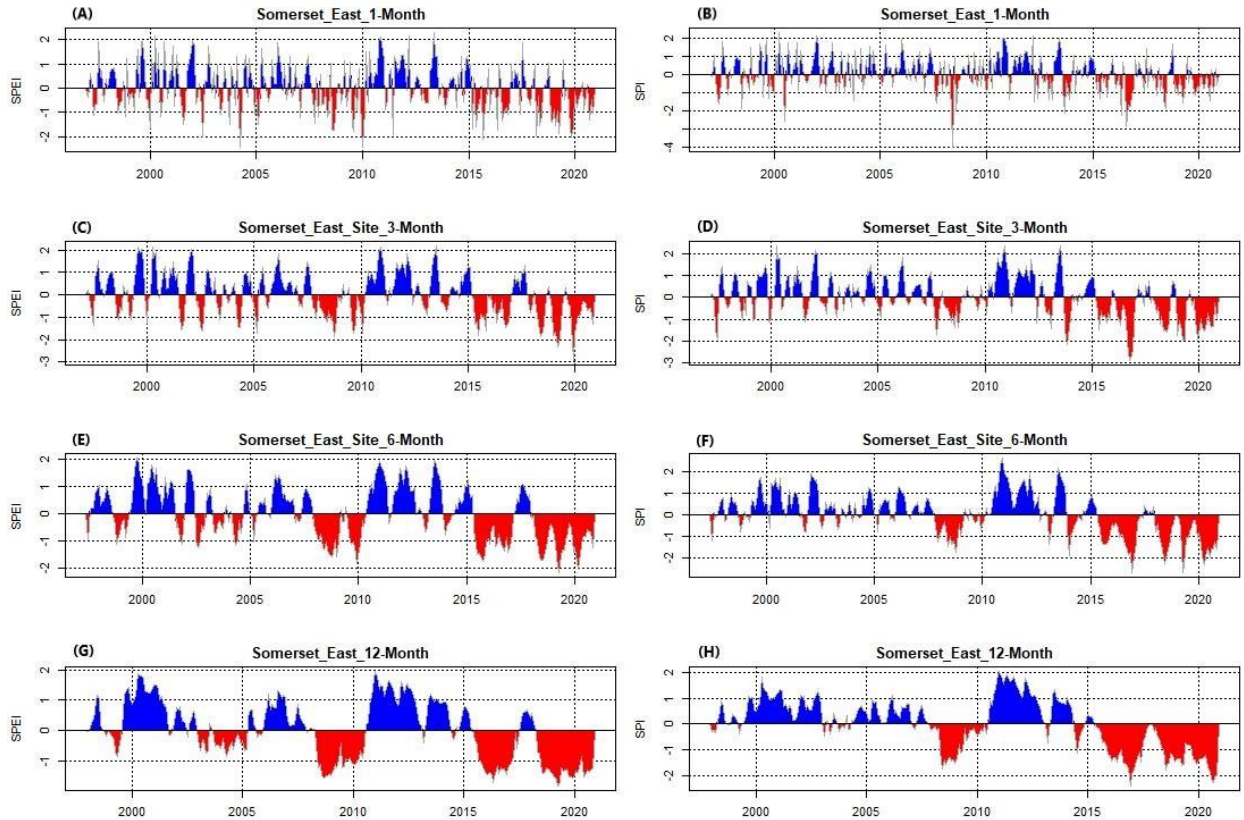


Figure 5: Standardized Precipitation Evapotranspiration Index (SPEI) and Standardized Precipitation Index (SPI) at 1-month (A, B), 3-month (C, D), 6-month (E, F), and 12-month (G, H) intervals, describing time series data for Somerset East weather station, the closest proximate to study site, in the Eastern Cape, South Africa. The red-coloured areas a points where the index (SPEI and SPI) has exceeded 0 threshold indicative of dry, arid conditions, while blue describe the converse indicating wet conditions. [Colour image on web; black and white for print]

In addition to presenting Standardized Precipitation Index (SPI) and Standardized Precipitation Evapotranspiration Index (SPEI) for the closest proximate weather station under the purview of the South African Weather Service (SAWS), this study included a temporal analysis of nearest proximate Metropolitan, Port Elizabeth. Additionally, this study included all major weather stations across the Eastern Cape Province (see Figure S1 for location) providing a spatially comprehensive account of drought, historically (Figures S2 and S3). The water deficiency trends observed for Somerset East (Figure 5) strongly agree with those exhibited for the major Metropol, Port Elizabeth (Figure 6), most significantly the protracted drought conditions initiated in 2015 continuing to present. There was, however, a notable reprieve of pre-2015 where both SPI and SPEI reached one of their respective highest values for the entire 50-year recorded period, denoting extremely wet conditions during this timeframe of the study.

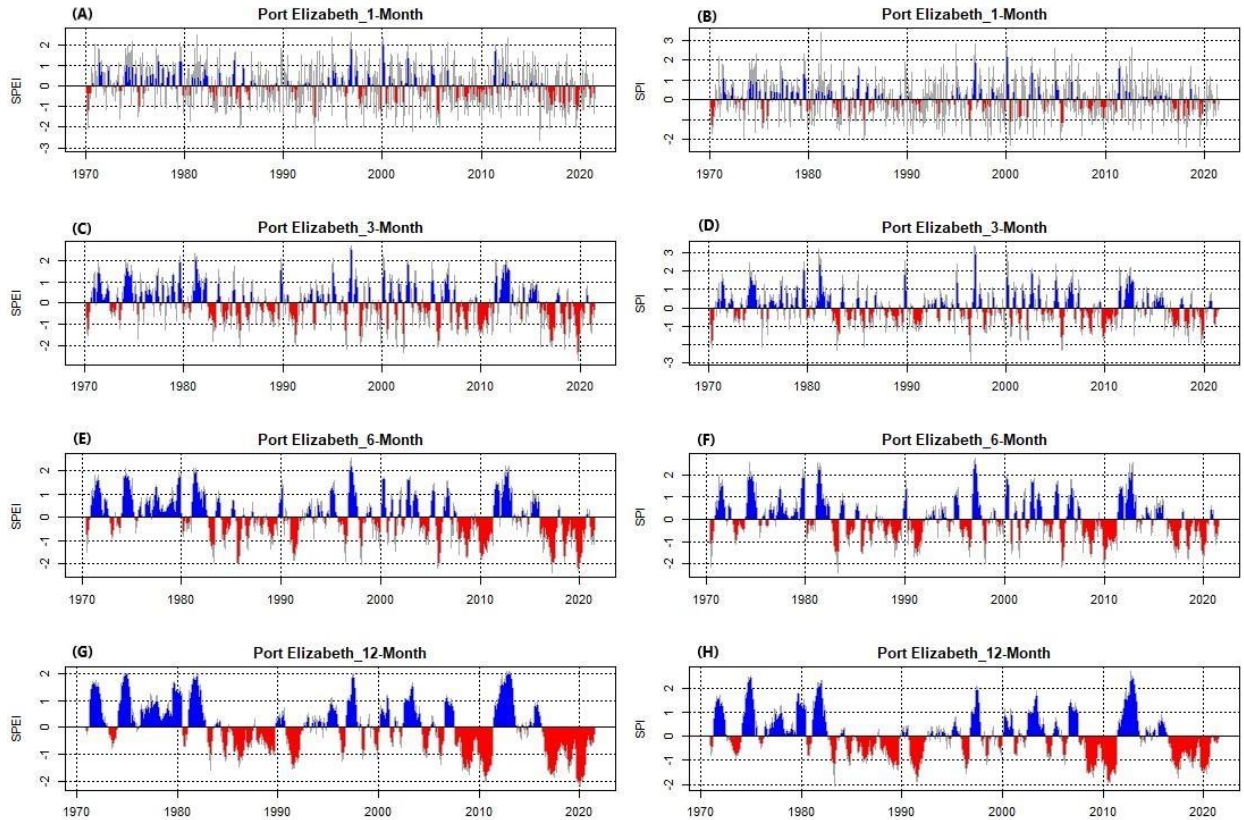


Figure 6: The Standardized Precipitation Evapotranspiration Index (SPEI) and Standardized Precipitation Index (SPI) at 1-month (A, B), 3-month (C, D), 6-month (E, F), and 12-month (G, H) intervals, describing time series data for Port Elizabeth, the nearest major Metropoli to study site, in the Eastern Cape, South Africa. The red-coloured areas a points where the index (SPEI and SPI) has exceeded 0 threshold indicative of dry, arid conditions, while blue describe the converse indicating wet conditions. [Colour image on web; black and white for print]

The study demonstrates temporal trend most characteristic of water deficiency changes and high frequencies of below zero SPI and SPEI for the region, which stress the appearance of drought and highlight its potential role as a source of considerable disturbance that may induce change in vegetation productivity for arid Subtropical Thicket of South Africa.

3.4.4. Spatiotemporal patterns and trends of remotely sensed indices

This study aggregated and generated spatial temporal distribution maps from NDVI (Figure 7) and EVI (Figure S14) for the study site enabling comprehensive monitoring of variations in vegetation conditional status and productivity. A pixel-based spatiotemporal analysis of NDVI for the period from 2001 to 2021 demonstrates vigorous growth and productivity for 2001-2003, 2008, and for 2012-2014 (Figure 7). These trends align with more wet conditions as indicated by more positive SPEI and SPI values which coincided with the incline in vegetation productivity as denoted by NDVI. In contrast, the NDVI values during the years 2004-2007, 2009-2010, and

2015-2021 exhibited considerably lower mean values compared to other periods due to severe reductions in water availability (SPI, Figure 5 B,D) characteristically drought years that occurred across the Eastern Cape (Figure S3, regional illustration of SPI demonstrating drought at major cities across the province) and encompassed the study area resulting a general decrease in productivity across the vegetation. The general browning trends observed during these latter mentioned years which coincided with negative SPEI and SPI values, indicative of dry-arid conditions, evidently demonstrates the significance of the link between drought and vegetation productivity, growth, and persistence (i.e., survival) of arid Subtropical Thicket in the Eastern Cape, South Africa. Moreover, considering the severity of observed browning trajectory from 2015 onwards to 2021, with NDVI values ranging between 0.14 to 0.45 and the strong agreement with declining trends in EVI (Figure S4) stresses the importance of interpreting the impacts and changes drought presents for this vegetation is paramount.

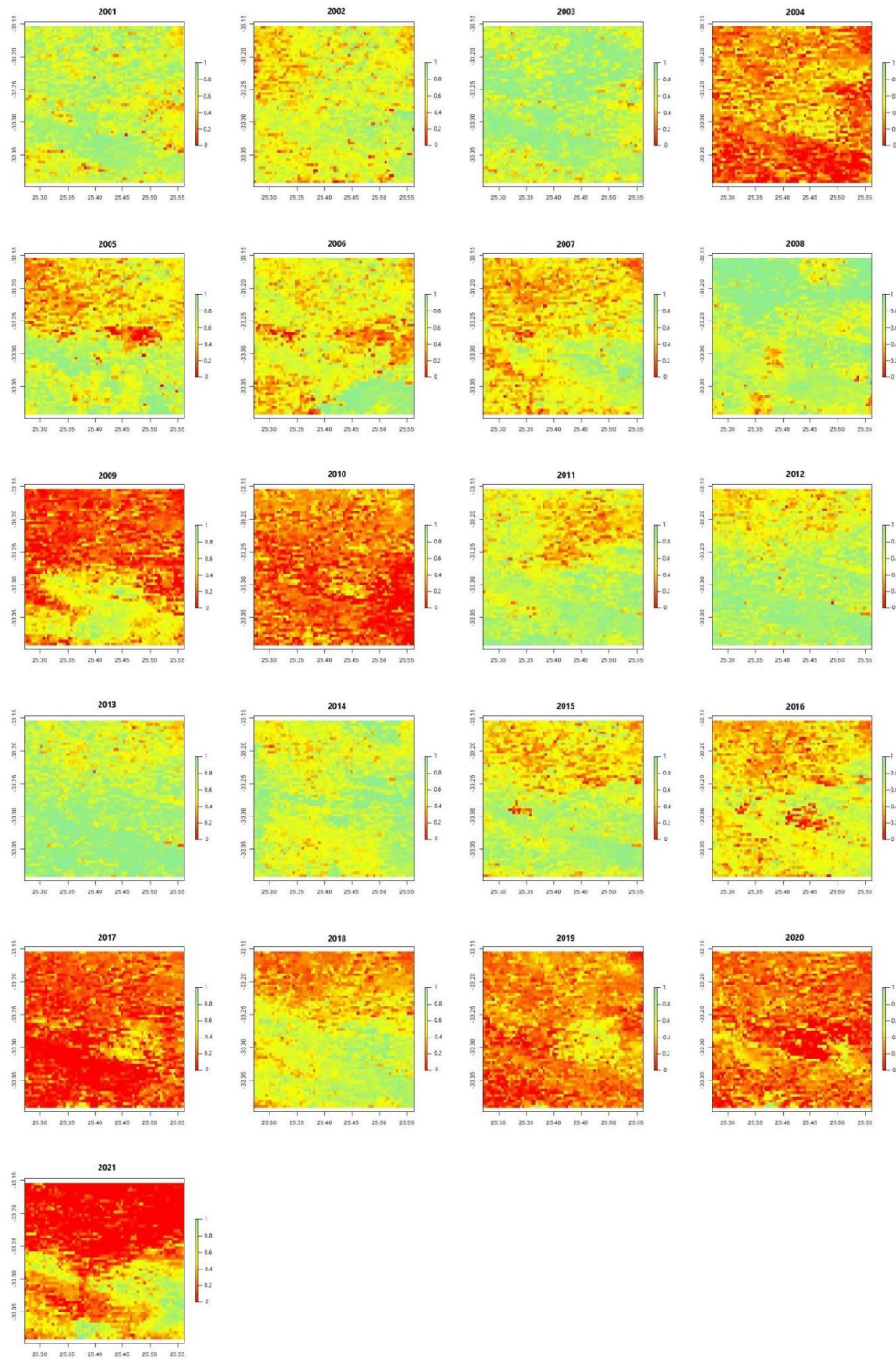


Figure 7: Spatiotemporal variability in Normalized Difference Vegetation Index (NDVI) for the area of interest for the period between 2001 to 2021. The images were MODIS derived satellite data

characterizing NDVI at commencement of each year (DOY 001) exhibiting changes in greenness throughout the study period. Colour scale delimit NDVI across the range threshold of 0 to 1. [Colour image on web; black and white for print]

To better understand the impact of drought in the Eastern Cape, South Africa the spatial distributions of Vegetation Condition Index (VCI) describing the conditional extent of the time based on the historical average state were assessed. Here the multi-year drought extent of 2015-to-present is compared with an average moist/wet year 2014 (Figure 8). The non-droughted year, 2014 depicts considerable growth and generally productive extent, although appearances of heterogeneously spatially explicit negative conditional status are apparent outside the distributional range of Subtropical Thicket (i.e., northern provincial reaches). These spatially distinct areas of negative conditional status demonstrate microtopographic complexity and influence of rainfall response. The vegetation response to the multi-year drought setting highlights the exacerbating impact that protracted arid conditions have had on the region. The response of vegetation to drought differs accordingly to biome, duration and severity of water availability, and seasonality, however, is strongly positively correlated with precipitation anomalous conditions for arid and semi-arid regions, demonstrating a robust correlation on interannual timescales for Subtropical Thicket (Lawal et al., 2019). Figure 8 describes the interannual shift in vegetation productivity in contrast to its historical average, as indicated by the Vegetation Conditional Index (VCI) per pixel. The north-eastern half of the region characteristically denotes grassland on the interior with Afromontane Forest along the coast. While the south-western extent of the province delimits Subtropical Thicket with scattered appearances of Karoo vegetation at interior ranges. Much of the south-western extent experiences all-year-rainfall, with great unpredictability (coefficient of variation >40%) progressively towards the interior characterizing arid thicket distribution, which is given precedence in this report. For 2014 this region illustrated widespread positive growth and productivity, as indicated by VCI (Figure 8). Throughout the multi-year drought extent, the south-eastern range, inhabited by Subtropical Thicket experienced the most intense declines in productivity, defoliation, and dieback over the region. This severe impact persisted, even during more favourable conditions of 2018 and 2021, where heightened precipitation regimes across grasslands prompted productivity across these ranges. However, throughout the remainder of the 7-year period the majority of the region experienced negative drought-induced impacts on vegetation. The most severe periods of negative anomalous conditions occurred in 2016, with riparian zones depicting drought refugia, and 2019, demonstrating acute drought strain across the coastal Afromontane Forest range. This demonstrates both the intensity of drought impacts during these years and the cumulative legacy effects of drought with near whole Subtropical Thicket range impacts in 2019 (i.e., coastal thicket refugia in 2020 and 2021) to protracted arid conditions extending from 2014.

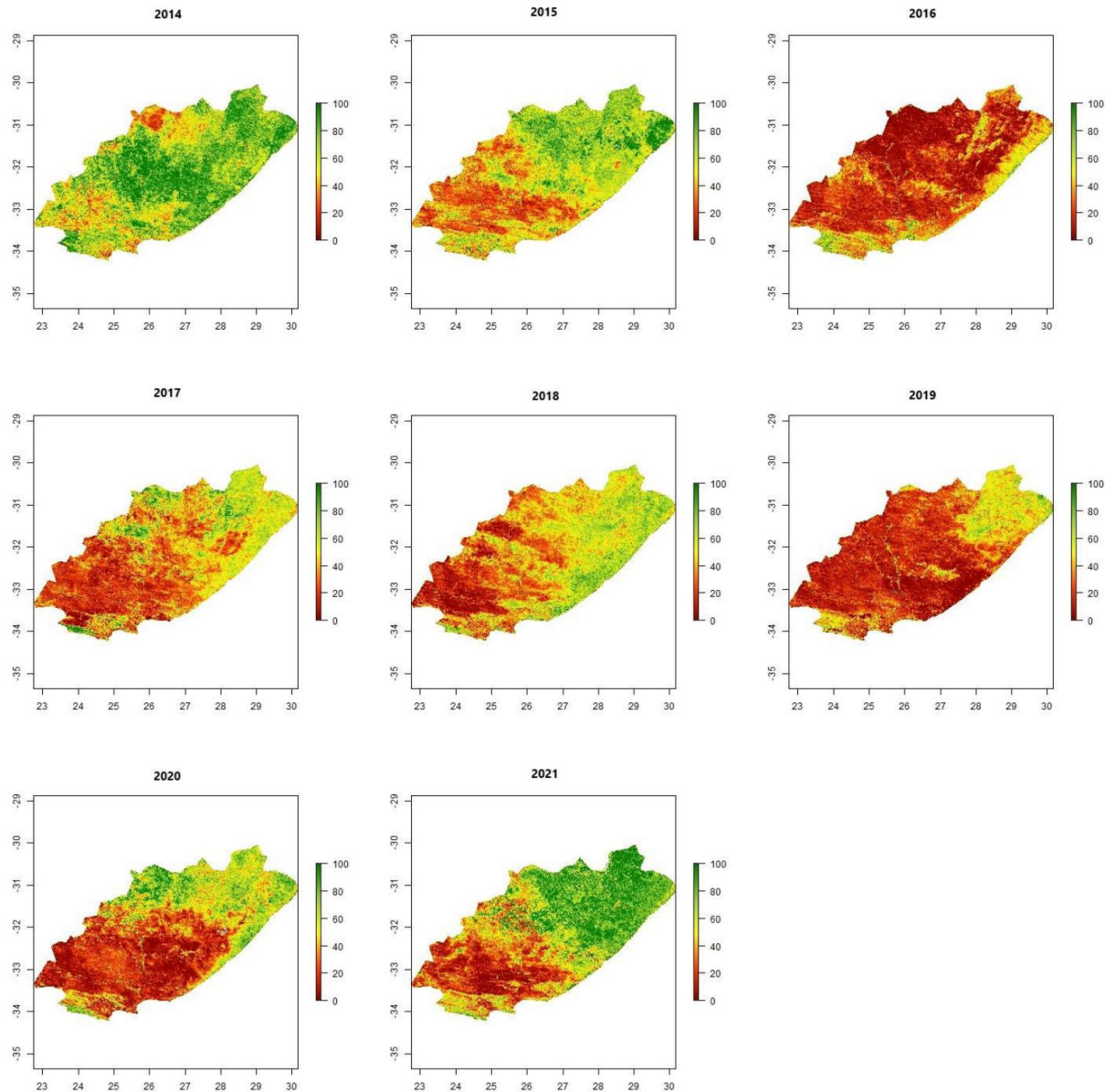


Figure 8: The spatial distribution of Vegetation Condition Index (VCI) trends for the Eastern Cape, South Africa under varying levels of water strain, with 2014 denoting a non-drought year with above average SPEI (<2). In comparison to VCI throughout the multi-year drought period commencing in 2015 until present (DOY 001). The drought event of 2020 coincided with canopy vitality sampling enabling the validation drought-induced vegetation impacts with ground-truth captured images and UAV photography. [Colour image on web; black and white for print]

3.5. Discussion and conclusion

The sudden and severe dieback and crown defoliation event of 2020 in arid thicket coincided with peak intensity of a multi-year drought in the southern cape of South Africa. This represents the first documented episode of this response associated with extreme climatic conditions in

Subtropical Thicket. A climatically comparable, though more acute, anomalous event was detected for the study area from 2009 to 2010, however, there were no reported cases of canopy dieback or crown defoliation during this period. Tree canopy dieback events have been recorded in few other biomes of South Africa and included semi-arid savanna where a record-high mortality was reported (Swemmer, 2020). Whilst fewer reports have been described elsewhere in other tree dominated biomes of South Africa, presenting a fundamental gap in ascertaining the vulnerability of these other systems under projected future increased frequencies of climatic variability and extreme anomalous events, such as drought.

Evidence of widespread or large-scale canopy dieback, crown defoliation, and tree mortality from severe drought conditions have been rarely documented or have seldom occurred in Subtropical Thicket. This suggests that tree species within arid thicket have evolved under these severe disturbances, enduring striking fluctuations and unpredictability in precipitation and temperature regimes, resulting in various responses and strategies of survival (Cowling et al., 2005; Bussotti and Pollastrini, 2020). The dominant species within these arid environments demonstrate strategies from succulence to sclerophylly (Vlok and Euston-Brown, 2002), with many trees suggested as having extensive root systems and storage organs facilitating resprouting which appears to be a dominant response of many species with arid thicket (Cowling et al., 2005). Although resprouting is advantageous strategy to disturbance there are considerable metabolic constraints, often offset by lower seedling recruitment, including greater allocation of carbon to roots compared to canopy and longer life expectancy to reach reproductive maturity (Liu et al., 2015). Hence, arid thicket productivity is suggested to demonstrate a long period of turnover with severe disturbances presenting a substantial threat to community composition and species survival.

To this end, this report counter argues the perceived response of trees by Archer et al. (2022) – one of the only studies considering the implications of the 2015 multi-year on vegetation in the Eastern Cape of South Africa. Whilst they found shrubs experienced near consistent mortality with background rates delimited from repeat photography and suggesting an analogous response by trees — the extension of a similar response to trees would be imprudent and present a false perception of expected response in a physiologically and architecturally divergent plant functional type. Moreover, height is a factor where shrubs are characteristically shrub growth form providing an inherent tolerance to water deficits as plant height is central to climate (Breshears et al. 2005). To the extent affecting water conduction, where drought can break the conductive stream introducing gas emboli and functionally impeding water conducting pathways of the plant (Scoffoni et al., 2017). A core architectural property defining species water conduction is xylem conduit diameter, with wider conduits maximizing productivity however are sensitive to embolism and water stress, while narrow conduits are more tolerable of embolism and drought however at the expense of productivity (Hacke et al., 2017; Savage et al., 2010). Taller plants consistently demonstrate wider conduits maximizing productivity at the expense of vulnerability to drought, whilst shorter plants frequently illustrate narrow conduits and are markedly more resistant to drought (Olson et al., 2018). This might account for the observed trend in minimal drought-related symptoms (defoliation) and mortality of shrubs by Archer et al. (2022). In contrast to the findings presented in this study on the first reporting of canopy dieback and crown defoliation of arid thicket tree species.

These evergreen sclerophyllous trees of arid thicket demonstrate several responses to disturbance the most characteristic being resprouting. The results presented in Table 1 illustrate

two species demonstrating a characteristic ability to resprout after and during a disturbance from the stumps, crown, basal roots were *Pappea capensis* and *Euclea undulata*. However, vigor success of resprouting shoots and foliage between these two species diverged, with *P. capensis* exhibiting severe functional loss symptoms, leaf discolouration and defoliation, of newly sprouted tissues, in contrast to those of *E. undulata* which illustrated great verve. This suggests that resprouting offered a strategy to avoid functional impediment to productivity and survival under drought through regrowth of dysfunctional tissues, however, if the species were inherently vulnerable to the existing water stress (i.e., surpassing embolism threshold) the resprouts would enviably suffer a similar outcome as the primary tissue. This outcome was observed in the woody canopy dominant *P. capensis* with epicormic resprouts from the stump and within the crown exhibiting signs of drought-induced stress, including discolouration, loss in leaf turgor, and senescence. Contrary to the resprouting vigor demonstrated by *E. undulata* seeming to suggest that it has an inherently greater tolerance to drought (resist embolism) in comparison to many of arid thicket tree species. Hence, in a scenario where drought episodes are more frequent and severe, a shift in species dominance leading to greater prevalence of *E. undulata* trees may be hypothesized. This pattern is reinforced by observed germination and survival resolve of *E. undulata* to disturbance in arid thicket (Lechmere-Oertel et al., 2005; Sigwela et al., 2009).

In contrast to observed trends in canopy dieback and crown defoliation of many arid thicket tree and shrub species, a markedly exceptional species was delimited exhibiting no early warning indicators of water stress or drought-induced mortality. *Boscia oleoides*, demonstrated no indication of dieback or water stress and unexpectedly illustrating new growth and foliage flushing under unprecedented drought conditions suggesting its tolerance to water stress. A similar outcome was suggested by Wand et al. (1999) where *Boscia albitrunca* behaviour to arid conditions was cited as being “highly unusual compared to other desert species and worth investigation”. Wand et al. (1999) described the capacity of *Boscia* in maintaining photosynthetic activity under high transpiration load and water stress experiencing water potentials of -6.3 MPa to its rather shallow and dually deep root system in contrast to the more monotopic approach by many other desert phreatophytes (Liu et al., 2013; Hultine et al., 2020). This suggested to Wand et al. (1999) that *Boscia* have evolved to become less dependent on deep water sources exploiting the surface water in the pulse rainfall arid environments it inhabits (Hultine et al., 2004). Additionally, suggesting the low leaf water potentials whilst sustaining photosynthesis was ascribed to the inherent cellular tolerance to water scarcity. This highlights the early sentiments of Palmer and Pitman (1972) asserting that “*Boscia* leaves present an intricate framework of durable hard cells which avert leaf collapsing during times of drought”. This intrinsic leaf cellular capacity of *Boscia oleoides* and structural tolerance may account for the observed trends in absent canopy dieback. Additionally, *Boscia* have characteristically dense primary stumps acting as water reservoir heightening the capacitance and ability of the tree to sustain a functional water column under periods of unprecedented water stress (Knipfer et al., 2017). These traits collectively might elucidate to the observed lack of defoliation or dieback in *Boscia oleoides* and account for the asynchronous phenological discrepancy (leaf flushing during drought) in comparison to that exhibited in other arid thicket trees (Kerley et al., 1995; Sakai et al., 2006).

The long-term patterns in NDVI, EVI, and VCI provide further insight into the response of arid thicket to water availability where contrasting trends might be attributed to changes in water balance. The influence drought appears to be more strongly persuasive across arid the regions highlighting the dependency on water availability of the vegetation in these regions compared to the less water-limited moist, humid regions of the north-east ranges of the Eastern Province in

South Africa (Figure 8). Importantly is the observed effect of contrasting VCI trends in the south-west ranges of the Eastern Cape in which Subtropical Thicket inhabits where the coastal thickets are largely buffered by the ocean and are intrinsically more moist than inland regions of arid thicket where water availability has a significant effect on productivity. The observed declining contrast in performance, where VCI values decrease, indicating a loss in green biomass, caused by intensified tree mortality or by canopy dieback and crown defoliation (Gonçalves et al., 2020; Janssen et al., 2021). With either canopy dieback or defoliation irrevocably leading to increased mortality as defoliation exacerbates carbon requirement of a stressed tree (Lotsch et al., 2005). For this reason, it is hypothesized that observed trend suggests elevated defoliation and drought-induced tree mortality in the arid interior of arid thicket. The field-based observations and survey of 2020 confirm attributed the declining VCI and NDVI values coinciding canopy dieback and crown defoliation which corroborates the inference of similar historical trends might suggest defoliation and mortality patterns in this region. Although from discussions with landowners similar drought-induced impacts and canopy vigor was not apparent in the earlier drought of 2008-2010. This would suggest that arid thicket exhibits a moderate tolerance towards short-term drought, however, is not fully adapted to withstand severe protracted climatic anomalous events nor adept to potentially endure consecutive periods of water stress.

The general decreasing productivity response of the region to drought was most severe during the multi-year drought of 2015-to-present with marked performance declines for the interior of the south-west region – with the largest extent of negative impact occurred in 2019 with the drought footprint extending across the entire Eastern Cape. Although, recovery and net positive values were recorded for the eastern extent of the region (grassland and coastal Afromontane Forest) following the peak severity of the drought. This contrasts with the consistent negative VCI and NDVI values experienced throughout the drought period for the south-west regions, describing Subtropical Thicket ranges and an all-year-rainfall regime with high unpredictability. It is hypothesized that the varying responses observed might be attributed to vegetation of each biome not exceeding the relative physiological (hydraulic) functional thresholds, hence water availability was not severe enough in the eastern extent for physiological dysfunction, however, was low enough to induce foliage damage such as defoliation although not severe enough to incite mortality. Whilst in the arid interior of the south-west region the vegetation would have surpassed critical physiological thresholds resulting in severe drought-induced symptoms and incipient mortality.

This hypothesis extends to suggesting the outcome of recovery trends following drought disturbance, which would anticipate more responsiveness and rapid recovery in the summer-rainfall east regions where Afromontane Forest and grasslands dominant. To the extent that reliability in rainfall of this region would facilitate rapid regrowth and recovery, as soil water availability and physiological functionality were not near critical thresholds due to precipitation consistency of the region. In contrast to the arid south-west region where rainfall unpredictability would have compounded recovery by prolonging water stress coupled with consecutive interannual precipitation shortfalls would increase the probability of arid thicket trees exceeding critical physiological functional thresholds elevating the vulnerability and drought-related impacts including defoliation and canopy dieback.

In addition to immediate drought-induced impacts, the more intangible aspect of drought legacy effects, where cumulative physiological damage must be recuperated are the metabolic impacts endured during previous drought periods. Such lagged responses to drought have been

previously documented in Colorado Rocky Mountains, where, proceeding a drought event, mortality rate of subalpine forests increased over the following 5-11 years across the region (Bigler et al., 2007). Such legacy effects have been observed across several water-limited biomes (Sarris et al. 2007; Richard et al., 2008; Peltier et al., 2016; Kannenberg et al., 2019; Bastos et al., 2020). To this end, it is hypothesized that several arid thicket species have surpassed critical physiological thresholds during first 3 years of the multi-year drought culminating in early physiological impairment, however not complete dysfunction, until the later years into 2020 were consecutive drought-induced physiological damage resulted in widespread defoliation and canopy dieback across arid thicket. Nevertheless, the ensuing canopy damage, dieback, and tree mortality has significant ramifications for community-level species compositional, and life form, dominance within arid thicket. Hence, yielding a similar outcome as described in Milton et al. (1995) following the 1990/1 drought where mortality of woody species far exceeded those of succulents, resulting in a dominant shift in species composition and growth form in the southern Nama-Karoo communities in South Africa. There is a high possibility that an analogous output might be observed for the arid thicket of the southern Cape, where the greater frequency of long-term droughts would potentially eliminate the more vulnerable woody canopy tree and shrubs across the region.

The mixed methods approach embarked on here provided a comprehensive understanding of the multi-year drought impacts of the Eastern Cape in South Africa, particularly for understudied tree dominant biomes on general canopy vigor and historical productivity. Recent research on climate change projects the increased irregularity of historically predictable meteorological patterns, exacerbated by predicted frequent severe droughts in the future (Pörtner et al., 2021). Hence, describing and understanding the vulnerability of communities and drought-related impacts provides a vital viewpoint on potential outcomes and appropriate management strategies in mitigating drought-induced vegetation changes. To this extent this study is the first to describe early warning symptoms of drought-induced mortality risk in arid thicket, filling the crucial gap of how southern Africa tree dominated biomes might respond to future drought and expectations for management. More importantly, and a theme raised throughout this discussion, is the significance of plant physiological functional thresholds in understanding the resistance and vulnerability of arid thicket to climatic anomalous events such as drought highlighting this as a research priority in arid thicket and other climatically susceptible ecosystems.

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3.7. Supplementary figures and tables

Table S1: Drought type classification based on Standardized Precipitation Index (SPI) and Standardized Precipitation Evapotranspiration Index (SPEI) as described by McKee et al. (1993) and Vinente-Serrano et al. (2010), respectfully.

SPI and SPEI value	Drought severity/category
0 to -0.99	Mild drought
-1 to -1.49	Moderate drought
-1.5 to -1.99	Severe drought
≤ -2	Extreme drought



Figure S11: Location of South African Weather Service (SAWS) automatic weather stations used to derive Standardized Precipitation Index (SPI) and Standardized Precipitation Evapotranspiration Index (SPEI) for this study. [Colour image on web; black and white for print]

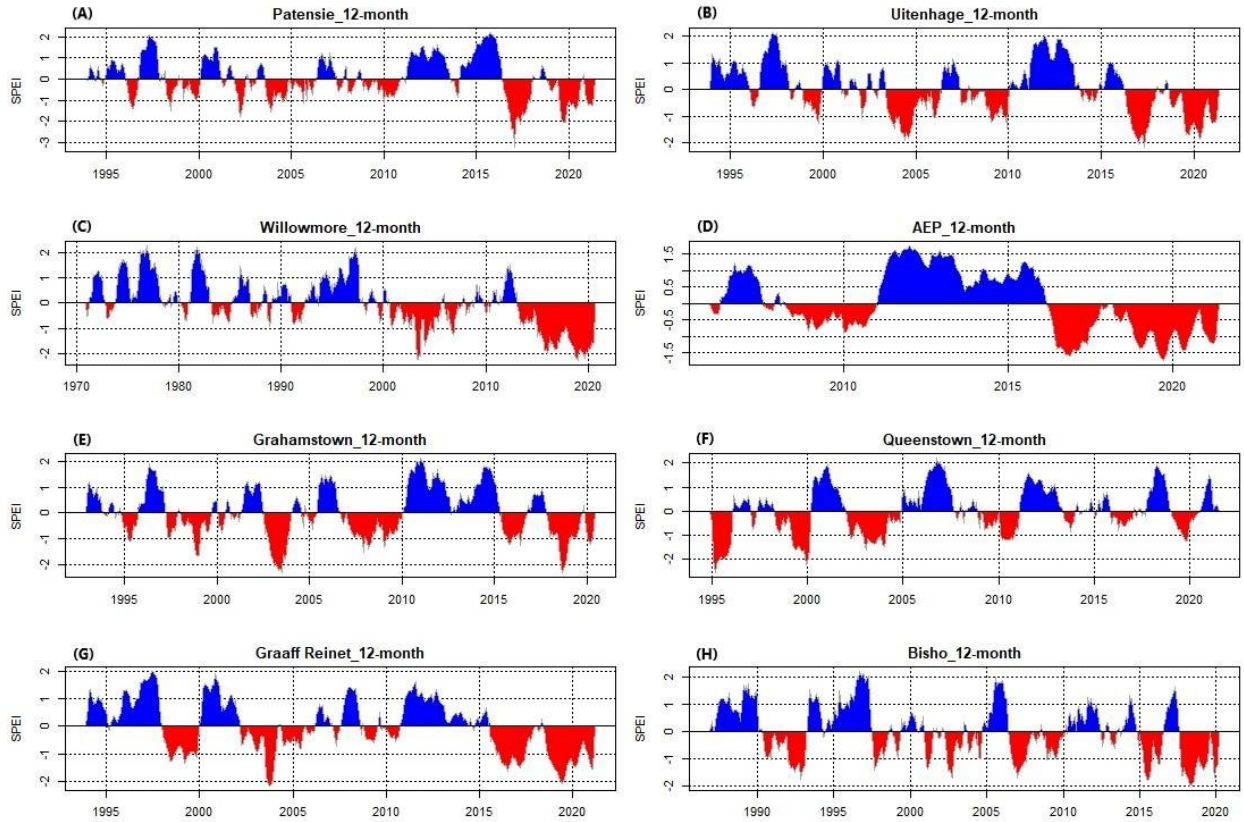


Figure S12: Standardized Precipitation Evapotranspiration Index (SPEI) at 12-month interval denoting temporal trends across major Metropolises of the Eastern Cape, South Africa. (A) Patensie, (B) Uitenhage, (C) Willowmore, (D) Addo Elephant National Park (AEP), (E) Grahamstown, (F) Queenstown, (G) Graaff Reinet, and (H) Bisho. Red coloured areas indicate dry, arid conditions while blue represent wet, humid climatic states. [Colour image on web; black and white for print]

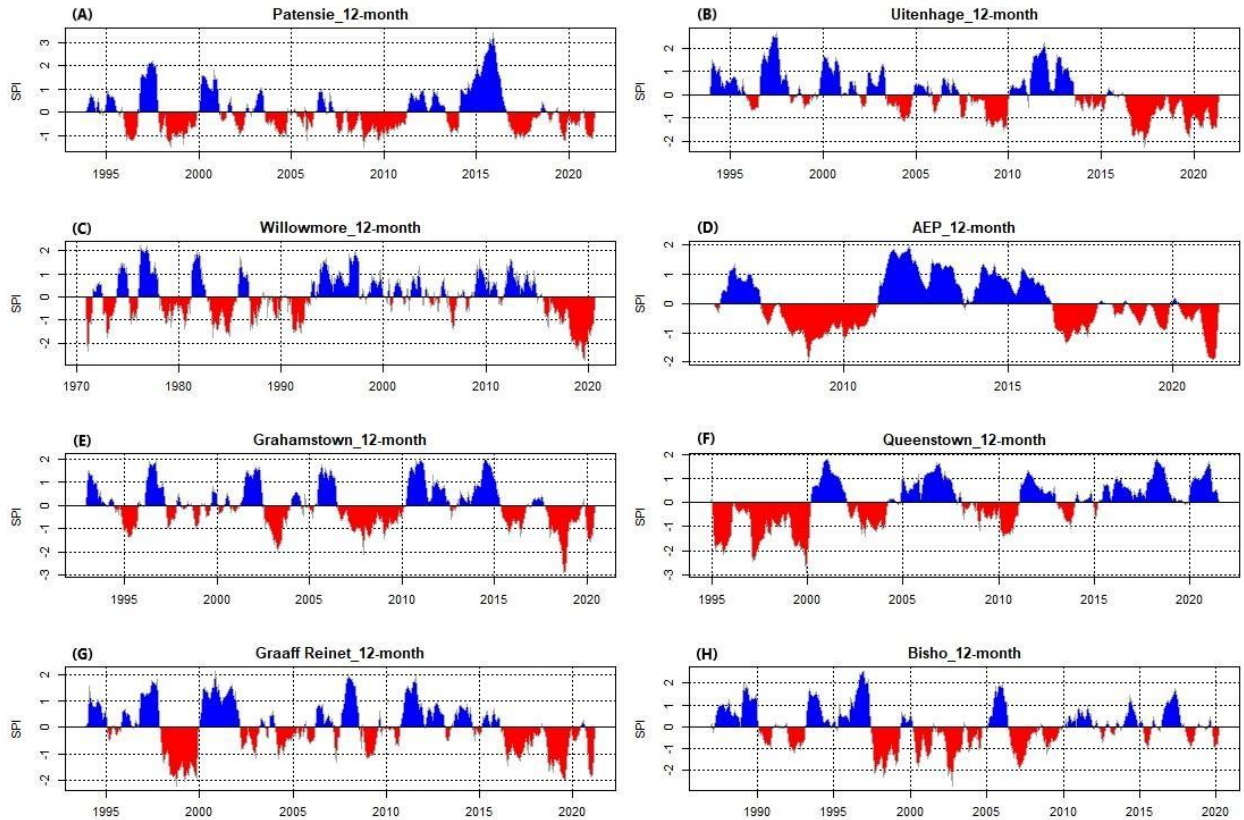


Figure S13: The Standardized Precipitation Index (SPI) at 12-month interval denoting temporal trends across major Metropolises of the Eastern Cape, South Africa. (A) Patensie, (B) Uitenhage, (C) Willowmore, (D) Addo Elephant National Park (AEP), (E) Grahamstown, (F) Queenstown, (G) Graaff Reinet, and (H) Bisho. Red coloured areas indicate dry, arid conditions while blue represent wet, humid climatic states. [Colour image on web; black and white for print]

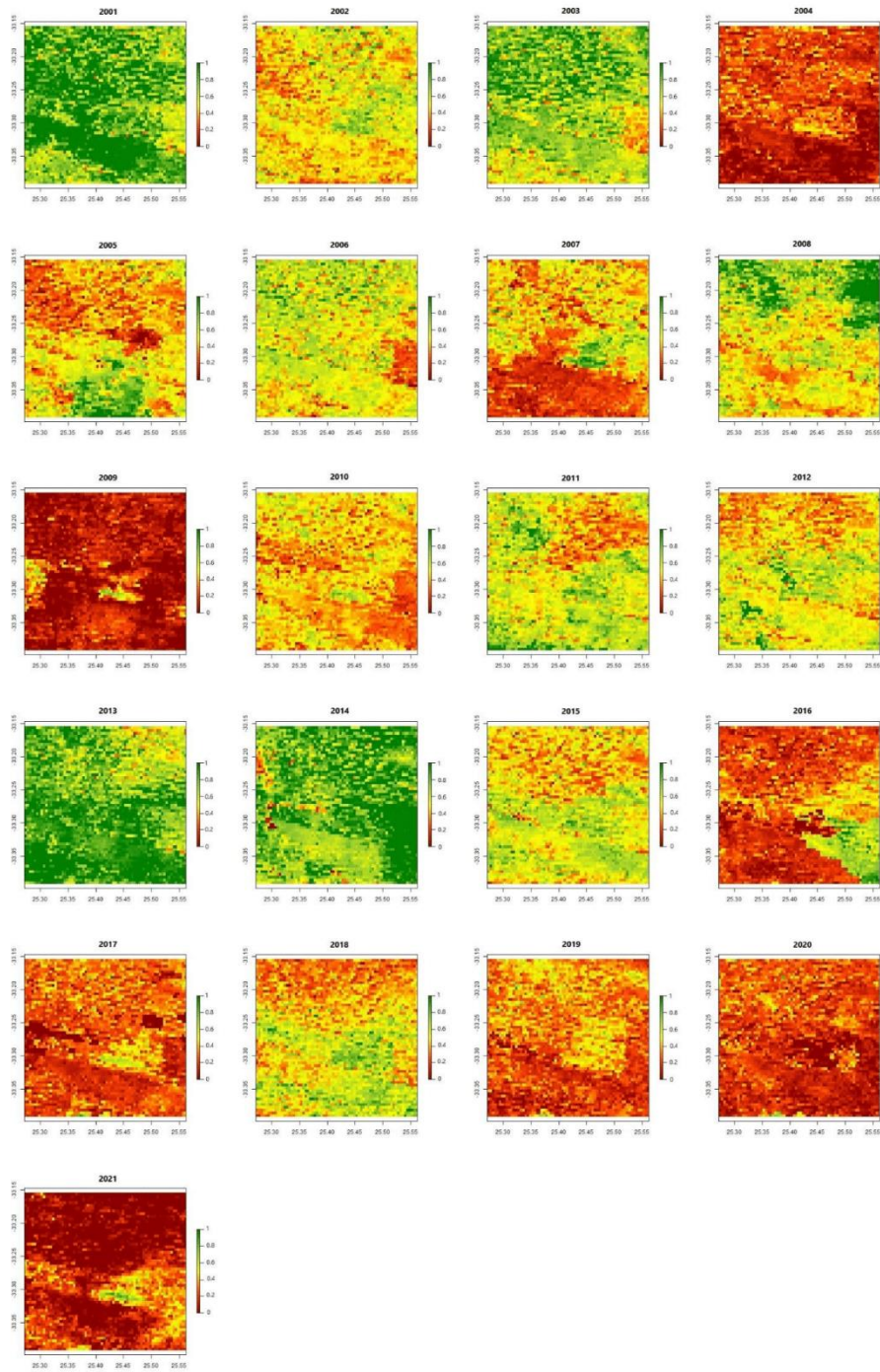


Figure S14: Spatial and temporal trends of Enhanced Vegetation Index (EVI) of the study site from 2001 to 2021 (DOY 001). Low EVI values post 2015 to 2021 were observed aligning to known drought years. The colour scale was threshold to EVI value range of 0 to 1. [Colour image on web; black and white for print]

Table S2: The crown attributes and scoring system for deriving canopy vitality conditional score based on ground-based observations in arid Subtropical Thicket of the Eastern Cape, South Africa. Scoring system was modified from Souter et al. (2010) and Stone et al. (2008).

Crown attribute	Score	Brief description
Crown extent and density	6	Maximum (91-100%)
	5	Major (76-90%)
	4	Moderate (26-75%)
	3	Sparse (11-25%)
	2	Minimal (1-10%)
	1	None (0%)
Epicormic and/or basal resprouting	4	Absent, effect is not apparent
	3	Scarce, effect not clear but is present in low frequency
	2	Common, effect is apparent throughout
	1	Abundant, effect dominates the trees appearance
Leaf dieback	4	Absent, effect is not apparent
	3	Scarce, effect not clear but is present in low frequency
	2	Common, effect is apparent throughout
	1	Abundant, effect dominates the trees appearance
Leaf damage	4	Absent, effect is not apparent
	3	Scarce, effect not clear but is present in low frequency
	2	Common, effect is apparent throughout
	1	Abundant, effect dominates the trees appearance
Leaf colouration	4	Green (healthy)
	3	Green/partial yellowing (moderate stress)
	2	Yellow/partial orange (severe stress)
	1	Orange/brown (dead, complete hydraulic impairment)
Branch condition	5	No dead/defoliated branches
	4	Dead/defoliated branch twigs (>5cm)
	3	Partial defoliated branches (>30cm)
	2	Whole branches defoliated
	1	Branches lost/senesced, decayed or any case worse than previous score
	4	Absent, effect is not apparent

Pest presence and severity of damage	3	Scarce, effect not clear but is present in low frequency
	2	Common, effect is apparent throughout
	1	Abundant, effect dominates the trees appearance

Note: Canopy vitality condition value is defined as the sum of the scores acquired for each of the eight crown attributes, with a maximum of 37 denoting the optimal condition of vigor and health of canopy tree and shrubs. While a value of 8 represents a damaged, worse case condition for canopy trees and shrubs in arid Subtropical Thicket.



Figure S15: Pest damaged individual *Pappea capensis* tree in arid Subtropical Thicket of the Eastern Cape, South Africa. Note areas where extensive bark removal has been incurred and while not visible borer beetle boreholes along the length of the bole and several primary and secondary branches were observed. (Image captured: 29 Sept. 2020). [Colour image on web; black and white for print]

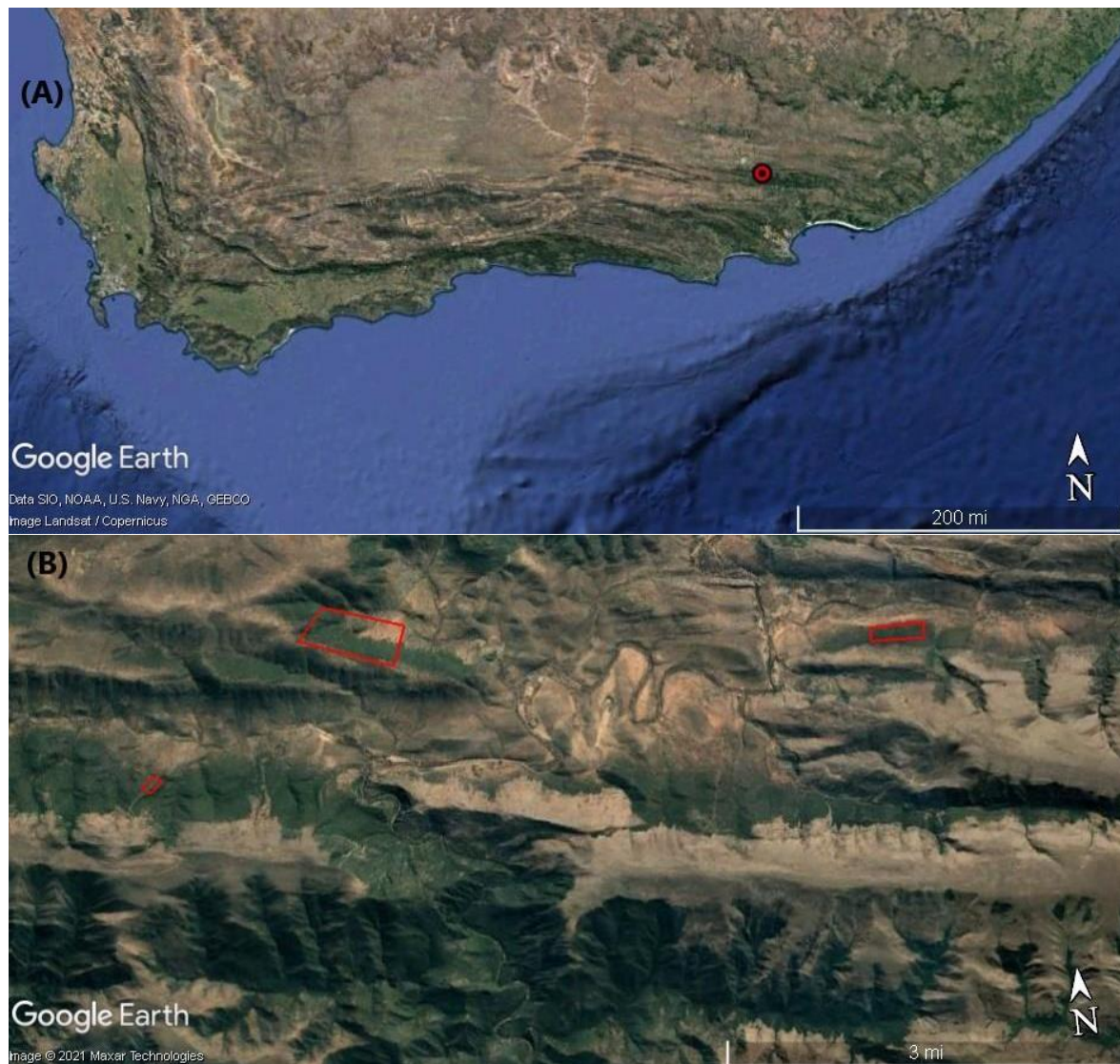


Figure S16: Satellite imagery capturing the location of (A) the regional position of the general study site, and (B) landscape positions of three long-term monitoring plots established on Kabouga Private Nature Reserve centred in Zuurberg mountain range of the Eastern Cape in South Africa (images acquired from Google Earth Pro).

Chapter 4

Hydraulic dysfunction in a non-seasonal, climatically variable southern African plant community, Subtropical Thicket under extreme drought.

4.1. Abstract

Understanding the vulnerability of trees to drought-induced mortality is essential for predicting the impact of water stress on tree dominant ecosystems. Comparative assessment of the physical tolerance thresholds of xylem to desiccation of different species can provide insight into their drought tolerance. This study examines xylem vulnerability to embolism of co-existing, dominant canopy trees and understory shrub species in a semi-arid sclerophyllous Subtropical Thicket community in South Africa. We also quantify *in situ* water stress under a multi-year drought, as well as leaf drought tolerance traits (e.g., turgor loss point, osmotic potential at full turgor). These data allowed us to calculate species-specific hydraulic and stomatal safety margins to predict hydraulic dysfunction during drought.

Species exhibited a broad range of xylem embolism resistance, with the water potential associated with 50% embolism (P_{50}) varying from -4.6 MPa (*Schotia latifolia*) to -7.89 MPa (*Euclea undulata*). Only two species had negative hydraulic safety margins (*Pappea capensis* and *Polygala myrtifolia*). Species with higher levels of *in situ* predicted native embolism (e.g., >80% in *P. myrtifolia*) exhibited the greatest degree of foliage damage and dieback. Damage also varied with species-specific hydraulic vulnerability to xylem embolism. Our results suggest that hydraulic dysfunction is a causal driver for canopy dieback and drought susceptibility in arid Subtropical Thicket.

Keywords

Hydraulic safety margins, hydraulic vulnerability, evergreen sclerophyllous Subtropical Thicket, xylem embolism, xylem, turgor loss point, drought resistance

4.2. Introduction

Drought is regarded as one of the most influential and environmentally extreme forces globally, shaping the composition of communities, affecting function and structure of ecosystems, and delimiting species distributional extents (Reichstein et al., 2013). However, there is considerable complexity in demographic predictions related to mortality rates or potential impact, even amongst co-occurring species, proposing that there is strong variability in traits delimiting drought response and plant strategies which ultimately affect survival and functional thresholds during drought (Allen et al., 2010; Hartmann et al., 2018; Johnson et al., 2018; Mackay et al., 2015). To this end, there is an urgent need to provide a mechanistic understanding of the causal processes and contrasting traits and thresholds affecting drought-induced mortality (Adams et al., 2017).

Drought-induced mortality is largely shaped by plants' capacity to avoid critical physiological thresholds associated with the breakdown of internal carbon dynamics or hydraulic function (e.g., water potential thresholds associated with air blockages in the xylem (McDowell et al., 2008; Sevanto et al., 2014; Choat et al., 2018).

In plant physiology, the minimum water potential (Ψ_{\min}) defines the greatest xylem tension an individual plant (or species or population) endures in the field and has demonstrated strong correlation with species drought tolerance (Martínez-Vilalta et al., 2021). Extremely low water potentials result in decreased turgor with associated membrane and metabolic disruption (Tyree and Jarvis, 1982), and eventually culminating in cellular death and irreversible functional decline (Tyree and Zimmerman, 2002; Bartlett et al., 2012). Hydraulic safety margin (HSM), often quantified as the difference between key functional thresholds (i.e., degree of embolism) and the Ψ_{\min} – is regarded as the standard metric defining plant drought vulnerability (Anderegg et al., 2016; Benito Garzón et al., 2018; Choat et al., 2012; Meinzer et al., 2009). The HSM integrates the absolute measure of functional tolerance to water stress with the quantified expected extremes experienced in the field presenting an indicator of hydraulic dysfunctional risk.

Drought-related hydraulic vulnerability is not solely dependent on xylem embolism resistance, rather integrating several physiological processes including stomatal regulation strategies. Where the stomatal safety margin (SSM) describes the role of stomatal control presents in maintaining plant water potential in relation to the water potential associated with embolism (Martin-StPaul et al., 2017). Much evidence indicates that many species exhibit a conservative stomatal strategy (i.e., positive SSM), suggesting that stomatal closure represents an essential component of avoiding drought-induced embolism (Creek et al., 2020; Martin-StPaul et al., 2017; Pivovarov et al., 2016).

Hydraulic dysfunction occurs under extreme water demanding conditions where a plant's water potential surpasses the threshold of air entry into and propagation through the xylem, reducing the plant's capacity to transport water, nutrients and carbon. This process culminates in diminished foliar productivity, foliar and/or branch dieback, and eventual whole-plant mortality (Brodribb et al., 2021). To avoid such critical water potentials stomata close to reduce water loss, but at the expense of carbon assimilation. Under long drought periods this can lead to the depletion of carbohydrate reserves exacerbating the risk of mortality (Sala et al., 2010; Hartmann et al., 2013). It has been shown that excessive declines in water potential is the primary trigger of plant mortality (Choat et al. 2018), while HSM from P50 are significant (but weak) predictors of cross-species patterns of mortality (Anderegg et al., 2015; Anderegg et al. 2016). Hydraulic dysfunction has been strongly associated with survival under extreme drought conditions for both angiosperms and gymnosperms (Choat et al., 2012), and ensuing xylem tensions resulting in nonrecoverable embolism-induce dysfunction (Choat et al., 2018). Thus, understanding xylem embolism tolerance capacity coupled with other plant water regulation traits is paramount for examining ecological hypotheses regarding drought tolerance within woody plant communities.

Hydraulic vulnerability to embolism varies widely across species and biomes, however, is strongly associated with mean annual precipitation at a region scale (Choat et al. 2012) and water availability at a local level – with more embolism resistant species occupying highly water-constrained environments (Skelton et al. 2021; Peters et al., 2021). This suggests that climate is a robust selective force influencing the evolution of embolism resistance, species distribution and performance (Larter et al., 2017; Maherali et al., 2004; Skelton et al. 2021). It has been reported that HSMs converge across several of the world's biomes (e.g., Choat et al. 2012). For example,

the semi-arid east coast woodlands of Tasmania where rainfall is predictable, subsequently none of the dominant woody species experienced near zero and/or negative HSM (Smith-Martin et al., 2020). A similar trend is seen in the United States chaparral where HSM ranged between positive 0.89 to 1.08MPa following four years of consistent, yet below average, rainfall (Paddock et al., 2013). It remains uncertain what patterns emerge in areas that are climatically heterogeneous. For example, in Subtropical Thicket biome region of South Africa there is a high coefficient of variation of rainfall (>40%) in rainfall (Vlok et al., 2003; Hoare et al., 2006; Cowling et al. 2005a). The evergreen, woody canopy trees and understory shrubs inhabiting this region are exposed to a heterogeneously variable climate, influenced greatly by local topography (Vlok and Euston-Brown, 2002; Vlok et al., 2003; Cowling et al., 2005a). Compounding the unpredictability in precipitation, are high temperatures, often in excess of 40°C, with frequent drought occurrences (Hoare et al., 2006). Few studies have examined plant water relations and hydraulic strategies or functional thresholds of South African woody shrub and tree species (e.g., Jacobsen et al., 2009; West et al., 2012) – with considerable absence of available or published data on underlying xylem embolism resistance in South African woody plant species.

Even less research has explored xylem embolism vulnerability in unpredictable rainfall ecosystems, such as those found in the Subtropical Thicket in southern Africa. This raises an intriguing question as to whether species experiencing uncertain water stress in any given year as a consequence of uncertainty in rainfall patterns tend to be more resistant towards xylem embolism, or do they take a more risky approach. In other words, do species that avoid plant water potentials associated with xylem embolism exhibit positive hydraulic and stomatal safety margins in drier years, meaning that they would be “overbuilt” for “normal” years? The hydraulic safety margins (HSM and SSM) are an ideal metric for predicting species vulnerability to drought by quantifying the proximity a species might approach irreversible hydraulic dysfunction (Blackman et al., 2014; Brodribb et al., 2020; Choat et al., 2018) in an irregular and variable rainfall region such as Subtropical Thicket.

The objective of this study was to examine drought tolerance of plants from semi-arid evergreen, sclerophyllous Subtropical Thicket. We asked whether species vary in (1) leaf-level drought resistance traits (e.g., turgor loss point, osmotic potential at full turgor), and (2) risk of hydraulic vulnerability to xylem embolism. In late 2020 to early 2021, the southern Cape of South Africa was at the peak of experiencing one of its driest periods on record, with the region facing its sixth consecutive year of drought (Archer et al., 2022). These exceptional conditions enabled us to capture extreme physiological extremes for Subtropical Thicket vegetation, which are crucial in characterizing the functional thresholds of populations and species (Martínez-Vilalta et al., 2021) and their potential susceptibility to future global climate change type events (Blackman et al., 2019; Bhaskar and Ackerly, 2006; Duursma et al., 2019).

4.3. Methods and materials

4.3.1. Study site

In situ measurements of plant water stress were conducted at Kabouga Private Nature Reserve, situated in the Zuurberg mountain range of the Eastern Cape. The site denotes a characteristically semi-arid to arid climate, receiving a mean annual rainfall of between 100-300 mm.yr⁻¹. The site is topographically and geologically variable with Witteberg shales dominating the valley slopes and quartzite-derived sandstone the bottomlands with subtle appearances on valley crests

(Johnson et al., 2006). The vegetation denotes a similar distributional complexity, although initially supposed to be edaphically driven only later to be suggested as climate driven (Becker et al., 2015; Coetsee et al., 2015), denoting Arid Thicket along the valley slopes and extending across the lowlands in certain areas, with Nama-Karoo vegetation dominating the valley floors and grassland interchanging with fynbos vegetation dominant across the valley crests.

4.3.2. Midday water potential

Leaf water potential was measured at midday (Ψ_{md}), specifically between the hours of 12:00 pm and 14:00 pm, during peak drought conditions in early October 2020 (i.e., commencement of dry season). Briefly, Ψ_{md} was measured on at least six representative individuals of each species. Samples of apical shoots from fully sun exposed branches were wrapped in moist paper towel, clipped and immediately sealed in a zip-lock bag. Prior to sealing, each bag was breathed into, humifying the bag, and thereafter samples were placed in a thermal regulated container for transport to the field site and subsequent *in situ* water potentials determined using a Scholander Pressure Chamber (Model 600; PMS Instruments Company, Corvallis, OR, USA). Water potential readings were taken without delay, with samples rarely spending longer than 5 min. in plastic bag before measurements were recorded.

4.3.3. Pressure-volume curve traits

A terminal branch, fully sun-exposed, was excised from each of the three fully hydrated individuals of each species to construct P-V curves to estimate the turgor loss point (Ψ_{TLP}), osmotic potential at full turgor (Ψ_{osm}) and bulk modulus of elasticity (ϵ). Sample branches were collected at sunrise and were 1 meter in length were cut from the main stem and the cut-end rapidly emersed in a bucket of deionized water. Where the stem was recut several times to remove any potential blockages, samples were thereafter transferred into a sealed plastic bag with moist paper towel for transport back to the laboratory. P-V curves were constructed using the bench dry method (Sack and Pasquet-Kok, 2011) by successively drying twigs (stem with one or two leaves attached) on laboratory bench measuring sequentially leaf water potential and mass at specified intervals. As such, a branchlet from each sample was excised and placed in a plastic bag (Whirl-Pak) to slow dehydration while on the benchtop. Samples remained in the plastic bag during successive water potential measurements using a Scholander Pressure Chamber (Model 600; PMS Instruments Company, Corvallis, OR, USA), preventing desiccation during pressurization with nitrogen gas while in the chamber. Leaf mass was coupled with each water potential measurement interval, with sample removed from plastic for measuring mass utilizing an analytical balance. Branchlets were dehydrated with mass and water potential being measured repeatedly until at least five measurements beyond the TLP were defined, i.e., linear trend was observed in data.

P-V curves were constructed for each sample by plotting the inverse of leaf water potential $\left(\frac{-1}{\Psi}\right)$ against relative water content (RWC) facilitating the determination of turgor loss point (transition between linear and non-linear trends), and osmotic potential at full turgor according to Bartlett et al. (2012). Relative water content (RWC) was calculated from Eq 1. below:

$$RWC = \frac{((TW - DW) - (FW - DW))}{(FW - DW)} \times 100$$

where, FW, DW, and TW describe the samples fresh, dried, and turgid weight, respectfully. Leaf capacitance was calculated from the change in volume per change in water potential above turgor loss point.

4.3.4. Optical vulnerability curves

Xylem embolism vulnerability in leaves was measured using the recently developed optical vulnerability technique (Figure1; Brodribb et al., 2016). Three healthy and well hydrated individuals for each of the six study species were selected to measure and construct leaf vulnerability curves to embolism. Branches were cut in the early hours (~6-7:00 am) ensuring greater hydration and minimal xylem tension. Whole branches were excised from the main bole, and only those exceeding 1 meter in length were selected [to avoid open vessel artefacts at distal ends], and swiftly transferred to a bucket of deionized water. The excised end of the branch was sequentially recut under water at 10 cm increments from the cut being recut at least twice. Branches were then transferred to plastic bags within which moist paper towels to reduce evapotranspiration and maintain hydration of the sample during transport back to the laboratory at Nelson Mandela University.

The optical vulnerability technique quantifies embolism resistance of leaf xylem tissue using optical light transmissibility changes. Briefly, embolism is identified and measured as a sample dehydrates by monitoring changes in light transmission through the xylem tissue of leaves. These changes in light transmission are due to the refractive properties of water, such that water-filled xylem conduits differ in transmissibility than gas-filled (i.e., embolized). The OVT involves capturing sequential observations of leaf xylem network (i.e., veins) during gradual desiccation of the sample which is attained using a scanner, light-transmission microscope, or image capturing photography. In this study leaf images were captured using custom-built 3D-printed (for an overview and details of methods and materials used see <http://www.opensourceov.org>) leaf clamp equipped with a x20 magnifier and 8-mega-pixel digital camera (Camera Module v2, Raspberry Pi Foundation, UK) and a LED light to illuminate the sample. The custom-built clamp assembly was interfaced with a Raspberry Pi Model 3 (Raspberry Pi Foundation, UK, <http://raspberrypi.org>) microcomputer programmed with the image capture sequence and storage protocols to be executed.

For each individual branch a healthy, fully expanded distal leaf was mounted in the clamp, reducing movement of the sample during image capture, with the abaxial side illuminated by the LED and the adaxial facing the camera module creating a transmitted image. Pre-capturing was undertaken to ensure main vein and at least several secondary leaf veins were imaged as the sample dehydrated in a dark room. The branch was left to dry-down under laboratory conditions, with the camera programmed to capture images at 5 min. intervals, until embolism events were no longer detected (c. 48-168 hours, dependent on species). Simultaneously, leaf water potential was monitored during dry-down using a psychrometer (ICT International, Armidale, NSW, Australia) installed within a 20 cm proximal area on the feeder stem of the leaf clamped. Leaf water potential was automatically logged at 10 min. intervals for the duration of dry-down. There

is the potential for gradients in water potential to exist between stem and leaf affected by cuticular conductance or stomatal leakage resulting in divergences of water potentials across plant tissues. To ensure the accuracy of recorded water potential values by the stem psychrometer cross-validation with periodic leaf water potential measurements using a Shcolander Pressure Chamber (Model 600; PMS Instrutments Company, Corvallis, OR, USA) were undertaken. Leaves in close proximity to clamped leaf were excised, wrapped in moist paper towel and place in a zip-lock bag and measured – with difference proving to be negligible between psychrometer and Pressure chamber readings (data excluded).

Embolism identification and quantification was carried out using the custom macro OVToolbox [and functions therein] in ImageJ (<https://imagej.nih.gov/ij/>) and followed that of Brodribb et al. (2016) and Brodribb et al. (2017). Briefly, images were stacked and subsequently the stack was run through an image subtraction algorithm of the OVToolbox macro using the “Image Difference function, which successive subtracted images identifying embolism as changes in pixel intensity (pixel values transformations). Manual filtering of pixel differences caused by noise artefacts (e.g. bug, leaf shrinkage or movement) were removed or images where there is no clear indication of embolism associated change (e.g., change in light in room). Leaf embolism was measured as the cumulative sum of embolized pixels of images divided by the total embolized pixels of the complete dried sample – providing a ratio at each time interval of the cumulative xylem embolism percentage. To determine the water potential associated with timing of embolism events, the use of time capture time and water potential were regressed (linear) was fitted. A full account of the procedures, software, and technique are available at <http://www.opensourceov.org>.

The percentage of leaf xylem embolized was plotted against water potential [at appropriate time intervals] and fitted using a sigmoidal function (Eq 2. below) – from which several thresholds were derived.

$$\text{Percent Embolism} = 100 - \frac{100}{(1 + e^{a(\Psi - P_{50})})}$$

4.3.5. Hydraulic safety margins

Hydraulic safety margins (HSM) define the scale of conservatism in plant hydraulic strategy, where negative HSM infers greater hydraulic impairment/failure risk in comparison to a safer positive HSM. In this study four approaches were taken in the calculation of the HSM: (1) the difference between point of incipient embolism, P_e (i.e., water potential initializing embolism), and turgor loss point (TLP HSM) was calculated, where the P_e and TLP derived from Optical Vulnerability Curve (OVC) and Pressure-Volume Curves of each species, respectfully. TLP HSM assesses the degree of stomatal control before the initiation of hydraulic functional loss and appearance of embolism, with broader TLP HSM indicative of responsive stomatal control protecting hydraulic function. (2) A second HSM was derived from the difference between 12% xylem embolized, P_{12} , and minimum water potential experienced in the field (Ψ_{\min}). The P_{12} HSM provides an indication of risk a species might approximate native embolism under drought conditions in the field. (3) A more conventional HSM was calculated as the difference between *in situ* minimum water potential (Ψ_{\min}) and P_{50} derived from the OVC, the P_{50} HSM describes how close a given species can hydraulically operate to the threshold (steepest, linear region of OVC) of catastrophic embolism. (4) A more conservative (i.e., and appropriate for angiosperms) critical

hydraulic threshold, P_{88} HSM was calculated using the Ψ_{min} and P_{88} derived from the OVC. The Ψ_{min} was measured for this plant community during severe decadal drought in the Eastern Cape.

4.3.6. Data analysis

For each species, the association between water potential and percentage xylem embolism was fit using a “Weibull” functional curve using the “fitcond” expression under the “fitplc” package in R (Duursma and Choat, 2017), which facilitated the determination of corresponding water potentials at P_{12} , P_{50} , and P_{88} , respectively. Moreover, for each species the use of corresponding vulnerability curves were employed to calculate the predicted native (i.e., *in situ*) embolism percentage induced by incidental minimum water potential quantified in the field as described by Uri et al. (2015). Differences amongst traits derived from hydraulic vulnerability curves (P_{12} , P_{50} , and P_{88}) and P-V curves (Ψ_{TLP} , Ψ_{osm} , and ϵ) between species was assessed using one-way ANOVAs with Turkeys HSD multiple *post-hoc* comparison tests. To ascertain whether a difference in HSM across species using independent ANOVAs to fit HSMs (P_{12} HSM, P_{50} HSM, P_{88} HSM, and TLP HSM) with Turkeys HSD *post-hoc* tests. Figures and analysis were the undertaken in R 4.0.3 (R Core Team, 2020).

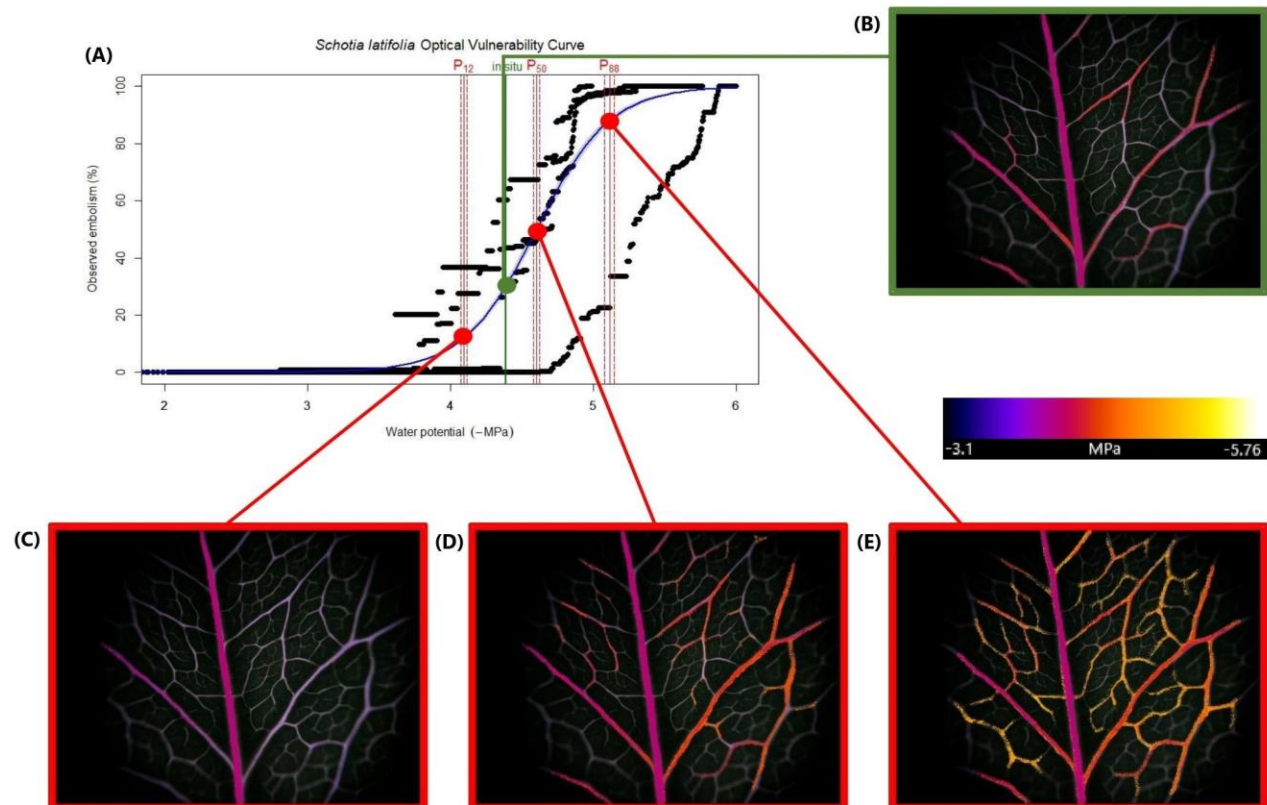


Figure 1: Xylem vulnerability to embolism of *Schotia latifolia* indicating critical water potential thresholds associated with varying amounts of predicted embolism. Black points denote individuals, blue line define the species mean. Critical points indicated on the mean curve are: the minimum water potential observed in the field (B., green); P_{12} (C., red); P_{50} (D., red); and P_{88}

(D., red). The actual observed embolism from a representative individual is shown by image insets (B-E). Scale bar describes associated water potential.

4.4. Results

4.4.1. Pressure-volume curve analyses

Pressure-Volume traits (Table 1) provided three metrics describing drought tolerance of the size angiosperm species under study. Across species the turgor loss point (Ψ_{TLP}) ranged from -1.33 to -3.68 MPa; the osmotic potential at full turgor (Ψ_{osm}) ranged between -0.93 to -2.92 MPa; and the bulk modulus of elasticity (ϵ) ranged from 3.48 to 12.43 MPa. *E. undulata* demonstrated the lowest values across all three traits with a Ψ_{TLP} of -3.81 MPa, a Ψ_{osm} of -2.92 MPa, and a bulk modulus of 12.43 MPa, depicting the most drought tolerant values of all species considered. A second species denoting extremely low values was *S. longispina* which differed most significantly in bulk modulus of elasticity which was approximately half of *E. undulata*. In contrast to the aforementioned tolerant species, two exhibited much higher trait values in turgor loss, full turgor solute potential (Ψ_{osm}), and bulk modulus of elasticity with *S. latifolia* ($\Psi_{TLP} = -1.33$; $\Psi_{osm} = -0.93$; $\epsilon = 3.87$ MPa) followed by *P. myrtifolia* ($\Psi_{TLP} = -1.66$; $\Psi_{osm} = -1.02$; $\epsilon = 3.48$ MPa).

Table 1: Pressure-Volume curve derived traits (mean \pm SE) quantified from well hydrated plant individuals (n = 3) of each species.

Species	Physiological metrics		
	Ψ_{TLP} (MPa)	Ψ_{osm} (MPa)	ϵ (MPa)
<i>Boscia oleoides</i>	$-1.95 \pm 0.19^{a**}$	-1.37 ± 0.11^a	$4.61 \pm 0.24^{a*}$
<i>Pappea capensis</i>	$-2.32 \pm 0.11^{b**}$	-1.45 ± 0.11^a	3.88 ± 0.88^b
<i>Euclea undulata</i>	$-3.81 \pm 0.04^{c**}$	$-2.92 \pm 0.13^{b*}$	$12.43 \pm 1.58^{c*}$
<i>Schotia latifolia</i>	$-1.33 \pm 0.08^{d**}$	$-0.93 \pm 0.09^{c*}$	3.87 ± 0.38^b
<i>Searsia longispina</i>	$-3.68 \pm 0.19^{e**}$	$-2.17 \pm 0.22^{d*}$	$6.14 \pm 0.94^{d*}$
<i>Polygala myrtifolia</i>	$-1.66 \pm 0.12^{f**}$	$-1.02 \pm 0.05^{e*}$	3.48 ± 0.85^b

Note: Letters assigned describe whether a significant difference between species was present at significance levels of $p < 0.05$ (*) and $p < 0.001$ (**).

4.4.2. Hydraulic vulnerability to embolism

Employing the optical technique, vulnerability curves for six Subtropical Thicket species were constructed for the first time (Figure 1) relating trends in embolism formation with associated water potential. The five tree species (Table S1) exhibited considerable variability in vulnerability to embolism during desiccation. The water potential at which 12% embolism (P_{12}) was observed varied amongst these tree species, with values estimated at -4.09 MPa for *S. latifolia*, -4.71 MPa for *P. capensis*, -6.87 MPa for *S. longispina*, -6.78 MPa for *E. undulata*, and -4.51 MPa for *B. oleoides*. Whereas water potentials describing the tolerance towards drought [defined by the steepest portion of the curve or P_{50}] demonstrated variance across these species with the more vulnerable species (*S. latifolia*: P_{50} of -4.6 MPa; *P. capensis*: P_{50} of -5.79 MPa; and *B. oleoides*: P_{50} of -6.14 MPa) predicted to have greater risk in loss of hydraulic function than the more resistant species (*E. undulata*: P_{50} at -7.86 MPa; and *S. longispina*: P_{50} of 7.79 MPa). Moreover,

the water potential at the critical threshold of hydraulic dysfunction (i.e., P_{88}) was greatest for *S. latifolia* ($P_{88} = -5.12$ MPa) and substantially lower for the four tree angiosperms (*P. capensis*: $P_{88} = -6.86$ MPa; *B. oleoides*: $P_{88} = -7.78$ MPa; *S. longispina*: $P_{88} = -8.72$ MPa; and *E. undulata*: $P_{88} = -8.93$ MPa) (Figure 2). In contrast to the five tree angiosperms, *P. myrtifolia* the understory shrub species demonstrated a protracted embolism accumulation response reaching critical thresholds of hydraulic dysfunction at appropriately 2 MPa intervals (Figure 2B). This shrub exhibited highest P_{12} value of all six species at -2.64 MPa, however illustrated xylem resilience to embolism with a correspondingly lower P_{50} and P_{88} values at -4.87 MPa and -7.09 MPa, respectively (Figure 2B).

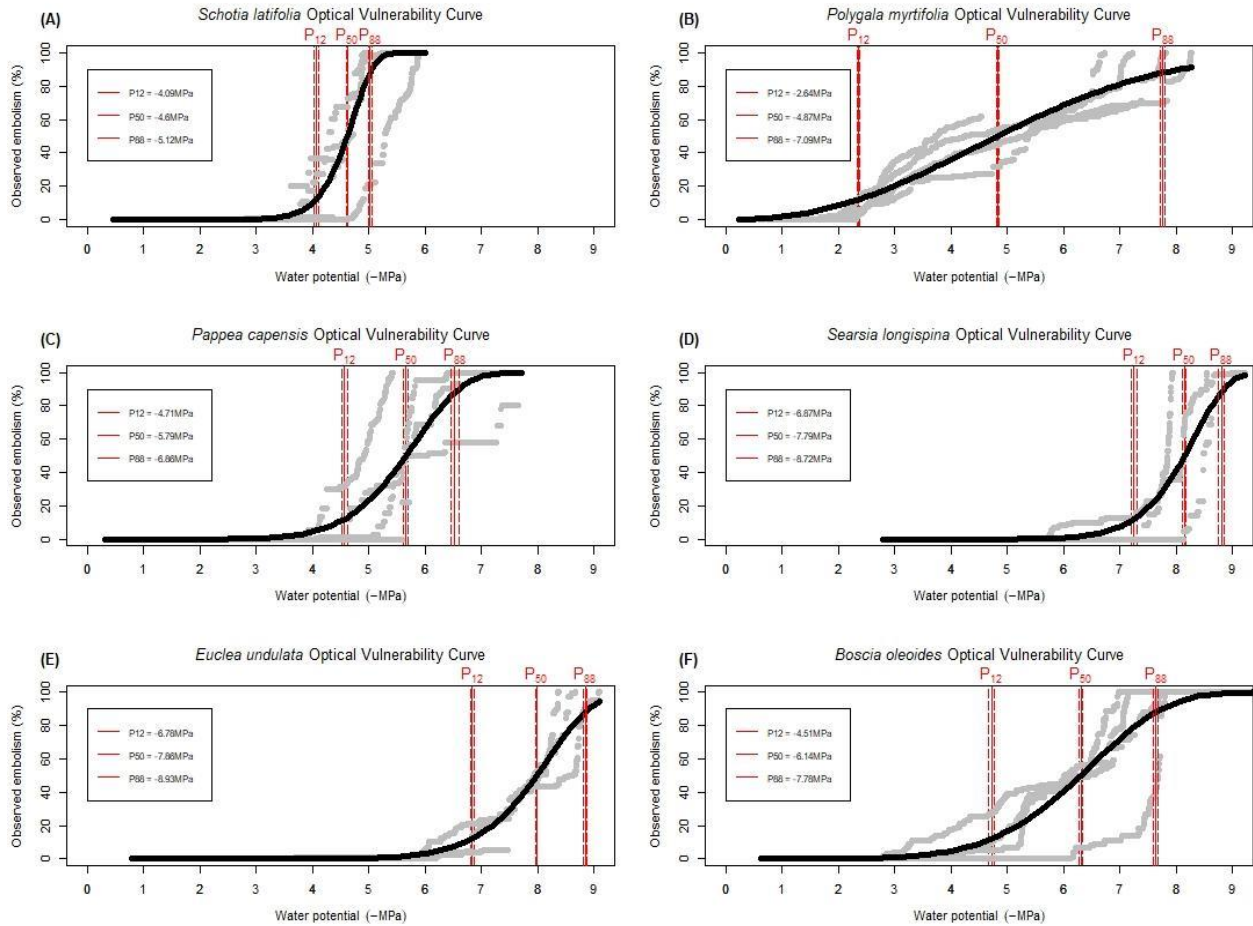


Figure 2: Optical vulnerability curves expressed as percentage of observed embolism as a function of stem water potential of (A) *Schotia latifolia*, (B) *Polygala myrtifolia*, (C) *Pappea capensis*, (D) *Searsia longispina*, (E) *Euclea undulata*, and (F) *Boscia oleoides*. Superimposed on the vulnerability curves are solid vertical lines representing the water potential corresponding to 12% (P_{12}), 50% (P_{50}), and 88% (P_{88}) embolism, respectively, with the dashed lines indicating the upper and lower 95% confident intervals.

A significantly robust correlation ($R^2 = 0.87$, $p < 0.001$) was observed between water potential at turgor loss point (Ψ_{TLF}) and 50% embolism induction within leaves of arid Subtropical Thicket

species (Figure 3A). Thereby illustrating a coordinated response to drought were stringent stomatal control or leaf solute driven processes increase xylem tolerance to desiccation. Additionally, a strong linear relationship was observed between P_{50} and Ψ_{osm} ($R^2 = 0.81$, $p < 0.05$; Figure 3B), highlighting a decline in hydraulic dysfunctional vulnerability accordingly with osmotic adjustments. Two species depicting considerably greater drought tolerance could be delineated, these species (*S. longispina* and *E. undulata*) demonstrated lower TLP and Ψ_{osm} concurrently in comparison to others.

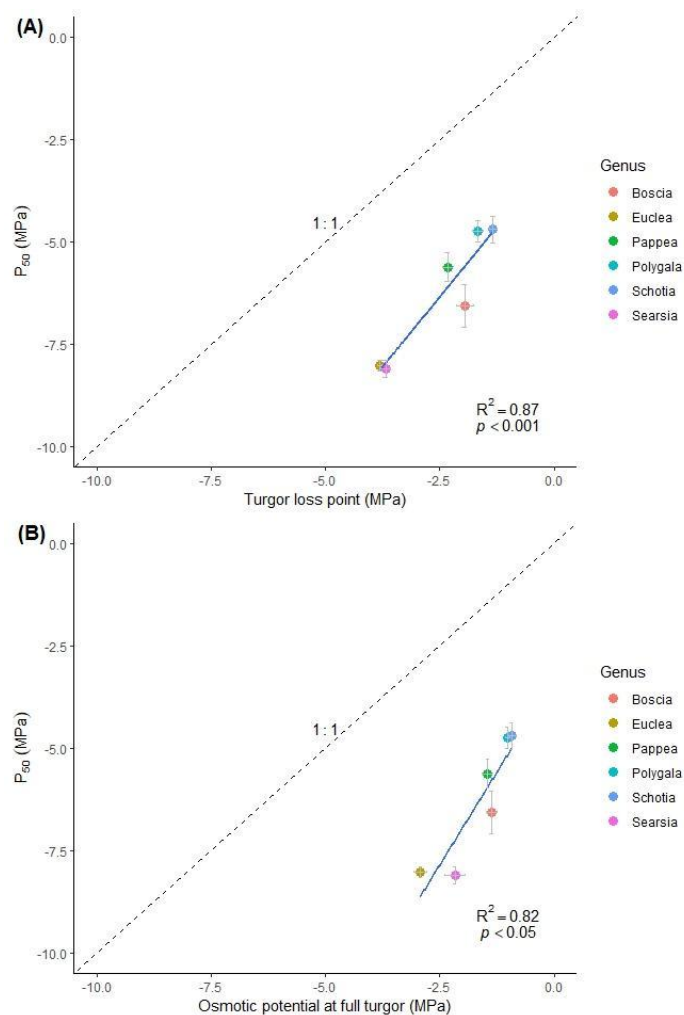


Figure 3: Correlation between leaf water potential inducing 50% embolism (P_{50}) and (A) turgor loss point (Ψ_{TLP}) and (B) osmotic potential at full turgor (Ψ_{osm}) in an arid Subtropical Thicket community. Each point denotes a species discriminated by corresponding colours. Dashed line represents a 1:1 relationship and the solid blue line depicts the independent linear regression.

4.4.3. Water potential analyses

There was a strong degree of dissimilarity between minimum water potentials across species ($p < 0.05$; Figure 4A). Where two of the six species under assessment demonstrated considerably

higher Ψ_{\min} values, with *B. oleoides* at -2.25 MPa and *S. latifolia* at -4.39 MPa, compared to the remaining four. Accordingly, these two species exhibited much lower plant water stress compared to the remaining four species (*P. capensis*, *E. undulata*, *S. longispina*, and *P. myrtifolia*) each demonstrating a Ψ_{\min} value exceeding -6 MPa (Figure 4A). The six species differed in the degree of hydraulic dysfunction, using the vulnerability curves and Ψ_{\min} the predicted native (i.e., *in situ*) embolism (%) of each species was estimated. There was considerable variation in predicted native embolism across species ($p < 0.05$: Figure 1B) with two species (*B. oleoides* and *S. longispina*) not having experienced any drought-induced emboli. The remaining species experienced varying degrees of functional loss induced by xylem embolism, with *P. myrtifolia* having lost entire function with 100% native embolism followed by *P. capensis* with 66.77%, and *S. latifolia* having partial loss with 41% predicted native embolism. In comparison *E. undulata* illustrated greater drought tolerance to abiotic conditions exhibiting a minimal loss in function with 6.21% native embolism.

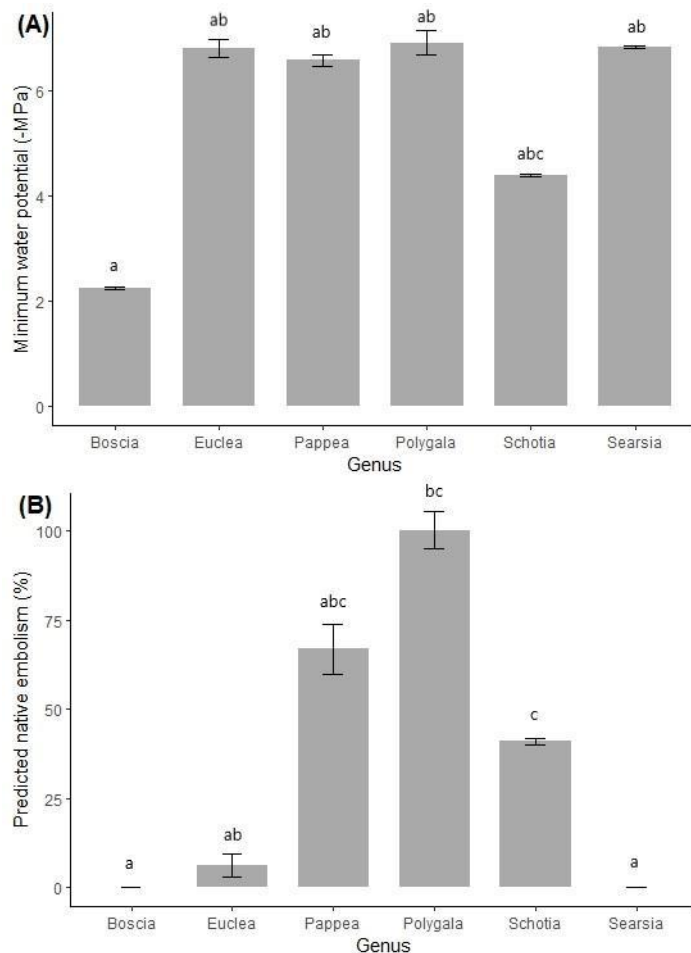


Figure 4: Bar plots describing the minimum water potential (A) and (B) the predicted native (i.e., *in situ*) embolism (%) experienced by each species (mean \pm SE) in the field. Letters differing above the plot indicate significant differences amongst species ($p < 0.05$).

There was considerable variance between conditional status of the vegetation and associated soil water potential, highlighting the role plant-induced adjustments have on surrounding edaphic properties (Figure S2). In this scenario under drought vegetation are abstracting significant volumes of edaphic water sources resulting in correspondingly lower, more negative, water potential of -7.78 ± 0.15 MPa. In contrast degraded vegetation denoted a much lower water holding capacity indicated by the higher water potential at -5.9 MPa ± 0.13 (Figure S2).

4.4.4. Hydraulic safety margins and drought tolerance

There was significant variability in P_{12} HSM amongst the species, depicting a wide range of hydraulic safety margins ($F = 38.28$, $p < 0.001$; Figure 5A). Three species (*P. capensis*, *P. myrtifolia*, *S. latifolia*) had HSMs exceeding zero indicating that they have surpassed the incipient threshold for embolism formation, with *P. myrtifolia* demonstrating the most negative HSM of around -4.21 MPa. Whilst, *S. longispina* and *E. undulata* illustrated near zero HSMs signifying that these two species have yet to incur functional xylem damage, however are operating close to their respective hydraulic thresholds. In contrast, *B. oleoides* has a broader range to incur xylem functional loss before the onset of embolism demonstrated by it's positive HSM of approximately 2.8 MPa. P_{50} HSMs exhibit a significant difference (Figure 5B) between species, denoting a broad range of embolism vulnerability, with most resistant species (*B. oleoides*) at 3.8 MPa to species that are more vulnerable, and in instances have surpassed critical thresholds, such as *P. myrtifolia* at negative HSM of -1.5 MPa. There was less variability between species considering the P_{88} HSMs (Figure 5C). Where *B. oleoides* denoted the least vulnerable of the species considered. The fourth HSM examines the difference between turgor loss point (TLP) and the point of incipient embolism (P_e), where five species (*B. oleoides*, *E. undulata*, *P. capensis*, *S. latifolia*, *S. longispina*) exhibit broad, positive HSMs indicating stringent stomatal control before initial embolism formation. In contrast, *P. myrtifolia* demonstrates the converse, with a near zero HSM (~ 0.8 MPa; Figure 5D) denoting full stomatal closure near the critical incipient embolism threshold.

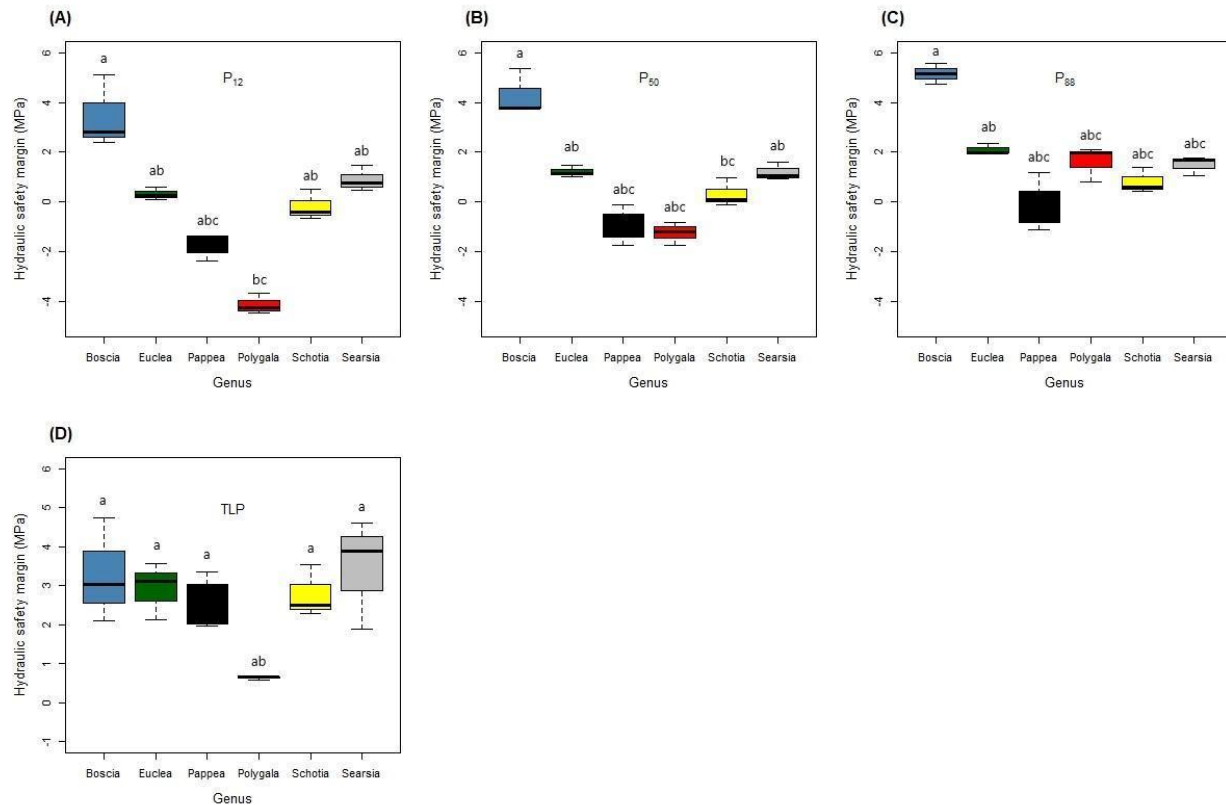


Figure 5: Boxplot illustrating the hydraulic safety margins formulated in this study as the difference between minimum water potential in the field and water potential associated with 12% (P_{12} : A), 50% (P_{50} : B), and 88% (P_{88} : C) observed leaf embolism derived from each species vulnerability curve. The fourth HSM calculated denoted the difference between turgor loss point and the incipient point of embolism (P_e : D). Letters above each plot indicate significant pairwise (Tukey's tests) differences amongst species ($p < 0.05$).

4.5. Discussion and conclusion

This study is one of the few exploring the diversity of hydraulic vulnerability of southern African species (Swemmer, 2020; West et al., 2012), with focus on arid Subtropical Thicket woody evergreen sclerophyllous canopy trees and shrub plant species. The six species evaluated in this study demonstrated a range of hydraulic tolerances and strategies to losses in hydraulic function due to xylem embolism. The species within this phylogenetically diverse, and climatically unique (Hoare et al., 2006), community were found to exhibit great resistance towards hydraulic dysfunction, with species denoting variance in their respective drought tolerance and proximity to surpassing critical thresholds of hydraulic impairment. Generally, the results illustrated a degree of convergence between species hydraulic thresholds facilitating the delimitation of more tolerant trees based on proximity to more extreme limits of species-specific hydraulic traits and functional retention (Ackerly, 2003; Larter et al., 2017). Moreover, this study is the first report quantifying xylem embolism resilience using the recently developed optical vulnerability technique (Brodribb et al., 2016) in a South African ecosystem, to examine the ecological and physiological significance underpinning Subtropical Thicket drought tolerance.

Studies which have quantified hydraulic dysfunction of plants in the field under drought conditions are of tremendous value (Urli et al., 2013; Li et al., 2018; Venturas et al., 2016), as such works capture the physiological extremes experienced by plants under these stochastically harsh events – presenting a rare opportunity to investigate underpinning mechanisms behind species tolerance and susceptibility. Many of these studies have observed a substantial rate of drought-induced native embolism across species, for example in *Populus tremuloides* stands in Northern Americas (Anderegg et al., 2012) to woodland communities of the southern Italy (Nardini et al., 2013), oaks of southern France (Urli et al., 2015) and Eucalypt forests of eastern Australia (Nolan et al., 2021). The prevalence of embolism formation, impeding water transport, has highlighted the significance of hydraulic dysfunction as a core driver for drought-induced functional loss and tree mortality. The results of this Chapter [i.e., chiefly predicted native embolism] strongly confer the implications of hydraulic impairment as a causal factor behind reductions in ground-based canopy vitality and overall dieback in arid Subtropical Thicket observed in Chapter 3 under extreme multi-year drought in the Eastern Cape, South Africa (Figure S1). The degree of predicted native embolism reported herein are consistent with the proposed theoretical threshold and predictor of cross-species trends in mortality linked to hydraulic dysfunction observed at P_{88} embolism occurrence corresponding to equivalent losses in conductivity in angiosperms (Urli et al., 2013; Choat et al., 2018). Given that a core gap in knowledge is the capability to accurately predict species mortality risk to drought, identifying critical physiological thresholds of plant water potential that coincide with, and are the cause, for declines in canopy health status and dieback is essential – stressing the significance of this thesis's findings (Chapter 3 canopy dieback linked to Chapter 4 physiological thresholds) and the influence drought presents to vegetation dynamics of Subtropical Thicket.

The severe drought of 2020 resulted in widespread canopy defoliation and dieback of several Subtropical Thicket woody tree and understory shrub species. The relative impact of this drought on varying species was strongly linked to each species-specific xylem embolism vulnerability. In the field, the species studied here seemed to diverge in the degree of functional loss to embolism in xylem conduits, where *in situ* measurements of minimum water potential (Ψ_{min} , quantification of water physiological stress) demonstrated a range of hydraulic impairment in species, including those experiencing little to no embolism (*S. longispina* and *B. oleoides*) to intermediate native embolism severities such as *E. undulata* with 6.21% and 41% for *S. latifolia*, to the most severely impacted species, *P. capensis* (66.77%) and *P. myrtifolia* exhibiting complete hydraulic failure at 100% embolism in many individuals. The significance of this finding was described by the linkage between observed trends in canopy dieback (Chapter 3) and native embolism in the field; and is well illustrated by one of the study species, *P. myrtifolia*, where extensive dieback is seen *in situ* which was anticipated given the considerable native embolism it experienced, exceeding critical functional thresholds, i.e., P_{88} . In contrast species experiencing far less native embolism had a corresponding more vigorous canopy (e.g., *E. undulata*), these species had not yet reached critical thresholds that translated into dieback symptoms or mortality. The species exhibiting less predicted native embolism demonstrated far less symptoms of canopy dieback and fatality (Chapter 3), indicating that P_{88} may be a more accurate representation of hydraulic impairment threshold for predicting mortality in angiosperms than the more conservative P_{50} as suggested by earlier works (Choat et al., 2012; Urli et al., 2013).

The vulnerability of species to hydraulic impairment is generally quantified as its P_{50} , defining either the water potential value causing 50% loss in conductivity or the point at 50% of the xylem vascular has been embolized – hence an indication of the intrinsic susceptibility of a species to

drought. Subtropical Thicket species varied significantly in hydraulic embolism vulnerability (Figure 4), with this being the first report on their physiological thresholds. The heterogeneity in embolism resistance was simplified by delimiting the six species into three groups (low, moderate, and high tolerance) based on the degree of xylem tolerance to embolism. The least tolerant were *P. myrtifolia* and *S. latifolia* with P_{50} values of -4.6 MPa and -4.87 MPa, respectively. While more moderately tolerant species based on P_{50} were *P. capensis* with a value of -5.79 MPa and *B. oleoides* (-6.14 MPa); and the most tolerant species were *E. undulata* (-7.86 MPa) and *S. longispina* (-7.79 MPa), respectively. The low embolism vulnerability of the latter two species explains their distributional extent ranging from the arid Subtropical Thicket into the Nama Karoo, facilitated by the species capacity to tolerate drought and periods of extreme water deficiencies (Jacobsen et al., 2009). In contrast, *S. latifolia* and *P. myrtifolia* denoted regional ranges limited to more mesic environments, increasing in abundance progressively eastwards to more forested landscapes. This illustrates the significance rainfall certainty and reliability presents for exceeding physiological functional thresholds. Consider Jacobsen et al. (2009) suggested that fynbos species avoid and rarely exceed 50% hydraulic vulnerability to embolism during drought, however, fynbos occupy a more reliable and consistent rainfall region in southern Africa. Studies such as these illustrate the vulnerability of species to hydraulic function is strongly affected by rainfall, hence can be valuable for distinguishing plant drought resistances (Anderegg et al., 2015; Choat et al., 2012). Subtropical Thicket, however, is characterized by an unpredictable rainfall which presents greater risks of vulnerability; whilst additionally selecting for generally more embolism resistant species in contrast to other southern African biomes.

The water potential at turgor loss point (Ψ_{TLP}) is a defining feature delineating species degree of drought tolerance as opposed to avoiding drought (Brodribb and Holbrook, 2003). In many of the studied species, it was observed that turgor loss occurred in close vicinity or before the incipient embolism, indicating the importance of embolism avoidance as an essential mechanism of tolerating drought in Subtropical Thicket. This finding agrees with those of other studies reporting on the importance of early stomatal closure as a strategy for the prevention of hydraulic impairment via embolism development in xylem vasculature. The finding of the large, positive hydraulic safety margins between Ψ_{TLP} and P_e is consistent with the results of recent study describing that only a small number of species exhibited small near equal values in safety margins with most species initiating stomata closure much earlier than incipient embolism (Martin-StPaul et al., 2017). To this end, Subtropical Thicket species prioritize hydraulic integrity as opposed to the maintenance of CO_2 assimilation under drought (Figure 3A) – stressing the significance of retaining functional embolism-free xylem. Moreover, this study agreed with the finding that safety margins increased with greater embolism resistance as suggested by Martin-StPaul et al. (2017), where *S. longispina* denotes a much greater safety margin than the more vulnerable counterpart, *P. myrtifolia* (Figure 4D). Furthermore, the results of this study demonstrated a coordinated link between intrinsic xylem properties and leaf physiological traits derived from pressure-volume curves (i.e., turgor loss point, osmotic potential at full turgor) denoting species-specific drought tolerances. Amid these linkages was the significant correlation between P_{50} , proxy of intrinsic xylem vulnerability, and both turgor loss point and osmotic potential at full turgor. This finding suggests hydraulic vulnerability illustrates a strong association with extra-xylary processes (osmoregulation) and cell structural properties (Scoffoni et al., 2017). Previous studies have demonstrated the heterogeneity in osmotic potential across tissues mitigating the proximity species might approach critical vulnerability thresholds, coupled with structural adjustments and lower turgor loss point would confer greater drought tolerances (Blackman et al., 2010, Guyot et

al., 2012). The relationships exhibited by P_{50} and pressure-volume curve parameters should be a future research priority to examine the role of that extra-xylary processes might play in species-specific drought tolerance.

Interestingly, the disparity of hydraulic vulnerability in this arid Subtropical Thicket community was driven chiefly by species-specific intrinsic embolism resilience as opposed to variation in experienced minimum water potential, as most species converged in the latter under drought conditions. This result deviates from the strong association observed between embolism tolerance and minimum water potential (Ψ_{min}) experienced by woody angiosperms of Tasmanian forests (Blackman et al., 2010); and where varying growth forms of species diverged in respective experienced minimum water potentials and drought-induced functional loss under drought (West et al., 2012). Two of the six species which experienced considerably greater minimum water potentials were *S. latifolia* (-4.39 MPa) and *B. oleoides* (-2.25 MPa), the derivation of which may be attributed to rooting depth. Where development of an deep reaching tap-root enables greater access to subterranean watercourses (Wand et al., 1999) of the former; and where a succulent architecture relieves water stain through increased hydrated tissue storage in the latter species (Domec and Gartner, 2001). Moreover, Palmer and Pitman (1972) described *Boscia* leaves as having “an intricate framework of very hard cells which prevent leaf collapse”; this cell wall strengthening would enable the plant to maintain turgor despite water loss (Lenz et al., 2006).

Lastly, this study investigated variation in species-specific embolism vulnerability within an arid Subtropical Thicket community, to provide a mechanistic interpretation of drought tolerance and a predictive basis by which to anticipated future changes in community composition and structure. Such a comprehensive account of species-specific drought resistance will be essential given the future forecast of increased global-change-type drought events (Breshears et al., 2005). To examine species most at risk of experiencing hydraulic damage under existing drought conditions this study retrospectively assessed the hydraulic safety margins defining the degree of hydraulic conservatism of species to drought (Johnson et al., 2016). Consequently, hydraulic safety margins should remain positive if hydraulic functionality is to be maintained under drought, the lower the value or in extremely dry conditions negative would indicate that the species has endure significant loss in function and experienced embolism. The results of this study suggested that two (*P. capensis* and *P. myrtifolia*) of the six Subtropical Thicket species have experienced embolism and considerable loss in hydraulic function denoting negative P_{50} and P_{88} HSMs (Figure 5B and C). These findings are supported the by previous observations made describing severe canopy dieback and defoliation (Chapter 3). This has implications for future community composition and structure shifting to more drought resistant species, such as *S. longispina* and *E. undulata*, becoming dominant in Subtropical Thicket under predicted escalations in drought frequency and severity – depreciating species diversity within this arid community. Such a response was observed in a similarly arid ecosystem the pinon-juniper woodlands in South-Western United States (Mueller et al., 2005). Where the co-dominant *Pinus edulis* had undergone extensive dieback compared to the more drought tolerant species, *Juniperus monosperma* (Mueller et al., 2005). With predictions of severe distributional contraction of the former pinon pine while conversely juniper expands throughout these woodland environments. Analogous trends in drought related replacement patterns have been observed globally (Batllori et al., 2020), stressing the implications of the findings presented in this Chapter. Additionally, stressing the importance of devising a mechanistic approach by which to anticipate demographic changes within communities induced by drought (Hartmann et al., 2018). While this outcome is doubtful in arid Subtropical Thicket, it's more probable that with continued global-change-type climatic

perturbations the most vulnerable canopy species will be filtered out leaving the more drought-tolerant species. However, this state would be aptly a transitional phase, where the dominance of drought tolerant species is driven by loss of species as opposed to recruitment given the prevailing life history strategy of resprouting in arid Subtropical Thicket (Cowling et al., 2005a).

This study is the first to document hydraulic vulnerability of embolism using the recently developed optical vulnerability technique in arid Subtropical Thicket community. The findings reported are the first estimate of drought-induced embolism in phylogenetically diverse Subtropical Thicket and is fundamental to understanding hydraulic adaptations of this community. The extremely low water potential at critical embolism thresholds, coupled with comparative low tissue dehydration tolerance traits (such as turgor loss point), emphasize the importance of drought-induced hydraulic functional impairment as a key component for the persistence canopy tree species and causal driver behind community dynamics within arid Subtropical Thicket. The strong association observed between vulnerability to embolism (i.e., HSM) and maintaining hydraulic function under climatic perturbations illustrates the possibilities xylem vulnerability presents to predicting species distributional extent and risk of mortality (Powers et al., 2020). To this end, demonstrating the fundamental significance that species-specific xylem vulnerability traits pose to community structure and compositional change in response to global-change-type drought events.

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4.7. Supplementary figures and tables

Table S1: A list of the six dominant woody canopy species present in arid Subtropical Thicket of the Eastern Cape, South Africa.

Species	Family	Growth form
<i>Schotia latifolia</i> Jacq.	Fabaceae	Tree
<i>Searsia longispina</i> (Eckl. & Zeyh.) Moffett	Anacardiaceae	Tree
<i>Pappea capensis</i> Eckl. & Zeyh.	Sapindaceae	Tree
<i>Euclea undulata</i> Thunb.	Ebenaceae	Tree
<i>Boscia oleoides</i> (Burch. ex DC.) Toelken	Brassicaceae	Tree
<i>Polygala myrtifolia</i> L.	Polygalaceae	Shrub

Note: The selected species comprised of the 5 dominant canopy tree species (all angiosperms) and a understorey, fringe-edge shrub species. All species denote evergreen phenology.



Figure S1: Branch dieback and retained dead foliage of entire canopies in *Polygala myrtifolia* (left image) and *Pappea capensis* (right image) individuals across Kabouga Private Nature Reserve.

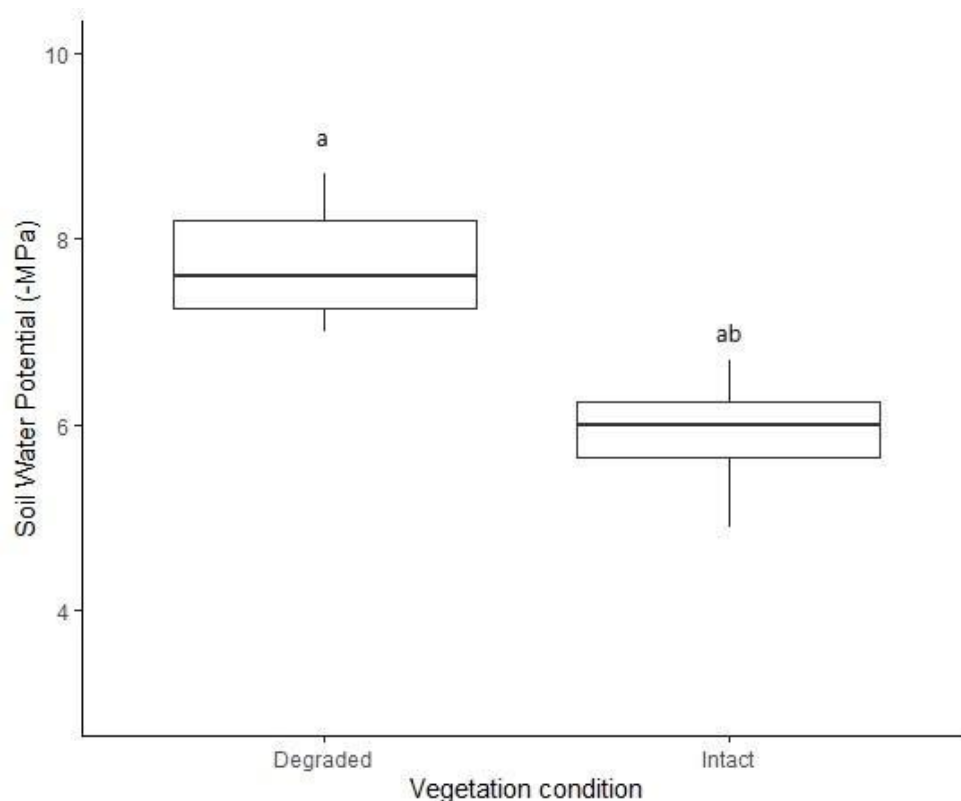


Figure S2: Soil water potentials, proxy of predawn available plant water, compared across vegetation conditional states of arid Subtropical Thicket. Letter differences indicate significant difference between vegetation condition ($p < 0.05$).

4.8. Supplementary methods

Soil water potential

Sediment samples were collected at sunrise (6:00 am) or just thereafter however, no later than sunrise at 6:30 am, and were characterized into two categories: “intact matrix” or “transformed” sediment. A total of 10 samples were collected for each treatment category, sediment was collected by transverse sampling at 5 cm depth from a dug core. The sediment was immediately transferred to a sealable plastic bag and placed in a cooled temperature regulated container to the laboratory. Storage of samples in airtight and cooled conditions was intended to reduce evaporative loss during transport, upon arrival soil water potential (Ψ_s) was measured using a WP4C Dewpoint Potentiometer (Decagon Devices Inc., Pullman, WA, USA). Soil water potentials (Ψ_s) were recorded and reported as MPa throughout the study. These measurements were documented as soil water potential is often inferred by way of proxy by means of predawn leaf water potential measurements describing soil available water to the plant. In this study, a direct measurement of plant-soil available water status is provided by synchronously documenting both plant water stress by midday water potential and available water by soil water potential.

Chapter 5

Synthesis and conclusion

“Can xylem vulnerability become a super trait with the capacity to globally predict species distribution and drought sensitivity”. Tim Brodribb (2017)

This research thesis was prompted by several interrelated lines of concern encompassing the broader ecological implications of drought, and how this persuasive disturbance might influence the vegetation dynamics of Subtropical Thicket, mainly the arid subtype. More importantly, how tolerant is this vegetation type to the physiological effects of drought, particularly addressing the maintenance of hydraulic integrity and general xylem function.

The detection and characterization of vegetation change over time is the first step toward identifying the causal forces and mechanisms behind this variation. This knowledge will be essential for predicting future trajectories in vegetation change, devising management strategies and monitoring practices to mitigate the potential ecosystem services and functions that may be lost depending on response to global-change-type disturbances, such as drought events. This study explored vegetation change within the semi-arid to arid Subtropical Thicket biome of South Africa, investigating both regional drought impacts and landscape scale effects. Leveraging climatological data (daily precipitation and temperature) of major weather stations situated throughout the Eastern Cape and remotely sensed physiological-based satellite data originated from Moderate Resolution Imaging Spectroradiometer (MODIS) Terra instrument platform (designation: MOD13Q1) – this study aimed to firstly, ascertain the response pathways accounting for inter-annual variability in vegetation change. Secondly, to determine the casual drivers underpinning vegetation change with focus on the role episodic climatic events, such as drought, and general variability influencing Subtropical Thicket in the Eastern Cape of South Africa. A core focus of this research was to delineate nonlinear patterns and identify structural breaks/shifts in vegetation indices enabling the detection of major ecological responses in the vegetation and prediction of future trajectories of the region.

To precisely delimit these shifts in vegetation productivity and general vigor this study employed physiologically relevant remote sensing spectral indices (Verbesselt et al., 2010) This provided a more accurate account of vegetation productivity changes spatially and temporally and detection of breaks in productivity by which to determine the defining ecological causes. In chapter 3, for the first-time widespread canopy dieback and crown defoliation was observed and documented in arid thicket coupled with meteorological indicators that illustrate a strong coherence between the timing of drought and vegetation-related impacts. A key finding of this study was the distinct spatial disparities in vegetation productivity through time. Additionally, the heightened spatiotemporal resolution of Chapter 3 facilitated a regional perspective of drought across the Eastern Cape Province of South Africa, which demonstrated a strong coastal-inland gradient in evapotranspiration demand and accompanying buffering effects of the coast towards drought-induced effects. This resulted in the greatest severity of drought influence being experienced inland, particularly westward. However, the recent drought acutely peaking in damage to vegetation productivity exhibited an alternative spatial trend with coastal extents progressing inland from a westerly approach was substantially impacted with extremely low values of EVI,

NDVI, and VCI, while eastern inland regions experienced positive productivity and growth conditions. Moreover, Chapter 3 explored the temporal trends in vegetation productivity using the two primary proxies NDVI and EVI. The findings illustrated the temporal heterogeneity of drought, coupled with metrics SPEI and SPI, highlight the complexity in parameterizing predictive models anticipating drought events. Furthermore, the results of this chapter stress the ecological significance of the current multi-year drought (2015-to-present), this protracted period of water deficit has had major implications on canopy vigor and general health of Subtropical Thicket, most significantly the woody species illustrated by landscape-level observed dieback and widespread crown defoliation. Most importantly, this study presents the first documented widespread drought-induced canopy dieback event in Subtropical Thicket, while historical anecdotal evidence has been suggested, the lack of quantitative data has limited analyses which is fulfilled in Chapter 3 capturing critical first-time data on the effects of drought in this characteristically arid biome. In addition to baseline data on the health, conditional status, and canopy vigor of dominant Subtropical Thicket woody canopy and shrub species, this study established three permanent long-term monitoring plots, approximately 2-3 ha founded during the first field-based surveys of September 2020 in intact vegetation on a private nature reserve. These plots would provide crucial data on the long-term trends in canopy health and productivity, where several measurements of canopy structure and vitality could be captured, in combination with aerial photography of the site enabling the characterization of whole-canopy condition and symptomology. Chapter 3 combined remotely sensed physiological based indicators, field-based canopy vitality metrics, and climatic water balance metrics (SPEI, SPI) to investigate the underpinning physiological processes for observed trends in vegetation change and spatiotemporal dynamics in arid thicket of South Africa.

In addition to characterizing the influence and consequences of drought on vegetation productivity and change for Subtropical Thicket, this study synchronously investigated the physiological mechanisms behind detected drought-induced productivity changes and mortality events in arid thicket during the peak of the multi-year drought (i.e., 2020). Since the advent of the “hydraulic and carbon framework” to elucidate the underpinning mechanisms of drought-induced mortality (McDowell et al., 2008), much research attention has given to attempt to ascertain significance and disentangle the concomitant impacts that each process, hydraulic dysfunction, and carbon starvation, contributed to observed mortality events (Hartmann et al., 2013; Anderegg et al., 2016). However, of the two, hydraulic dysfunction is currently the most well supported physiological mechanism underpinning drought-induced mortality with several lines of evidence to substantiate its effect on vegetation dieback (Hartmann et al., 2013; Hartmann et al., 2015; Hartmann et al., 2018; Rowland et al., 2015; Sevanto et al., 2014; Venturas et al., 2017). These lines of evidence include the general observation that whole-plant mortality driven by desiccation is explicitly connected to the biophysical water potential stress induced on the vasculature, characterizing thresholds by which impaired function may ensue through embolism resulting in fatal consequences. To the extent that the strength of this trend in embolism driven fatality and associated limitations at specific hydraulic functional thresholds facilitates the delimitation of a potential “tipping point trait” such that mortality trends can be defined by key thresholds delineated by this trait, e.g., P_{50} , P_{88} (Anderegg et al., 2016; Choat et al., 2018). Thus, providing a reliable metric or “super trait” as suggested by Brodribb (2017), for predicting species survival and performance under water stress. Furthermore, research investigating carbohydrate dynamics coupled with hydraulics have generally concluded the plants depicted dieback exhibit clear reductions in hydraulic functioning (and greater embolized xylem conduits) with high variability in

non-structural carbohydrate concentrations (Anderegg et al., 2012; O'Grady et al., 2012; Sala et al., 2012). Hence, stressing the significance hydraulic failure as an immediate and direct impact of water-related stress presents to whole-plant physiological function. This illustrates the complexity behind drought-induced mortality mechanisms, however much of this evidence was obtained from environmentally controlled laboratory seedlings and potted experiments, with field-based observations of mature plants remains limited.

In this thesis, the association between hydraulic impairment and canopy dieback is investigated, based on field observations of arid thicket in the Subtropical Thicket biome, leveraging the peak of current multi-year drought encapsulating the Eastern Cape, South Africa (Chapter 4). The findings reported in this thesis exhibit the high diversity of hydraulic vulnerability, and by association strategies, to xylem embolism and general functional loss under drought in Subtropical Thicket. Chapter 4 presents an innovative perspective for this research, being the first of its kind to apply the latest method in deriving hydraulic vulnerability curves – the recently developed optical vulnerability technique (Brodribb et al., 2016) – outputting crucial hydraulic traits to characterize the functional thresholds of dominant Subtropical Thicket species. The focus species of this thesis comprised of the two most abundant remnant canopy woody tree species (*Pappea capensis* and *Euclea undulata*) persisting within a matrix of bare soil and karroid shrubs representing an alternative stable state transition between intact and fully desertified land of arid thicket, with an additional three commonly found species in pristine Subtropical Thicket (*Searsia longispina*, *Schotia latifolia*, and *Boscia oleoides*), and lastly one understory canopy fringe species (*Polygala myrtifolia*). This study revealed, counterintuitively however, that many of these subtropical lineages demonstrated great resistance to desiccation (e.g., P_{50} in excess of -3MPa), retaining functionality at relatively extreme levels of water deficiencies — highlighting the general drought tolerance and convergent evolutionary role aridity presented to this vegetation (Larter et al., 2017). Interestingly, the variation in hydraulic dysfunction, and conversely functional retention, was determined by the intrinsic capacity of each species to tolerate xylem embolism formation as opposed to variation in native minimum water potential (i.e., a measure of infield-instantaneous experienced water stress).

Hydraulic impairment is a major evidentiary process explaining canopy dieback and whole-plant mortality under water strenuous states or protracted periods of drought (Choat et al., 2018). Recent research has identified critical thresholds of functional retention, i.e., embolism levels of 50% in conifers and 88% in angiosperms are non-recoverable, if exceeded result in mass mortality and dieback events (Anderegg et al., 2012; Urli et al., 2013). To this end, assessing the degree of native embolism experienced by a plant under drought coupled with defined xylem functional thresholds is vital to evaluate and parametrize models on the impact of water deficiencies on species demography, community structure and composition. The findings of Chapter 4 observed two species were at greater risk of experiencing hydraulic dysfunction by xylem embolism to drought than the remainder, which translated in severe symptoms of canopy dieback, defoliation, foliage discolouration, and whole-plant mortality detected in these two species (*Schotia latifolia* and *Polygala myrtifolia*). Characteristically more mesic (e.g., broad, large, and thin leaves) than the other sclerophyllous species investigated in this chapter, the decline of these two could lead to shifts in community-level species composition under predicted increased frequency and severity of future droughts. This highlights the significance climatic shifts and/or perturbations, such as drought and aridity, as key ecological drivers of change in Subtropical Thicket. This study demonstrated that resistance to xylem embolism formation is a reliable proxy describing the tolerance and persistence of species under drought. The variance in

hydraulic trait strategies of angiosperms, combined with embolism resistance, is critical for predicting species drought survival.

By combining detailed field-based surveys of canopy condition and vitality (Chapter 3) with quantification of xylem hydraulic vulnerability and predicted native embolism (Chapter 4) of dominant Subtropical Thicket species captured during a regional specific dieback event this thesis stresses the suitability and significance of thresholds for xylem embolism and hydraulic safety margins as valuable proxies for interpreting and predicting drought-induced mortality. These proxies can be used to parameterize species-specific mechanistic models of carbon-water dynamics and algorithms predicting mortality, providing a forecast, and understanding, of vegetation productivity, community composition and structure in response to future climates. This highlights the importance of characterizing mechanistic traits over more universal, and easily acquired functional trait correlates, such as specific leaf area (SLA), wood density and other foliar traits. Whilst these traits vary across broad environmental gradients, they are rather representative of a “trait syndrome” and are an output of several interacting ecological processes acting synchronously to influence trait expression. For consideration, SLA, the most widely applied metric used in functional trait-based ecology is largely influenced by light-exposure, nutrient availability, leaf lifespan and ontogeny (Poorter et al., 2009), all of which may act in synchrony, independency, or bilaterally resulting in varying explanatory and correlation strength depending on the processes acting on SLA at a given point in time and particular environment. While there have been suggestions as to the suitability of these “trait syndromes” to drought tolerance (Niinemets, 2001) and embolism resistance (Hacke et al., 2001) the correlation strength remains relatively weak, although significant, highlighting the multi-variant nature of these relatively easily measurable traits. The reduced correlation strength has been demonstrated in several studies (Fortunel et al., 2013; Maréchaux et al., 2015; Powers et al., 2020) reducing the practicality and suitability of using these traits for predictions of drought vulnerability or parametrizing hydraulic models.

This draws into question the suitability of underlying traits used to describe functional ecological principles and paradigms, for example the “leaf economic spectrum” and other morphological traits that have no clear mechanistic linkage to physiological fitness or performance of species in predicting drought vulnerability – and highlights the significance of mechanistic hydraulic traits as a research priority in forecasting future community dynamics to climate change and drought. This emphasizes the opening quote by Tim Brodribb and the underlying sentiment for a concept of the mechanistic doctrine in ecology. While the complexity of biological life makes the identification of a ‘super trait’ somewhat of a challenge, providing a mechanistic perspective, one in which a few key traits, no more than a handful, that are directly linked physiological fitness is not as implausible and will provide a basis by which to predict survival and success of species.

5.1. Future research recommendations

This thesis furthers the understanding of landscape-level vegetation change response in the context of drought in Subtropical Thicket of the Eastern Cape, South Africa. This information is becoming increasingly more significant for monitoring and adaptive management of this species-enriched biome under forecasted climate change scenarios of prolonged, severe drought in this region. Importantly, the findings of this thesis not only provide first documented account of widespread canopy dieback and crown defoliation, additionally provides a perceived

understanding of the physiological underpinnings explaining these observed trends while delimiting key functional thresholds that can parameterize models and predict species-specific vulnerabilities to drought.

Future research directions should integrate a multi-structure framework in vegetation monitoring that combines both field-based and remotely sensed surveyance, *ad hoc* plant climate response experiments, and the establishment of several permanent research plots for the collection of long-term data, both biotic and abiotic as seen in other vulnerable ecosystems across the globe (Pollastrini et al., 2019; Brun et al., 2020; Schuldt et al., 2020; Senf et al., 2020; Walthert et al., 2021). Furthermore, future research should expand on the data collected herein, capturing mechanistic physiological traits across a diverse array of species from across all major biomes of South Africa. Importantly, given future predictions regarding drought and aridity for southern Africa, more research is needed on species-level hydraulic vulnerability to drought-induced mortality. This thesis recommends employing the optical technique for capturing large datasets of critical functional physiological thresholds to characterize species-specific vulnerabilities to drought. This innovative optical technique for evaluating xylem vulnerability to embolism has broaden the possibilities of capturing essential mechanistic trait data at lower costs, additionally the non-invasive nature reduces introduced noise to the experiment while providing accurate estimates of xylem resistance to embolism and general drought tolerance (Brodribb et al., 2017; Gauthey et al., 2020).

It is essential that future research capturing canopy vitality and health condition be undertaking in Subtropical Thicket, with the establishment of long-term monitoring plots across the spatially heterogenous distribution of this vegetation. Future research questions should address concerns around: (1) the spatial the extent and severity of climate impacts to identify hotspots where resources should be prioritized and baseline assessments of species-level vulnerabilities can to targeted; (2) establishing long-term research plots and monitoring networks across South African biomes to capture community and species scale drought response and implications on ecosystem functions and services; (3) collating existing data into pre-existing climate and vegetation models to discover potential knowledge gaps and areas of importance enabling research to focus attention on these core aspects and permit greater parameterization for future models and simulations on drought vulnerability in semi-arid and arid ecosystems.

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