Hydraulic trait variation in *Protea repens* with change in climate in space and time.

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Abstract

Global climate change and atmospheric CO_2 concentrations are affecting all levels of biodiversity in a number of ways. For example, the unique vegetation of the Cape Floristic Region (CFR) is expected to experience increased temperatures while rainfall becomes more seasonal, resulting in stronger summer drought with greater hydraulic stress in plants. Increased CO_2 concentrations, on the other hand, are expected to relieve hydraulic stress in plants that utilize the C_3 pathway for photosynthesis (most fynbos species), by reducing the amount of time they have to keep their stomata open to take up the CO_2 they require. Observed weather data suggest that rainfall has remained relatively stable over the last 21 years, while temperatures for the region have increased marginally. Here I explore variation in the hydraulic traits (leaf and xylem anatomy) of *Protea repens* (L.) across a spatial climatic gradient in the CFR relative to a common garden experiment. I then compare the contemporary trait-climate relationships with a 21 year old xylem anatomy dataset.

In the common garden experiment I explore xylem and leaf trait variation in *P. repens* from thirteen populations representing a gradient in temperature and mean annual precipitation. Because trait-gradient relationships can be confounded by genetic differences between populations along the gradient, I used a common garden experiment to test the degree to which trait variation was genetically constrained among populations. My results show that xylem vessel diameters and an estimate of hydraulic conductance increased with increases in maximum temperature and soil moisture days across the spatial gradient. My results for the common garden experiment does however show genetically constrained intra-specific differences in xylem vessel morphology between populations. Despite this, differences in xylem vessel and leaf morphology between plants in the common garden and their source locality demonstrate that *P. repens* has some ability to respond to changes in the environment through phenotypic plasticity.

To determine the response of *P. repens* to changes in climate over the past 21 years, I compared contemporary xylem anatomy to an existing dataset collected from the same sites in 1994. My results show no significant change in vessel diameters since 1994 even though temperatures and atmospheric CO_2 have increased, with no change in rainfall amount. These results suggest that either *P. repens* is not experiencing increased drought stress under current climate conditions, or that xylem vessel anatomy is not a good proxy for small changes in

drought stress in this species. The effect of increased drought stress due to higher temperatures and associated evaporative demand may be alleviated by increased atmospheric CO_2 reducing the amount of time the plants have to keep their stomata open to take up the CO_2 they require. Similarly, it is possible that drought stress has not changed substantially over this period, because a reduction in wind run across the CFR may have balanced the increase in evaporative demand created by higher temperatures. At a plant level, *P. repens* may not respond to small increases in drought stress by utilising deep water. In addition, *P. repens* is potentially able to reduce stomatal conductance thereby alleviating xylem anatomical responses to the small change in temperature since 1994.

Taken together, my results demonstrate that while there is some phenotypic plasticity there is also strong genetic structuring among populations, with populations being highly adapted to their local climatic conditions. I also found little evidence for change in xylem anatomy of *P. repens* in response to changes in climate over the past 21 years. These results suggest that the different populations of *P. repens'* will respond uniquely to rapid climate change. With each population highly adapted to local site conditions, the degree to which each population can respond to rapid environmental change is reduced, risking rapid loss of populations and greater extinction risk for the species as a whole.

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Chapter 1: Introduction

Detecting the impacts of global change on biodiversity requires monitoring at scales from genes to landscapes, because the spatial and temporal sensitivity to change typically varies with the scale of observation (Noss, 1990). Population and species-level studies are perhaps the most sensitive for detecting change, but for observed responses to be meaningfully interpreted as global change impacts they require a well-developed understanding of the biology of the species, and the ability to predict how it may respond to a particular environmental stressor. Here I examine variation in the hydraulic traits (leaf and xylem anatomy) of *Protea repens* (L.) across climatic gradients in the Cape Floristic Region (CFR) and use these spatial trait-climate relationships to inform my expectation for a comparison of trait-level responses to climate change by comparing contemporary trait values with a 21 year old dataset. While a recent analysis has shown that rainfall has remained relatively constant across the CFR over the last 30 years (Hoffman *et al.,* 2011), temperature has increased, potentially causing higher evaporative demand and increasing plant water stress. Conversely, higher atmospheric CO_2 may be alleviating plant water stress by reducing the time plants have to keep their stomata open to take up the CO_2 they require.

Climate and vegetation of the Cape Floristic Region

The CFR of South Africa, with its predominantly winter rainfall, is home to the unique fynbos vegetation, comprised of a rich array of plant species (Myers *et al.,* 2000). More than 9000 vascular plants occur in the region, with approximately 68% being endemic (Manning & Goldblatt, 2012). The Ericaceae, Restionaceae and Proteaceae plant families' best epitomize the dominant growth forms in the region (Linder, 2003; Manning & Goldblatt, 2012). The Ericaceae is the largest genus with ±680 species, of typically small to medium, fine-leaved schlerophyllous shrubs. Restionaceae, with 342 species, are thin reed-like monocotyledons (Manning & Goldblatt, 2012), while the Proteaceae (333 species) are perhaps the most striking, with large, broad-leaved shrubs that form much of the Fynbos overstorey (Rebelo, 1995; Manning & Goldblatt, 2012). Given the variability in hydraulic traits among the different families and species as demonstrated by West *et al.* (2012), my study focuses on a single species, *Protea repens*, within one family, the Proteaceae.

There has been a lot of research on the ecology and distribution of *P. repens*, also known as the Common Sugarbush (Rebelo, 1995; Protea Atlas, 2008). The species is extremely varied in its habitat, occurring between altitudes of 20 to 2000 m above sea level, on gentle to steep slopes across the entire CFR (Protea atlas, 2008, Fig. 1.1 and 1.2). This unique distribution contains both a South – North gradient of increasing aridity and a West – East seasonality gradient, moving from predominantly winter to year-round rainfall (Midgley *et al*., 2005).

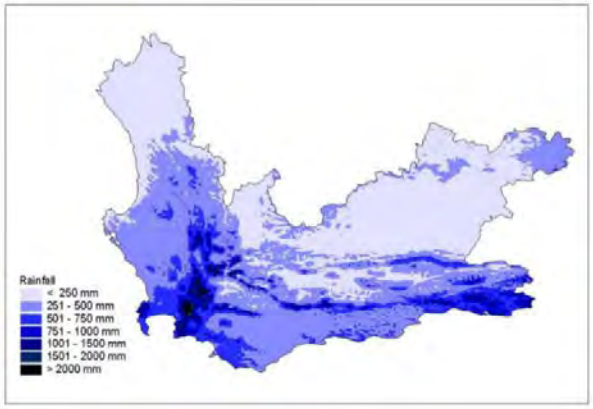

Figure 1.1 Mean annual rainfall of the Western Cape (Midgley et al.,2005)

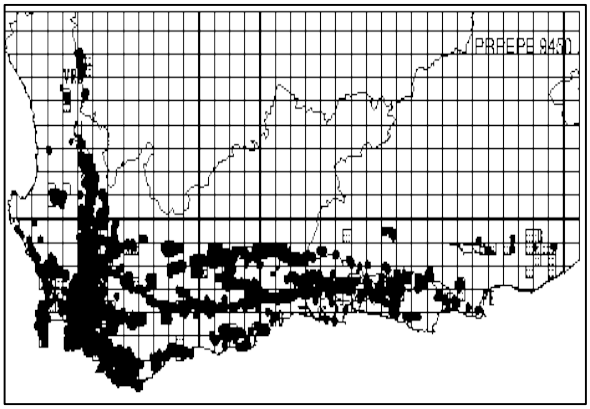

Figure 1.2 Protea repens distribution across the CFR (The Protea Atlas, 2008)

Climate and CO_2 effects on hydraulic traits

Water movement through woody plants, such as *P. repens,* is influenced by plant hydraulic architecture, which varies with water availability (Tyree & Ewers, 1991). Xylem vessel diameter and frequency form part of this architecture, and affect plant hydraulic efficiency and conductance (February, 1994, 1995; 1999a; Tyree & Zimmerman, 2002; Jacobsen *et al.,* 2007). Conductance increases as xylem vessel diameters increase, but increased xylem vessel diameters also increase a plants' vulnerability to drought induced cavitation (Tyree & Ewers, 1991; Tyree & Zimmermann, 2002). Generally, however, xylem vessel diameters increase with higher rainfall or available water (February, 1994; Tyree & Zimmermann, 2002; Jacobsen *et al*., 2007). Carlquist (1966) was one of the first to show this trend in a study on the *Asteraceae* in Australia. Using *Protea roupelliae,* February (1994) found the same relationship along a rainfall gradient from 760 mm in Musina to 1665 mm in Umtamvuna in South Africa, with corresponding mean vessel diameters increasing from 46 µm to 62 µm. While there have been very few studies in this regard, xylem vessel diameter and frequency

may also be affected by elevated temperatures (Tyree & Zimmermann, 2002; Kilpeläinen *et al*., 2007).

Most literature on the temperature effects on xylem anatomy are restricted to the Northern hemisphere, where very cold temperatures increase xylem vulnerability to freezing, selecting for smaller vessel sizes (Cavender-Bares *et al.,* 2005). Applying the centrifuge method to account for freezing induced cavitation in xylem, Davis *et al.* (1999) and Pittermann and Sperry (2003) found that vulnerability to freezing-induced cavitation increases significantly as vessel diameters and hydraulic conductance increase. Notwithstanding, in areas where temperatures are too high for freezing-induced cavitation, and if water availability is not limiting, plants will respond to an increase in temperature by increasing transpiration (Lafta & Lorenzen, 1995; Maherali & DeLucia, 2000) and therefore increasing xylem vessel diameters to account for the demand in conductivity. Such an increase in xylem anatomy has been demonstrated by Carlquist (1966) who showed that plants from cooler coastal temperate regions had smaller vessel sizes (51 μm) and increased number of vessels per transverse area of stem than plants from warmer tropical regions (65 μm). In contrast to the expectation of increasing vessel diameter with increased temperature and greater transpiration, higher temperatures for a given rainfall will affect water availability by increasing evaporative demand (Midgley *et al.,* 2005), creating greater drought stress and ultimately selecting for smaller xylem vessel diameters.

In contrast to increasing temperatures increasing potential drought stress, increased atmospheric CO_2 concentrations are expected to relieve hydraulic stress in plants with a C_3 photosynthetic pathway (Midgley *et al.,* 2005), which includes most fynbos species. This should occur because the amount of time plants need to keep their stomata open to take up the same amount of CO_2 is reduced, thus reducing transpirational demand and water use (Morison, 1985; Nowak *et al.,* 2004; Franks & Beerling, 2009; Bond & Midgley, 2012). Studies that characterize variation in xylem anatomy under elevated CO_2 have primarily been performed in greenhouses and growth chambers where CO_2 enrichment can be controlled. One such study measured stem hydraulic conductivity in *Quercus robur L.,* to determine the effects of increased CO_2 concentrations on the relationship between stem growth, xylem vessel size and frequency as well as stem transport capacity (Atkinson & Taylor, 1996). This study showed that xylem vessel diameters in seedlings increased from 16 μm at ambient (350 ppm) CO_2 concentrations to 19.9 μm at extremely elevated (700 ppm) CO_2. Long term studies found that mean vessel diameters and water use efficiency increased significantly while vessel density decreased with increased CO_2 concentrations (Medeiros & Ward, 2013;

Kostiainen *et al.*, 2014). Unfortunately, there are no studies of the effects of elevated CO_2 on the xylem anatomy of plants from the CFR. Experiments performed on four *Leucadendron* species showed little consistent response to CO_2 concentrations double that of ambient (700 ppm), and suggested that photosynthetic acclimation was at play (Midgley *et al.*, 1995, 1999), but this merits further investigation because these studies had low sample sizes and all plants were well watered. I do however, hypothesise that with an increase in both temperature and CO_2 there will be no change in xylem anatomy because plants will be able to obtain the same amount of carbon while reducing the amount of water lost through stomatal regulation. The increase in temperature will therefore not result in an increase in evaporative demand and greater drought stress.

Leaf anatomy is also expected to respond to changes in atmospheric CO_2 concentration and plant available water by altering resource allocation to tolerate stress or increase productivity. Plants in more arid areas may produce thicker, smaller leaves to adapt to the increased tension on the water column, while plants in more mesic areas with lower water stress have high conductivity and transpiration rates and thus larger leaf sizes (Atkinson & Taylor, 1996, Tyree & Zimmermann, 2002). Thuiller *et al.* (2004) found that leaf size decreased significantly with water availability across 88 *Leucadendron* species. In addition, species occupying areas of low soil water availability were found to adopt thick, heavy leaves with lower specific leaf area (SLA) to withstand the increased tension on the water column, while in warm, moist areas the reverse was evident (Thuiller *et al.,* 2004; Yates *et al.,* 2010a; Carlson *et al.,* 2010). Elevated atmospheric CO_2 concentrations allow plants to take up the CO_2 they require more rapidly with reduced stomatal conductance and transpiration rates (Midgley *et al.,* 2005; Nowak *et al.,* 2004; Franks & Beerling, 2009; Way & Oren, 2010). I hypothesise that if water is limiting a reduction in stomatal aperture, and consequent reduction in transpiration rates will mean that less water will be lost in producing the same amount of photosynthate. This will result in smaller leaf size and lower SLA. Alternatively, if resources are not limited, plants may increase productivity under elevated CO_2 by having larger leaves with higher SLA.

Constraints on trait response to abiotic factors

Plant responses to changes in abiotic factors, such as water availability, temperature and CO_2 concentrations, depend on the genetic variation within the species and the phenotypic plasticity of individuals in response to environmental stimuli. Changes in plant anatomy,

morphology or physiology are commonly expressed through phenotypic plasticity whereby the traits expressed by an individual are largely dependent on the conditions of the environment in which they grow (Bradshaw, 1965; Schlichting, 1986; Alpert & Simms, 2002; Valladares *et al*., 2007b). Plasticity allows plants and populations to respond rapidly to environmental change (De Witt *et al*., 1998). Over time, interactions between genotypes and environmental conditions create genetic variations that lead to the development of selectively advantageous phenotypic plasticity within individuals (Schmalhausen, 1949; Via & Lande, 1985). Populations or species that have little phenotypic plasticity, depend on genetic variation among individuals to persist through changing environmental conditions. In this case, only individuals with genes for the appropriate trait values survive. With isolated populations highly adapted to their local site conditions and low levels of gene flow between populations, the degree to which each population can respond to rapid environmental change is reduced, risking rapid loss of populations and greater extinction risk for the species as a whole.

I explore the degree of phenotypic plasticity in hydraulic traits within populations, and genetic structuring among populations, because it has strong bearing on the ability of *P. repens* to survive changing environmental conditions. To do this I compare trait data measured in the field to that collected from plants grown in a common garden. Trait differences among plants sourced from different populations and grown under identical conditions in the common garden would indicate intraspecific variation and genetic structuring among populations. Significant differences in traits between individuals from the same population grown in the field and individuals grown in the common garden would reflect phenotypic plasticity. Although studies on hydraulic functioning across populations are relatively scarce, especially in Fynbos, previous studies on the Proteaceae indicate strong genetic structuring among populations (Latimer *et al.,* 2009; Prunier *et al.,* 2012; Carlson *et al.,* 2010, 2015). If *P. repens* has strong genetic structuring among populations and lacks phenotypic plasticity, the species would be highly vulnerable to changing environmental conditions.

Predictions

One expects trait variation in a species that spans large temperature and rainfall gradients to reflect trade-offs between maximizing conductance and maintaining safety against cavitation across these gradients, and that this should give us an indication of how traits may shift with

changing climate through time. I propose that *P. repens* populations from more mesic environments should grow larger leaves and have fewer, larger xylem vessels (Thuiller *et al.,* 2004, February *et al.,* 1994; 1995; 1999a). Since rainfall appears to have remained relatively constant for the study area over the last 30 years (Hoffman *et al.,* 2011), elevated temperature and atmospheric CO_2 are likely to be the main drivers affecting change in hydraulic traits over time. As higher temperatures may be creating greater soil moisture stress by increasing evaporative demand, I expect plants to have smaller leaves, lower SLA and greater numbers of xylem vessels with narrower diameters, to decrease vulnerability of xylem to cavitation with increased temperature and drought stress. Alternatively, increased atmospheric CO_2 concentrations may relieve water stress by decreasing stomatal conductance and transpiration, thereby increasing safety to cavitation, which will result in an increase in xylem vessel diameter and leaf size. Similarly, if plants are not near their hydraulic limits and growth is constrained by temperature, then increased temperatures should result in larger xylem vessels. If traits are constrained by genetic structuring among populations, and plants grown in a common garden reflect similar traits to their source populations, *P. repens* could be limited in its ability to respond to rapid climatic change.

In chapter 2 I explore trait variation in 13 *P. repens* populations across the CFR and compare these to a common garden experiment where individuals were grown from seed collected from each population. The common garden thus serves as a control that allows me to determine genetic structuring of trait variation among populations by examining how hydraulic traits may differ between populations grown under the same climatic conditions. The common garden also allows me to test for phenotypic plasticity within populations, by exploring differences in trait expression between plants from the same population relative to their source locality. Greater plasticity allows rapid responses to environmental change with a possible increased resilience to climate change. Strong genetic structuring among populations limits a species' ability to respond to rapidly changing environmental conditions across its range.

To determine the response of *P. repens* vessel morphology to changing climate and increased atmospheric CO_2, in chapter 3 I compare contemporary trait data to historical data recorded from the same source populations 21 years ago. I conclude my thesis with a synthesis chapter exploring the implications of my results for the resilience of plant species in the CFR to future scenarios.

Chapter 2: Hydraulic trait responses to climate gradients and genetic constraint in a widespread woody shrub.

Introduction

The Cape Floristic Region (CFR) of South Africa supports an extremely large number of plant species arrayed across steep rainfall, temperature and seasonality gradients (Linder, 2003; Manning & Goldblatt, 2012). Weather records in the region show strong trends (New *et al.,* 2006; Lumsden *et al.,* 2009; Hoffman *et al.,* 2011) and Global Circulation Models predict dramatic shifts in climate over the next 30 to 50 years (Midgley *et al.,* 2005; IPCC AR5, 2014), posing a threat to species diversity (Midgley *et al.,* 2002, 2003, 2005). Plant physiological traits, such as leaf or xylem vessel morphology, are typically highly adapted to environmental conditions (Dudley, 1996; February & Manders, 1999a; Thuiller *et al.,* 2004; Yates *et al.,* 2010a). These traits have been used as proxies for determining historical climate change (February *et al.,* 1994; 2000; Fonti *et al.,* 2010). Developing an understanding of how traits vary along spatial climatic gradients allows us to predict how we would expect these to respond to changes in climate through time. In addition, species resilience to climate change can be inferred by examining the extent to which traits can respond, either through phenotypic plasticity or genetic variation within and among populations.

The steep moisture gradients, linked to elevation and distance to the coast, and the clear West-East seasonality gradient in the CFR (Midgley *et al.,* 2005; Schulze, 2007; Wilson & Silander 2013), result in considerable variation in vegetation composition and plant diversity (Cowling & Lombard, 2002). A study examining the morphology of 88 different *Leucadendron* species found that water availability is a strong determinant of species distributions and leaf morphology, with species in areas with less water having smaller leaf sizes than those in areas with more water (Thuiller *et al.*, 2004). Yates *et al*. (2010a) found further support that Proteaceae species with larger leaves are associated with higher precipitation and lower temperatures in the CFR. Similarly, a number of Proteaceae and other CFR species have wood anatomy adapted to mitigate drought stress through increased resistance to cavitation (Jacobsen *et al.,* 2005; 2007; Crous *et al.,* 2012; Pratt *et al.,* 2012).

Several studies have shown that woody plants may cope with changes in water availability by changing the number and diameter of their xylem vessels (February, 1994; 1995; 2000; Tyree & Zimmermann 2002; Jacobsen *et al.,* 2007; Crous *et al.,* 2012). February and Manders (1999a) found that xylem vessel diameter in *P. repens* and *P. neriifolia*

increased from 42.3 μm to 63.8 μm and from 36.1 μm to 67.7 μm respectively, while number of vessels decreased from 117.8 mm^{-2} to 60.7 mm^{-2} and 99.2 mm^{-2} to 44.9 mm^{-2}, as available water increased. Species with few large vessels are typical of mesic environments, and have the ability to rapidly conduct large amounts of water, while species growing in drier environments tend to have many small vessels that are less vulnerable to cavitation under drought stress, but have lower conductance (Hacke & Sperry, 2001; Tyree & Zimmermann, 2002; Jacobsen *et al.,* 2007; Pratt *et al.,* 2012). Water transport efficiency is generally increased by producing larger xylem vessels, while xylem vulnerability to cavitation is reduced by having narrower vessels (Tyree & Zimmermann, 2002). The relationship between vessel diameter and conductance is expressed mathematically by the Hagen-Poiseuille equation, which states that the conducting efficiency of a conduit is proportional to the fourth power of the vessel diameter (Tyree & Zimmermann, 2002). This means that a small increase in vessel diameter will result in a greater proportional increase in conducting efficiency (Hacke & Sperry, 2001; Tyree & Zimmermann, 2002). In the CFR, Jacobsen *et al.* (2007) found that species with larger vessel diameters had increased xylem specific conductivity.

A species' resilience to climate and environmental change depends on its ability to adapt to and withstand these changes. This depends on the degree of phenotypic plasticity within individuals, the amount of genetic variation in the species, and the structuring of this genetic variation within versus between populations. Phenotypic plasticity is the ability of an individual organism to change its phenotype in response to altering environments, allowing rapid response to environmental change in the short term. The traits that individuals have, and the degree of phenotypic plasticity they display, depend on their genotype (Nicotra *et al.,* 2010). Greater genetic variation within populations increases a species' fitness under changing conditions, because there is a greater probability that some individuals will be suited to the new conditions (Via *et al.,* 1995; Nicotra *et al.,* 2010). Genetic variation is often partitioned among populations such that particular genotypes dominate in environments where they convey greater fitness (Nicotra *et al.,* 2010) and could drive a species to occupy extensive areas (Ackerly *et al.,* 2000). However, should genotypes be strongly partitioned among populations, and little gene flow occur between them, then the species will be more vulnerable to changing conditions, because each population will be closer to it's tolerance limit than expected based on variation across the species entire range. Since global climate change is a dynamic process, the demand for plants to respond rapidly to environmental change is high (Parmesan, 2006; Valladares *et al.,* 2007a; 2007b). Greater population level phenotypic plasticity or genetic variation in important traits such as those relating to leaf or

wood anatomy enable species to respond to climate and environmental changes more rapidly (Nicotra *et al.,* 2010).

Common garden experiments help determine the extent to which trait differences in plants reflect responses to the environment from which they originated (Ackerly *et al.,* 2000; Reich *et al.,* 2003) and the evolutionary processes (genotypic constraints or phenotypic plasticity) responsible for such variation (Prunier *et al.,* 2012). Turreson (1922) was the first to use common garden experiments to test the adaptive ability of population divergence (Ackerly *et al.,* 2000). Since then, many researchers have used such experiments to explore differences in traits (e.g. Prunier *et al.,* 2012; Carlson *et al.,* 2010, Carlson & Holsinger, 2012).

There are several considerations for predicting *P. repens* xylem anatomical and leaf morphological adaptations across an environmental gradient.

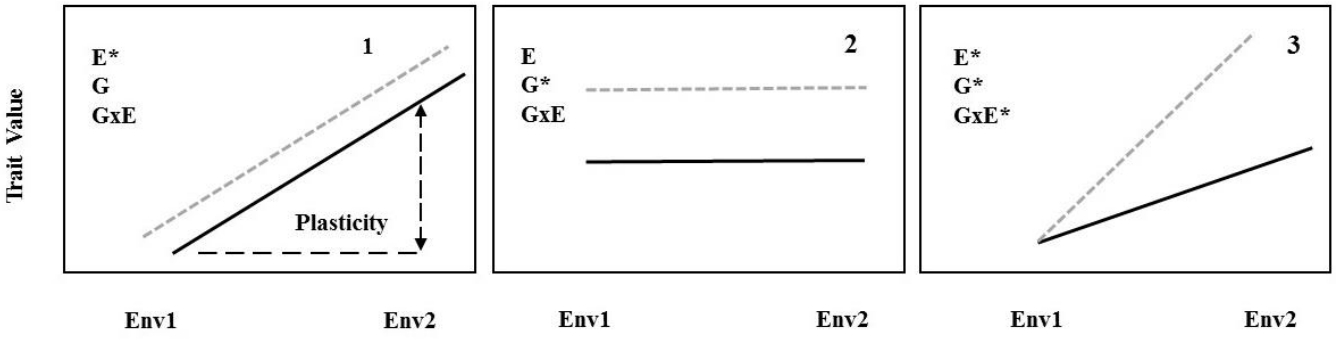

Figure 2.1 Varying responses to different amounts of available water. The grey dashed and black solid lines indicate the reaction norms of two different genotypes responding to a change from high water stress (Env1) to low water stress (Env2). The extent of phenotypic change in response to a signal is its phenotypic plasticity. Asterisks in the panels denote whether there is a significant effect of environment (E) or genotype (G), and whether there is a significant genotype by environment interaction (GxE) (redrawn from Nicotra et al., 2010).

The first expectation is a strong plastic response with no genotype effect resulting in no correlation between the traits expressed in the common garden and climate at the source population locality (Fig. 2.1, Scenario 1). All populations planted in the common garden should therefore have similar traits, with large increases in xylem vessel diameters and conductance for arid-adapted populations in the more benign garden. Secondly, no plasticity and strong genotype effects would imply that no significant differences will be visible between *P. repens* anatomy in the common garden and their wild parents, but there will be strong differences between populations (Fig. 2.1, Scenario 2). This will make *P. repens* highly vulnerable to change in climate, because there will be no short term plastic response to

change, and the species will require gene flow between isolated populations to adapt. Lastly, *P. repens* grown in the common garden could show strong genotype effects with some phenotypic plasticity (Fig. 2.1, Scenario 3). In this case all populations will show an overall increase in vessel sizes and conductance when grown in a more favourable common garden, but there will be significant differences between populations and smaller changes visible for populations from higher rainfall areas.

Here, I describe variation in leaf and wood anatomical traits between 13 *P. repens* populations across a rainfall and temperature gradient, and explore the degree to which this variation is constrained by genetic differences between populations (Table 2.1). I do this by comparing traits from individuals in wild populations sampled across the climatic gradient with those from a common garden experiment, with uniform water availability and soil conditions, grown from seed collected from the wild populations. Specifically, I ask whether xylem vessel morphology and specific leaf area (SLA) of *P. repens* correlate with climatic gradients, whether they differ between wild populations and the common garden (suggesting phenotypic plasticity), and if the traits of individuals grown in the common garden differ among source populations and reflect the climate of their source site (suggesting genetic constraint).

Methods

Common Garden and study region

A common garden was established at Kirstenbosch Botanical Gardens at Newlands, Cape Town (S33.993643; E18.428657; Carlson & Holsinger, 2012). Kirstenbosch is situated in the winter rainfall region of South Africa, with mean annual precipitation of 1447 mm, mean annual temperature of 16.5ºC and an elevation of 175 m (Table 2.1). Soils are relatively nutrient rich (total P 12 ppm, total K 171 ppm, total N 0.86%; Carlson & Holsinger, 2012). Seeds from 13 populations of *P. repens* from sites with similar sandstone derived soils from across the CFR were propagated in the common garden (Table 2.1). The driest site, Baviaanskloof, has a mean annual precipitation (MAP) of 376 mm while the wettest site, Ceres, has a MAP of 997 mm (Wilson & Silander, 2013). After germination, in a greenhouse, seedlings were transplanted in a randomized layout in the garden in mid July 2011 (Carlson *et al.*, 2010). Each plant was allocated a unique label that could be traced to both the population and parent plant from which the seed was originally sourced. At the time of sampling, plants were at least two years old. Six individuals, each from a unique parent, were

selected from each source population and position within the common garden to create a spatially random distribution of samples in consideration of potential edge effects or slight differences in water and shading.

Interpolated daily meteorological observations (1 arc-min grid resolution) of minimum and maximum temperatures, precipitation and summer and winter soil moisture days for the CFR were used to determine the spatial climate variation between source locations and the common garden from 1999 to 2009 (Wilson & Silander, 2013). Rainfall, temperature and seasonality play a pivotal roll in the development and support of biodiversity in the GCFR and are the key drivers of biological processes in this region. Soil moisture days in winter and summer obtained from Merow *et al*. (2014) were used as an indication of rainfall seasonality and/or drought.

Table 2.1 Location and climate statistics for Protea repens populations sampled across the CFR and the common garden (Kirstenbosch) with acronyms for each population, as represented in the subsequent graphs. Climate data for each site include, maximum temperature (Tmax), minimum temperature (Tmin) and mean annual precipitation (MAP) from Wilson and Silander (2013).

Source Population	Acronym	Latitude	Longitude	Tmax	Tmin	MAP
Anysberg	ANY	-33.49369	20.70409	20.31	8.25	456.56
Baviaanskloof	BAV	-33.49336	23.63356	21.93	7.71	376.68
Bredasdorp	BRD	-34.54593	20.0386	22.83	11.90	539.89
Cederberge	CDB	-32.40640	19.10676	16.00	7.84	737.81
Ceres	CER	-33.36542	19.276	21.61	7.35	997.13
Garcias pass	GAR	-33.96789	21.21982	24.42	10.13	681.05
Kleinmond	KLM	-34.33109	19.03363	20.39	12.05	931.02
Kirstenbosch	KBS	-33.99364	18.42866	21.45	12.68	1446.80
Klein Swartberg	KSW	-33.45617	21.28479	23.50	10.69	410.43
Potberg	POT	-34.41555	20.60545	23.98	11.54	476.53
Riviersonderend	RND	-34.10228	19.99314	23.41	11.11	539.89
Swartberg Pass	SWA	-33.34953	22.04591	20.01	6.05	694.83
Uniondale	UNI	-33.72581	23.05253	20.51	8.56	401.47
Van Rhynsdorp	VAN	-31.37083	19.0225	24.79	10.30	471.17

Trait data collection

Six plants from each of the 13 populations (78 plants) in the common garden were harvested at 1 cm above the soil surface on clear warm days between November 2013 and April 2014.

At the time of harvesting, I recorded stem diameter. Wild populations were sampled in April 2014, following a similar procedure. Where wild populations differed in age to the common garden we collected branches with diameters between 8 mm and 20 mm to match the range sampled from the garden. After collection, harvested branches were covered individually with a polyethylene bag with the cut stem in a bucket of water to keep the plant fully hydrated. In the laboratory total leaf area for each branch was determined using a Li-3100 leaf area meter (Li-cor Inc., Lincoln, Nebraska, USA). Total leaf wet weight was ascertained, after which the leaves were oven dried for at least 48 hours at 70° C before determining dry leaf mass and calculating specific leaf area (SLA) by dividing leaf area by dry leaf mass (mm^2/mg; Cornelissen *et al.,* 2003).

I determined xylem vessel diameter and frequency in a modification of the methods used by February *et al.* (1995) and February and Manders (1999a). In the laboratory a 2 cm segment was cut from the base of each sample, incorporating both the pith and the cambium. Transverse sections of between 20 and 30 μm thick were cut using a Reichert-Jung base sledge microtome (Reichartd-Jung, Austria). The resulting sections were stained with a mixture of alcohol, glycerol and safranin red and mounted in Kaiser's gelatin-glycerin on glass microscope slides. These were photographed at a magnification of 40x using a Leitz Laborlux K incident light microscope (Wetzlar, Germany) with a mounted Canon EOS 600D camera (Canon, Japan). Eight non-overlapping digital photographs of equivalent cross-sectional area were taken from the outermost 2 mm section of wood (most recent growth) for all stem sections. A graticule was also photographed at the same magnification to calibrate measurements on the photographs. Vessel diameters were measured using open-source image analysis software (ImageJ; http://imagej.nih.gov/ij/). Radial diameters were measured for the 30 biggest vessels encountered in the 8 photographs per section of wood. Measurements were taken at the widest part of the vessel opening as it is these vessels that are the most efficient conductors and most vulnerable to hydraulic dysfunction, indicating stress at the onset of adverse environmental conditions (Hacke & Sperry, 2001). Vessel density was determined by counting the number of vessels (n) for all 8 photographs, expressed per unit area (mm^{-2}).

Calculations of hydraulic variables

Mean vessel diameter is not a proper indicator of potential conductance due to the disproportional increase in conductance between small and large vessel diameters and individual plants optimising conductance and resistance to cavitation by investing in a

combination of both small and large vessel diameters (Hagen-Poiseuille; Hacke & Sperry, 2001; Tyree & Zimmermann, 2002; Pratt *et al.,* 2012, Tyree & Zimmermann, 2002). Several equations can be used to estimate conductance from xylem vessel diameter, including mean hydraulic diameter (D_H) and hydraulic vessel diameter (D_S or d_h), (Tyree & Zimmermann, 2002).

Mean hydraulic diameter is expressed as:

$$D_H = [(\Sigma d^4)/n]^{0.25} \qquad \text{(eqn. 1).}$$

This calculation incorporates the d^4 relationship as the sum of the vessel diameters (d) to the fourth power divided by the number of vessels (n) to the fourth root.

Sperry *et al.,* (1994) defined hydraulic vessel diameter (D_S or d_h), with the formula:

$$D_S = \Sigma d^5/\Sigma d^4 \qquad \text{(eqn. 2).}$$

Although this equation is closely related to D_H (eqn. 1), weighting vessel diameter by its hydraulic contribution, D_S is not considered as meaningful as D_H (Tyree & Zimmermann, 2002). It is important to note that these measures provide an estimate of the potential conductance of the stem assuming that all xylem vessels are functional, which is not often the case (Tyree & Zimmermann, 2002). It should thus be considered an estimate of maximum potential conductance.

Another valuable calculation to determine how sufficiently stems supply water to leaves is the Huber value (H_V) that is obtained by dividing active cross-sectional sapwood area (m^2) by the leaf area (m^2) distal to the stem (Tyree & Zimmermann, 2002). The advantage of using H_V is that the equation takes active leaves into consideration, allowing direct comparison between measurements taken at any point on the plant. Large ranges in stem diameters do however make H_V comparisons between species difficult. Plants in drier climates usually have higher H_V (Tyree & Zimmermann, 2002) since both leaf sizes (Thuiller *et al.,* 2004) and vessel diameters are generally smaller. As the Huber value does not take vessel diameters into consideration and stem diameters varied between the different sampling areas to some degree, I use only D_S and D_H.

Similar to xylem vessel diameters, leaf sizes increase with water availability, because larger leaf areas with an increased amount of anastomosing tissue increases a plant's ability to transport nutrients and water (Chave *et al.,* 2009; Thuiller *et al.,* 2004; Carlson *et al.,* 2010). Leaf specific conductivity (LSC or K_L) is often used to determine how efficiently

xylem vessels transport water to the leaves (Tyree & Ewers, 1991; Tyree & Zimmermann, 2002). K_L is derived from hydraulic conductivity (K_h) per leaf area (A_L) distal to the stem segment (Tyree and Zimmermann, 2002):

$$K_L = K_h/A_L \quad \text{(eqn. 3)}$$

From:

$$K_h = F(L/\Delta P) \quad \text{(eqn. 4)}$$

Where hydraulic conductance (K_h) is the flow rate (F) times the length of the segment (L) divided by the pressure drop (ΔP). The estimate for potential conductance D_H is considered to be a logical equivalent (rather than mathematical equivalent) to K_h, as results are expected to react in similar manner, indicating larger values as conductivity increases. Based on this assumption I use D_H as a proxy for K_h since hydraulic conductivity (K_h) was not measured directly. Leaf specific hydraulic diameter (D_{HL}) can therefore be calculated as an equivalent to K_L:

$$D_{HL} = D_H/A_L \quad \text{(eqn. 5)}$$

Where the estimate of conductivity, hydraulic diameter (D_H), is expressed per distal leaf area (A_L), representing how much water and nutrients the stem could potentially supply to the leaves. Leaf and wood anatomical trait differences, as determined by D_H and D_{HL}, should reflect climatic differences among *P. repens* populations. Determining these relationships are important for understanding potential responses to the changing climate being experienced by the plants in the CFR (Lumsden *et al.,* 2009).

Statistical Analysis

To ascertain whether there is genetic constraint on trait expression among populations (*sensu* Fig. 2.1), I used one-way ANOVAs to test for differences in functional traits between populations when grown in the common garden. Furthermore, to test for evidence of phenotypic plasticity within populations, revealed by differences in traits between the source population and common garden, I fitted a linear mixed effects models for each trait with source population versus common garden as a fixed effect and population as a random effect. Models were fit in R using the library nlme (Pinheiro *et al*., 2015).

To explore the climatic drivers of trait variation I fit linear mixed effects models with each trait (D_H, SLA, D_{HL}, mean xylem vessel diameter, xylem vessel density) modelled as a function of mean annual maximum temperature (Tmax), mean annual minimum temperature (Tmin), mean annual precipitation (MAP) and soil moisture days in winter (SMD WIN) and summer (SMD SUM). These variables were included as fixed effects, while population was included as a random effect to account for pseudoreplication of individuals within populations. Stem diameter was included to test and account for variation in traits with plant size. Separate models were fit for the source populations and the common garden to see if the traits expressed in the common garden reflect environmental conditions at the site of the source population.

The global model was: (G.M.)

Trait ~ maximum temperature + minimum temperature + mean annual precipitation + smdwin + smdsum + stem diameter, random=~1|population.

All possible model subsets were explored using the *dredge* function in the library MuMin (Bartoń, 2015), following the method of Burnham & Anderson (2002). The Akaike Information Criterion (AIC) was used to compare between all possible combinations of the global model in order to select the 'best' set of predictor variables based on the likelihood of the data corrected for the number of parameters (Zuur *et al.,* 2009; Nakagawa & Schielzeth, 2012). Conditional R-squared (R^2_c) and marginal R-squared (R^2_m) were determined using the method of Nakagawa & Schielzeth (2012). R^2_c report the variance explained by both fixed and random effects, while R^2_m considers only fixed effects. All linear mixed effects models were fit using maximum likelihood (Zuur *et al.,* 2009) in R 3.2.2 (The R Foundation for Statistical Computing 2015).

Results

Climatic differences between source populations

The interpolated climate data from the population localities indicate great variation in temperature and rainfall across the CFR (Fig. 2.2). The highest mean annual maximum temperatures were at Van Rhynsdorp in the Northwestern border of the CFR and at the southern Cape sites, Garcias Pass and Potberg (Fig. 2.2). Lowest mean annual minimum temperatures were at high elevation sites situated far from the coast (Swartberg Pass, Ceres, Baviaanskloof, Cederberge and Anysberg). The common garden at Kirstenbosch experienced

more benign conditions than most field sites, with higher rainfall and warmer minimum temperatures than all sites, and cooler maximum temperatures than most (Fig. 2.2).

Trait differences among populations in the common garden

A comparison of D_H (Tyree and Zimmermann, 2002) and D_S (Sperry *et al.,* 1994) showed near identical results, I therefore only report results for D_H. The one-way ANOVA comparing individuals between populations in the common garden (Table 2.2) shows that D_H, mean vessel diameters, vessel densities, and D_{HL} are all significantly different between populations. There are however no significant differences in SLA between populations in the common garden.

Table 2.2 One-way ANOVA indicating significant differences between populations in the common garden for each trait.

Trait	df	Sum Sq	Mean Sq	F-Value	P-value
D_H	12	4260	355	10,25	< **0.001**
Xylem Diameter	12	3929	327,4	9,834	< **0.001**
Vessel Density	12	32432	2702,7	2,95	< **0,005**
SLA	12	0,0018	0,0002	1,597	0,114
D_{HL}	12	0,0198	0,0017	2,401	< **0,05**

Trait differences between source populations and the common garden

A comparison of hydraulic traits between the source population and the common garden using a linear mixed effects model show that mean vessel diameter and D_H of all the populations increased significantly in the common garden (Fig. 2.3 and Table 2.3; $p < 0.001$, slope = 6.942), while vessel density decreased significantly (Fig. 2.3 and Table 2.3; $p < 0.001$, slope = -134.712). Similarly, SLA was greater in the common garden than at the field sites (Fig. 2.3 and Table 2.3, $p < 0.001$, slope =1.87). There were varied responses for D_{HL} between the common garden and source populations as most populations (8/13) have

increased in D_{HL} in the common garden and the overal difference was significant at the second decimal place (Fig. 2.3 and Table 2.3, p = 0.052, slope = 0.008).

Table 2.3 Linear mixed effects model to test for phenotypic plasticity of each trait within populations, revealed by differences in traits between the source population and common garden.

Trait	Slope	Std. Error	t value	P-value	Multiple R^2
D_H	6,759	1,323	5,109	< **0,001**	0,145
Xylem Diameter	6,274	1,279	4,906	< **0,001**	0,135
Vessel Density	-134,712	8,927	-15,09	< **0,001**	0,596
SLA	1,87	0,149	12,56	**<0,001**	0,506
D_{HL}	0,008	0,004	1,962	0,0515	0,024

Abiotic drivers of trait differences between populations

The results of the linear mixed effects models exploring the environmental correlates of each trait are presented in Table 2.4. Only models with an AIC less than 2 units from the best model or identical R^2 values as determined by both fixed and random effects ($R^2_m < R^2_c$) were retained. These models, show that maximum temperature and soil moisture days in winter were important in impacting both D_H and mean vessel diameter in the common garden (CG; $R^2_c = 0.628$ for D_H and $R^2_c = 0.641$ for mean vessel diameter; Table 2.4) and in the source populations (SP; $R^2_c = 0.467$ for D_H and $R^2_c = 0.472$ for mean vessel diameter; Table 2.4). Interestingly, in the model for source populations, D_H and mean vessel diameter show significantly negative trends with mean annual precipitation and significantly positive trends with soil moisture days in summer. Xylem vessel densities were poorly predicted by the models (CG; $R^2_m = 0.269$, SP; $R^2_m = 0.215$) and showed no significant relationship with the climate variables at their source populations or in the common garden. Mean annual precipitation ($p < 0.05$; slope = -20,146) and minimum temperatures ($p < 0.05$; slope = -5,092) indicated near significant reductions in vessel densities in the common garden.

Models for SLA include large variation in the combination of climate variables both in the common garden (CG) and at the source populations (SP; Table 2.4). At their source populations SLA is however correlated with soil moisture days in winter (SMD WIN; $p < 0.01$; slope = -0.021) and mean annual precipitation ($p < 0.01$, slope = -0.677) while soil

moisture days in summer weakly affects SLA (SMD SUM; $p < 0.05$; slope = 0.053; Table 2.4).

The combination of climate variables varied greatly for D_{HL}. At the source population D_{HL} is significantly affected by both mean annual precipitation ($p < 0.001$; slope = 0.017) and soil moisture days in winter ($p < 0.001$; slope = 0.001; Table 2.4). In the common garden Mean vessel diameters and hydraulic diameters positively correlated with stem diameters ($p < 0.01$), while D_{HL} decreased significantly with stem diameters ($p < 0.001$; Table 2.4). Vessel densities ($p < 0.01$) and D_{HL} ($p < 0.001$) in the field (SP) showed negative correlations with stem diameters, while SLA showed weakly significant increases with stem diameter ($p < 0.05$; Table 2.4).

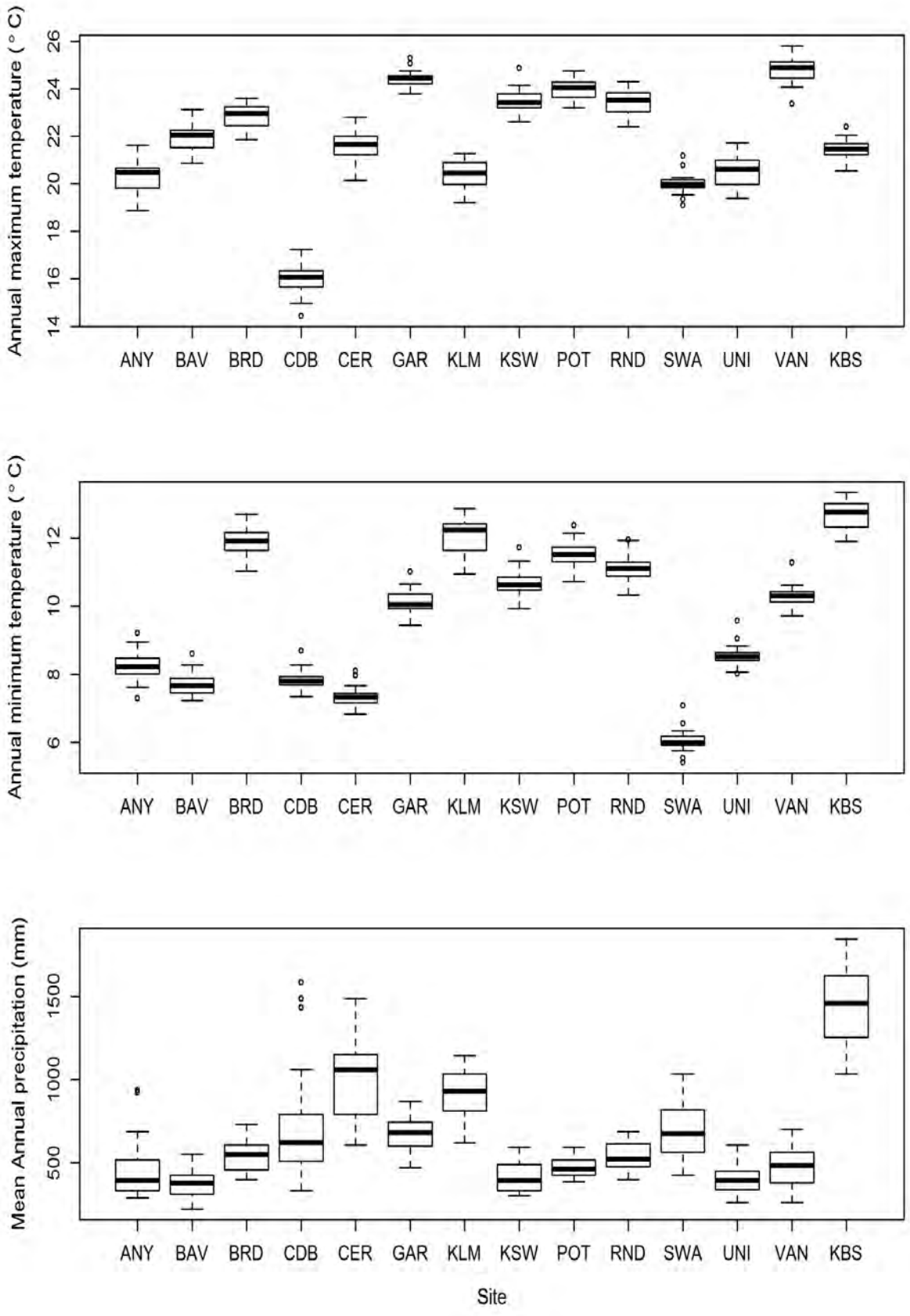

Figure 2.2 Median mean annual maximum and minimum temperature (ºC) and mean annual precipitation (mm) for source populations for the period 1990 to 2009 (source: Wilson & Silander 2013).

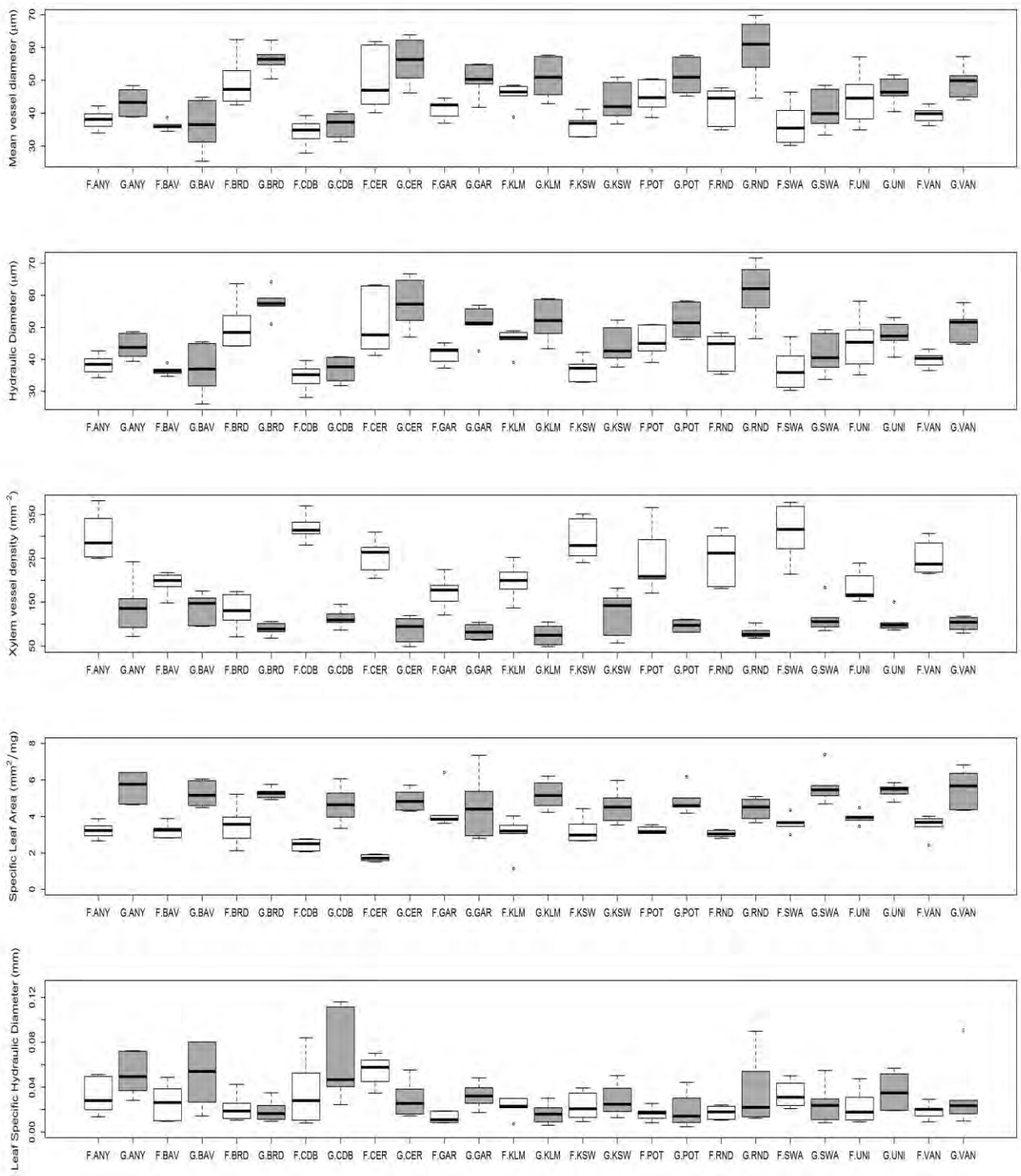

Figure 2.3 Intraspecific differences of hydraulic traits between field (F, white boxes) and garden (G, grey boxes) samples. Results of a one-way ANOVA and a linear mixed effects model to test for phenotypic plasticity are reported in Table 2.2 and Table 2.3 respectively. Outliers for SLA (G.ANY = 9 mm^2/mg) and DHL (G.BAV = 2.4 mm) were excluded.

Table 2.4 Evaluation of linear mixed effect models indicating the effects of environmental factors on Hydraulic diameter (D_H), Specific leaf area (SLA) and Leaf specific hydraulic diameter (D_{HL}) for both the common garden (CG) as well as the source populations. Values are the slopes for the specific variables. Models ordered by AIC Weight.

	Hydraulic diameter											
Treatment	Intercept	Stem Diameter	Tmax	Tmin	MAP	SMD WIN	SMD SUM	df	AIC	AIC Weight	R^2m	R^2c
SP	-10,142NS	0,463NS+	1,507**	-1,152NS+	-16,746**	0,652**	0,768**	9	492,403	0,5	0,467	0,467
SP	-15,005NS+	-	1,849**	-1,161NS+	-17,511**	0,700**	0,877**	8	494,287	0,26	0,439	0,439
CG	-11,462NS	0,568**	1,933**	-	-	0,172*	-	6	510,310	0,39	0,506	0,628
CG	-10,025NS	0,577**	1,908**	-	-3,451NS	0,252NS+	-	7	511,622	0,16	0,514	0,627
CG	-10,304NS	0,554**	1,765*	0,335NS	-	0,163*	-	7	512,091	0,13	0,511	0,630
CG	-13,791NS	0,555**	1,954**	-	-	0,181*	0,087NS	7	512,147	0,13	0,510	0,630
CG	-18,180NS	0,529**	1,967**	-	-8,424NS	0,407*	0,378NS	8	511,737	0,12	0,544	0,632

	Mean Vessel Diameter											
Treatment	Intercept	Stem Diameter	Tmax	Tmin	MAP	SMD WIN	SMD SUM	df	AIC	AIC Weight	R^2m	R^2c
SP	-11,699NS	0,486NS+	1,589**	-1,258NS+	-17,658**	0,686**	0,797**	9	497,110	0,58	0,472	0,472
SP	-16,803NS+	-	1,948**	-1,267NS+	-18,461**	0,736***	0,912**	8	499,134	0,28	0,444	0,444
CG	-13,158NS	0,587**	2,016**	-	-	0,183*	-	6	513,345	0,4	0,516	0,641
CG	-11,825NS	0,596**	1,994**	-	-3,218NS	0,257NS+	-	7	514,789	0,16	0,523	0,640
CG	-15,862NS	0,573**	2,040**	-	-	0,193*	0,101NS	7	515,139	0,13	0,522	0,642
CG	-12,049NS	0,575**	1,855*	0,319NS	-	0,174*	-	7	515,159	0,13	0,521	0,642
CG	-20,203NS	0,548**	2,053**	-	-8,340NS	0,417*	0,389NS	8	514,941	0,11	0,552	0,645

	Xylem vessel density											
Treatment	Intercept	Stem Diameter	Tmax	Tmin	MAP	SMD WIN	SMD SUM	df	AIC	AIC Weight	R^2m	R^2c
SP	416,074***	-5,667**	-	-11,092NS	-	-	-	5	846,164	0,16	0,215	0,590
SP	315,673***	-6,054**	-	-	-	-	-	4	846,602	0,15	0,078	0,564
SP	457,876***	-5,673**	-6,739NS	-	-	-	-	5	847,320	0,09	0,169	0,587
SP	454,111***	-5,488**	-	-11,301NS	-	-	-2,191NS	6	847,331	0,07	0,255	0,597
SP	350,231***	-5,934**	-	-	-	-	-2,062NS	5	847,985	0,06	0,119	0,573
CG	201,508***	-1,343NS	-	-5,092*	-20,146*	-	-	6	760,817	0,14	0,269	0,269
CG	206,724***	-	-	-6,708**	-24,300**	-	-	5	761,276	0,13	0,246	0,246
CG	245,957***	-	-2,198NS	-5,328*	-27,000**	-	-	6	761,845	0,08	0,259	0,259
CG	233,720***	-1,207NS	-1,775NS	-4,141NS	-22,747*	-	-	7	761,8825	0,07	0,278	0,278

Specific Leaf Area

Treatment	Intercept	Stem Diameter	Tmax	Tmin	MAP	SMD WIN	SMD SUM	df	AIC	AIC Weight	R^2m	R^2c
SP	3,696***	0,060*	-	-	-	-0,021**	-	5	165,291	0,15	0,303	0,460
SP	2,737***	0,052NS+	-	-	-0,677**	-	0,053*	6	165,559	0,11	0,344	0,472
SP	3,129***	0,055*	-	-	-	-0,019**	0,028NS	6	165,727	0,1	0,339	0,469
SP	3,271***	0,055*	-	0,054NS	-	-0,022**	-	6	166,517	0,07	0,324	0,469
SP	2,668***	0,048NS+	-	0,057NS	-	-0,019**	0,029NS	7	166,758	0,05	0,360	0,476
SP	3,661***	0,059*	-	-	0,158NS	-0,025NS+	-	6	167,168	0,05	0,306	0,461
CG	5,934***	-	-	-0,088NS	-	-	-	4	228,416	0,11	0,027	0,074
CG	5,410***	-0,025NS	-	-	-	-	-	4	229,090	0,08	0,014	0,082
CG	6,004***	-	-0,041NS	-	-	-	-	4	229,528	0,06	0,009	0,073
CG	5,390***	-	-	-	-0,179NS	-	-	4	229,544	0,06	0,009	0,073
CG	6,360***	-	-	-0,095NS	-0,220NS	-	-	5	229,544	0,05	0,040	0,074
CG	5,277***	-	-	-	-	-0,003NS	-	4	229,847	0,05	0,004	0,073
CG	5,160***	-	-	-	-	-	-0,003NS	4	230,037	0,05	0,0003	0,073
CG	5,963***	-0,014NS	-	-0,073NS	-	-	-	5	230,156	0,04	0,031	0,082
CG	6,061***	-	-	-0,086NS	-	-0,002NS	-	5	230,260	0,04	0,030	0,074
CG	6,006***	-	-	-0,088NS	-	-	-0,004NS	5	230,387	0,03	0,028	0,074

Leaf Specific Hydraulic Diameter

Treatment	Intercept	Stem Diameter	Tmax	Tmin	MAP	SMD WIN	SMD SUM	df	AIC	AIC Weight	R^2m	R^2c
SP	0,038***	-0,003***	-	-	0,017***	-	-	5	-407,302	0,2	0,358	0,358
SP	-0,0005NS	-0,003***	0,002NS	-0,003NS+	-	0,001***	0,001NS+	8	-406,894	0,09	0,402	0,402
SP	0,027*	-0,003***	-	-	-	0,001***	0,001NS	6	-405,740	0,08	0,361	0,361
SP	0,037***	-0,003***	-	-	0,014NS	0,001NS	-	6	-405,635	0,07	0,360	0,360
SP	0,042**	-0,003***	-	-0,001NS	0,017***	-	-	6	-405,548	0,07	0,360	0,360
SP	0,028NS	-0,003***	0,001NS	-	0,018**	-	-	6	-405,515	0,07	0,359	0,359
SP	0,037***	-0,003***	-	-	0,017***	-	0,001NS	6	-405,33	0,06	0,358	0,358
SP	0,037**	-0,003***	-	-0,001NS	-	0,001***	0,001NS	7	-405,644	0,06	0,377	0,377
CG	0,089***	-0,004***	-	-	-	-	-	4	-377,676	0,21	0,499	0,499
CG	0,111***	-0,004***	-0,001NS	-	-	-	-	5	-376,747	0,11	0,505	0,505
CG	0,084***	-0,004***	-	-	-	0,001NS	-	5	-376,464	0,1	0,504	0,504
CG	0,095***	-0,004***	-	-	-	-	-0,001NS	5	-376,335	0,09	0,503	0,503
CG	0,085***	-0,004***	-	-	0,003NS	-	-	5	-376,085	0,08	0,501	0,501
CG	0,088***	-0,004***	-	0,0001NS	-	-	-	5	-375,687	0,07	0,499	0,499

*NS = non-significant; + = p < 0.1; * = P<0.05; ** = P<0.01; *** = P<0.001; "–" implies that the specific variable was not included in the model.*
Treatment (SP = Source population; CG = Common Garden), Maximum Temperature (Tmax), Minimum Temperature (Tmin), Mean Annual Precipitation (MAP), Degrees of Freedom (df), Akaike Information Criterion (AIC), conditional R-squared (R^2_c) and marginal R-squared (R^2_m)

Discussion

In this study I explored trait variation in leaf morphology and wood anatomy between *P. repens* populations along a spatial climatic gradient. I also explored evidence for genetic constraint and differences in phenotypic plasticity among populations by comparing wild populations with a common garden experiment. The effects of water availability on plant hydraulic traits have been well established (e.g. Carlquist, 1966; February *et al.,* 1994, 1995 & 1999; Carlson *et al.,* 2012), while little attention has been given to the effect of differences in temperature on xylem vessel anatomy in the southern Hemisphere. Several studies have also demonstrated widely diverse levels of heritability in species' phenotypic trait responses to altered conditions (Carlson *et al.,* 2010; Nicotra *et al.,* 2010).

The common garden in Kirstenbosch experienced the most favourable climate relative to the 13 field sites, as minimum temperatures were higher, maximum temperatures lower and precipitation was higher. Significant increases in xylem vessel diameter and SLA in plants grown in the common garden indicates that there is some degree of phenotypic plasticity within each population (Table 2.3). In addition, there were significant differences in xylem vessel diameters between populations in the common garden, indicating that genotypic differences among populations constrain the expression of hydraulic traits in *P. repens*. No differences in SLA between populations in the common garden (ANOVA, Table 2.2) suggest that *P. repens* have strong phenotypic plasticity in leaf traits. These results suggest that variation in both leaf and wood anatomical traits in *P. repens* are determined by a combination of genetic constraint and plasticity in response to environmental conditions consistent with scenario 3 in Fig. 2.1. This is in agreement with Carlson *et al.* (2010) who show that Proteaceae species show changes in leaf traits with environmental conditions within a heritable range.

Larger xylem vessel diameters and a concurrent decrease in vessel density found in the common garden, relative to those of their source populations, confirms that higher precipitation and change in maximum temperature experienced at the common garden significantly influences the development of xylem vessel diameter. Several studies have however shown that nutrient limitation decreases plant water use efficiency (Raven *et al.,* 2004; Cernusak *et al.,* 2007; Matimati *et al.,* 2013) and fynbos soils are known to nutrient poor. The soils of the common garden at Kirstenbosch are more nutrient rich than typical fynbos soils, this increase in nutrients may therefore have played some part in contributing to my results.

My models suggest that both D_H and mean vessel diameter increase with higher maximum temperatures and greater numbers of soil moisture days in winter. This reflects both their expected dependence on water availability and possibly the role that temperatures play in regulating growth rates (February *et al.,* 2007; Way & Oren, 2010) and ultimately conduit diameter (Tyree & Zimmermann, 2002). Plant growth in *Protea* species have previously been found to be constrained by the minimum temperature of the coldest month (Carlson *et al.,* 2010). It is also possible that rare episodes of frost may cause freezing-induced embolism within the lifetime of a plant, creating a strong selective force limiting xylem vessel sizes (Davis *et al.,* 1999; Pittermann & Sperry, 2003). The rugged topography of the mountains in the CFR mean that they can frequently suffer localized sub-zero temperatures and frost events, but regional gridded climate models such as Schulze (2007) and Wilson and Silander (2014) do not reflect this, because these models average over this variability (Slingsby *et al.*, unpublished data). The negative correlation between both D_H and mean vessel diameter and mean annual precipitation in the field may reflect an interaction between mean annual precipitation and the measures of soil moisture days within the model. These covariates were correlated with an R^2 of 0.0005 and 0.776 for summer and winter soil moisture days respectively. Alternatively, that soil moisture days significantly influences xylem vessel diameters, D_H and SLA (Table 2.4) suggests that the reliability of rainfall may be more important than amount of rainfall. Sites with higher mean annual precipitation may perhaps have more irregular rainfall patterns and therefore not influence hydraulic traits as much.

My results show that the SLA of *P. repens* populations in the field decrease significantly as soil moisture days in winter increase, while soil moisture days in summer had a weakly significant positive affect. The inconsistent results for D_{HL} are possibly a result of a highly plastic response in leaf size to environmental conditions. Since leaf traits (e.g. SLA) are highly labile, some populations will show decreases in D_{HL} while others will increase.

In all, *P. repens'* shows distinct differences in hydraulic traits along environmental gradients and populations are able to occupy different geographical areas with contrasting available water and temperature regimes. This is likely due to local adaptation and high intra-specific genetic variation between populations. While the distribution of *P. repens* is limited by areas of climate extremes (Merow *et al.,* 2014), we found evidence for limited phenotypic plasticity, suggesting the species has some ability to rapidly respond to climate change in the short term. Despite this plasticity, each population is highly adapted to their local climatic conditions, and it appears that there is little gene flow between them. This suggests that as

climate changes each population will reach its own physiological limits and be threatened with local extinction far sooner than one would expect on the basis of the climatic tolerance of the species as a whole. The majority of species distribution models are based on the assumption that the current range is indicative of the species' climatic tolerance, and no distinction is made among populations. My results demonstrate that *P. repens* is likely far more sensitive to climate change than these models would suggest. In Chapter 3 I explore this further by determining how xylem vessel morphology in *P. repens* populations have responded to climate change over the last 30 years.

Chapter 3: The effects of anthropogenic climate change on the xylem anatomy of *Protea repens.*

Introduction

Most studies investigating species responses to climate change predominantly focus on shifts in species ranges (Parmesan & Yohe, 2003) or phenology (Walther *et al.,* 2002; Root *et al.,* 2003). These approaches are data and effort intensive, requiring either spatial or temporal sampling, or both, over a long time. A third approach involves examining plant trait responses to climate change, which can be done by examining changes in the range, diversity or average values of traits in multi-species communities (Mouillot *et al*., 2013), or by examining temporal changes in traits that are known to be sensitive to spatial climatic gradients within focal species (Sasaki & Lauenroth, 2011). Of the trait-based approaches, examining temporal changes in traits within a focal species can be rapid and cost-effective, evading many community data collection issues such as species detection probability, taxonomic changes, identification error, and trade-offs in trait sampling whereby high species numbers limit the numbers of samples that can be processed per species. In addition, examining traits for a single species eliminates the complexities of controlling for diverse community assembly processes. Here I examine temporal changes in the xylem anatomy of *Protea repens* (L.), a common shrub in the Cape Floristic Region (CFR), over a 21 year period, 1994 to 2015.

Environmental conditions affecting plant water stress play a pivotal role in the development of wood anatomy, enabling xylem vessels to feature as proxies for determining climatic variations or indicating species' response to altered climate (February & Manders, 1999a, Jacobsen *et al.,* 2007). Xylem anatomy has long been used as a proxy for reconstructing changes in climate in palaeorecords (February, 1994; Verheyden *et al.,* 2005; Fonti *et al*., 2010), and a comparison of wood anatomy from multiple species across the globe found that xylem vessels predict previous climatic conditions fairly accurately (Wiemann *et al.,* 1998). February (1994) demonstrated that vessel diameters in two Proteaceae species, *Protea caffra* and *Protea roupelliae* were positively correlated with rainfall along a gradient from 760 mm at Musina to 1665 mm at Umtamvuna, while vessel density declined along the same gradient. These relationships of how xylem anatomical features change along a climatic gradient in space were used to reconstruct changes in historical climate through time.

The strong relationships that have been established between xylem anatomical features, such as vessel diameter, and water availability exist because xylem anatomy is optimized to suit the conditions in which the plant grows (Carlquist, 1966; Xinying *et al.,* 1988; February *et al.,* 1995; Villar-Salvador *et al.,* 1997). Plants are able to enhance their conductivity by investing in larger xylem vessel diameters or increasing the number of vessels (i.e. density) (February & Manders, 1999a, Tyree & Zimmermann, 2002). This relationship is best explained by the Hagen-Poiseuille equation for the flow of liquid through a conduit that shows that the efficiency of transport increases with the fourth power of the diameter of the conduit (d^4; Tyree & Zimmermann 2002). This means that a small increase in vessel diameter will result in a disproportionally greater increase in conducting efficiency (Hacke & Sperry*,* 2001; Tyree & Zimmermann, 2002; Pratt *et al.,* 2012). Stems with a few large diameter vessels have high hydraulic conductivity and do well in wet conditions, but wider conduits have a greater risk of cavitation when under drought stress (Hacke & Sperry*,* 2001; Tyree & Zimmermann, 2002). By contrast, stems of equivalent size with many vessels with smaller diameters cannot take advantage of wet conditions, but are less vulnerable to cavitation under drought stress (February, 1994; 1995; 2000; Hacke & Sperry*,* 2001; Jacobsen *et al.,* 2007; Pratt *et al.,* 2012). Kavanagh *et al.* (1999) showed that tracheid diameters between populations of the Douglas fir (*Pseudotsuga menziesii*) were larger in more mesic environments along the coast (± 422 µm) than the drier interior (± 172 µm). Several plant species have displayed similar changes in xylem anatomy with temporal change in climate over the last 300 years (Fonti *et al.,* 2010). In the CFR, February and Manders (1999a) experimentally showed that vessel diameters of *P. repens* and *P. neriifolia* increased from 42.3 µm to 63.8 µm and from 36.1 µm to 67.7 µm respectively, along a gradient of increasing water availability. They also showed that xylem vessel density for *P. repens* decreased from 117.8 mm^{-2} to 60.7 mm^{-2}, while *P. neriifolia* decreased from and 99.2 mm^{-2} to 44.9 mm^{-2}.

Precipitation in the CFR has remained relatively stable over the last 30 years (Hoffman *et al.,* 2011), while temperatures and atmospheric CO_2 have increased significantly during this period (New *et al.,* 2006; Lumsden *et al.,* 2009; Hoffman *et al.,* 2011). Minimum temperatures at selected sites have increased by 0.58ºC on average, while maximum temperatures have increased by 0.98ºC between 1974 and 2005 (Hoffman *et al.,* 2011). Atmospheric CO_2 concentrations measured at sea level have increased by more than 82 ppm since the onset of CO_2 recordings in 1959 at Mauno Loa, including more than 40 ppm between 1994 and 2015 (NOAA, 2015). The relatively stable rainfall suggests that any changes in xylem anatomy

should primarily be due to changes in evaporative demand due to changes in temperature or wind, or changes in plant physiology due to elevated atmospheric CO_2.

While there is general agreement on the effects of changes in water availability on plants, research on the effects of elevated CO_2 have yielded contradictory results. Bond and Midgley (2012) argued that woody biomass in southern Africa is increasing due to the alleviating effect that increased CO_2 has on drought stress for most C_3 photosynthetic plants. Under elevated CO_2 concentrations, less stomatal conductance and transpiration are needed to take up the CO_2 required by the plant (Franks & Beerling, 2009; Warren *et al*., 2011), leading to increases in water use efficiency (Warren *et al.,* 2011; Bond & Midgley, 2012). Kgope *et al.* (2010) found that the water use efficiency (WUE) of two *Acacia* tree species (*A. karroo* and *A. nilotica*) increased up to a saturation point under increased CO_2 concentrations. They also found that total stem diameters increased with higher CO_2 concentrations as the plants were able to assimilate more carbon. If increased atmospheric CO_2 concentrations are relieving hydraulic stress in plants that employ the C_3 photosynthetic pathway (Midgley *et al.,* 2005), this should lead to plants developing fewer, larger vessel diameters per unit stem area.

Experimental studies exploring the effect of CO_2 on xylem anatomy have produced contradictory results (Pritchard *et al.,* 1999; Telewski *et al.,* 1999). Many of these studies found either no change or an increase in vessel diameters with increased CO_2, while few show decreases. Reviews by Telewski *et al.* (1999) and Pritchard *et al.* (1999) found that the few studies that examined changes in xylem anatomy with elevated CO_2 concentrations found little significant change in vessel attributes. However, Atkinson and Taylor (1996) found that vessel diameters in seedlings of the pedunculate oak, *Quercus robur*, increased from 16 µm at ambient CO_2 concentrations (350 ppm) to 19.9 µm at extremely elevated (700 ppm) CO_2. Similarly, Kostiainen *et al.* (2014) and Medeiros and Ward (2013) found significant increases in mean vessel diameters and water use efficiency while vessel density decreased. Notwithstanding, most studies suggest that resource availability (nutrients and water) does influence a plants ability to respond to increases in CO_2 (Nowak *et al.* 2004). Unfortunately, there are no studies of the effects of elevated CO_2 on the xylem anatomy of plants from the CFR.

Global mean temperature increases up to 1.4°C are expected over the next 50 years (IPCC, 2014) which, with the continued rise in atmospheric CO_2 concentrations (NOAA, 2015), emphasize the need to determine plant responses and resilience to changing temperature and CO_2. The shrub *Protea repens* (L.) provides a good opportunity to explore these effects in

the CFR, because it is widely distributed throughout the region and a dataset representing historical xylem anatomy for the species was collected from multiple sites in 1994. Here I sample the same populations to compare contemporary *P. repens* vessel morphology with the historical dataset, representing 21 years of change in climate and CO_2 (1994 to 2015). I compare these changes with rainfall and temperature records for the area between 1990 and 2015. I hypothesise that rainfall has remained constant, as suggested by Hoffman *et al.* (2011), but temperatures have increased over this period. Under these weather conditions VPD and evaporative demand should have increased resulting in smaller vessel diameters (Tyree & Zimmermann, 2002).

Methods

Study site

In September 2015 I sampled *Protea repens* individuals from three populations, Anysberg (-33.49369 S; 20.70409 E), Attakwasberg (-33.8584 S; 22.03807 E) and Besemfontein (-33.3635 S; 21.45388 E), that had been sampled by E.C. February in 1994. Besemfontein in the North has the lowest rainfall (277.58 mm) of the three sites at an elevation of 1145 m. Further South, Attakwasberg experience the highest rainfall (783.64 mm) at the lowest elevation (680 m). Unfortunately, while E.C. February had sampled additional sites, these had all recently burnt and no individuals of a suitable size were available.

Data collection

I sampled 10 stem sections from each population, varying in diameter between 15 and 30 mm to exactly replicate the historic collection. These samples were processed in precisely the same manner as for chapter 2 according to a modification on the methods used by February *et al.* (1995) and February and Manders (1999a). Stem sections between 20 to 30 µm thick were cut using a base sledge microtome (Reichartd-Jung, Austria) stained with a mixture of alcohol, glycerol and safranin red and mounted in Kaiser's gelatin-glycerin on glass microscope slides. Glass microscope slides of the thin sections from the 1994 collection had been prepared using the same methods, but to preserve the slides the cover slips were sealed with nail varnish around the edge and stored at the Iziko South African Museum , Cape Town. Eight non-overlapping digital photographs of equivalent cross-sectional area were taken from the outermost 2 mm section of wood (most recent growth) for all historic and contemporary stem

sections. Photos were taken at a magnification of 40x using a Leitz (Wetzlar, Germany) Laborlux K incident light microscope with a mounted Canon EOS 600D camera (Canon, Japan) and vessel sizes measured using open-source image analysis software (ImageJ; http://imagej.nih.gov/ij). The 30 vessels with the largest radial diameters encountered in the eight photographs for each stem section were identified for subsequent analyses, because larger vessels contribute disproportionally to sapflow, as dictated by the Hagen-Poiseuille equation (Tyree & Zimmermann, 2002). Vessel density was determined by counting the number of vessels for each photograph area (for which magnification and size was fixed) and expressed per unit area (vessels/mm^2).

Calculations and Statistical Analysis

Since the trade-off between vessel size and frequency is nonlinear and plants are not limited to any particular combination (large vessels or many vessels; Tyree & Zimmermann, 2002), average vessel diameter for *P. repens* populations is unreliable as an estimate of hydraulic conductance. I therefore calculate Hydraulic Diameter (D_H), based on the Hagen-Poiseuille equation (Tyree & Zimmermann, 2002), as an estimate of conductance for each individual. D_H is calculated as:

$$D_H = [(\Sigma d^4)/n]^{0.25} \qquad \text{(eqn. 1).}$$

and is the sum of the tangential vessel diameters (d) to the power of four, divided by the number of measured vessels (n) to the fourth root. D_H should reflect variation in xylem vessel diameter between and within *P. repens* populations (see Chapter 2 for further explanation).

Linear mixed effects models were used to test for differences in hydraulic diameter, mean xylem vessel diameter and vessel density between 1994 and 2015 (Zuur *et al.,* 2009). The models included year as a fixed effect, while population was included as a random effect to account for differences between individual sites. Models were run twice, including and excluding stem radius as an additional fixed effect, to account for any effects of potential biases in the size of the stems sampled, as the size of stems was found to be important in Chapter 2. The assumption of homogeneity of variance across sites was verified by testing whether model residuals were normally distributed. All analyses were done in R 3.2.2 (The R Foundation for Statistical Computing 2015) using the package nmle (Pinheiro *et al.,* 2015).

Climate and CO_2 trend analyses

I obtained rainfall records for rainguages at the sampling sites from the Western Cape Provincial conservation agency, Cape Nature and monthly meteorological observations for weather stations from three towns in the region from the South African Weather Services (SAWS, 2015). From these weather data I determine any temporal trends in annual precipitation and mean annual minimum and maximum temperatures from 1990 to 2014. Unfortunately, no wind data were available. Linear regression models were fit to the data. While temporal autocorrelation violates the assumption of independence of observations required by these models, they do give a quick assessment of trends in the data and have been used elsewhere (e.g. Hoffman *et al.,* 2011). Interpolated temperature data for each sampled population obtained from Wilson and Silander (2013) is reported in Table 3.1, because there are no temperature records for the sites. The primary observations demonstrating the increase in CO_2 are from the Mauna Lao observatory in Hawaii, because this is the longest record and these values are similar throughout the world (February & Stock 1999). A linear regression show the change in CO_2 over time.

Table 3.1 Sampled Protea repens populations across the CFR with acronyms for each population, as represented in the subsequent graphs, GPS coordinates, maximum (Tmax) and minimum temperature (Tmin), mean annual precipitation (MAP) and height above sea-level. Temperatures from Wilson and Silander (2013), MAP from SAWS (2015).

Source Population	Acronym	Latitude	Longitude	Tmax	Tmin	MAP	Elevation
Anysberg	ANY	-33.49369	20.70409	20.31	8.25	287.36	1152m
Attakwasberge	ATK	-33.8584	22.03807	20.31	7.90	783.64	680m
Besemfontein	BES	-33.3635	21.45388	17.43	8.00	277.58	1145m

Results

Climate and atmospheric CO_2 trends since 1990

My analyses show no significant trends in mean annual maximum temperature for Ladismith or Oudtshoorn between 1990 and 2014. Riversdale however show significant increases for this period (Fig. 3.1, $p < 0.01$). Rainfall from the regional weather stations show no significant change since 1990, confirming the results from previous research (Hoffman *et al.,* 2011). Similarly, observations of mean annual rainfall from each of the study sites indicate no

significant change since 1990 (Fig. 3.2). CO_2 concentrations as recorded at the Mauna Loa Observatory in Hawaii (NOAA, 2015) have increased by more than 40 ppm in the period 1990 to 2015 (Fig. 3.3).

Xylem characteristics

Xylem vessel diameters and hydraulic diameter (D_H), showed no significant change between 1994 and 2015 for any of the three sites (Fig. 3.4 and Table 3.2; p = 0.338). Vessel density did not change during this period either (Fig. 3.4 and Table 3.2; p = 0.658). Stem radius had no significant impact on xylem anatomy, its inclusion did not alter the significance of the estimates of the effect of year, and AIC scores showed a marginal preference for the models for which it was not included, suggesting that any slight differences in stem radius have not biased our results (Table 3.2).

Table 3.2 Linear mixed effects models weighted by AIC for Hydraulic diameter (D_H), mean vessel diameter and vessel density with and without stem radius.

Trait	Intercept	Stem Radius	Year	df	AIC	R^2m	R^2c
D_H	44,082***	0,051NS	-1,157NS	55	371,197	0,012	0,266
D_H	44,566***	-	-1,182NS	56	369,217	0,012	0,269
Mean Vessel Diameter	43,638***	0,054NS	-1,286NS	55	369,957	0,016	0,256
Mean Vessel Diameter	44,148***	-	-1,312NS	56	367,981	0,015	0,259
Vessel Density	382,948***	-4,969NS	5,996NS	55	702,697	0,011	0,554
Vessel Density	336,145***	-	8,401NS	56	701,480	0,001	0,568

*NS = non-significant; + = p < 0.1; * = P<0.05; ** = P<0.01; *** = P<0.001; "–" implies that the specific variable was not included in the model.*
Year (1994 vs 2015), Degrees of Freedom (df), Akaike Information Criterion (AIC), conditional R-squared (R^2_c) and marginal R-squared (R^2_m) are reported.

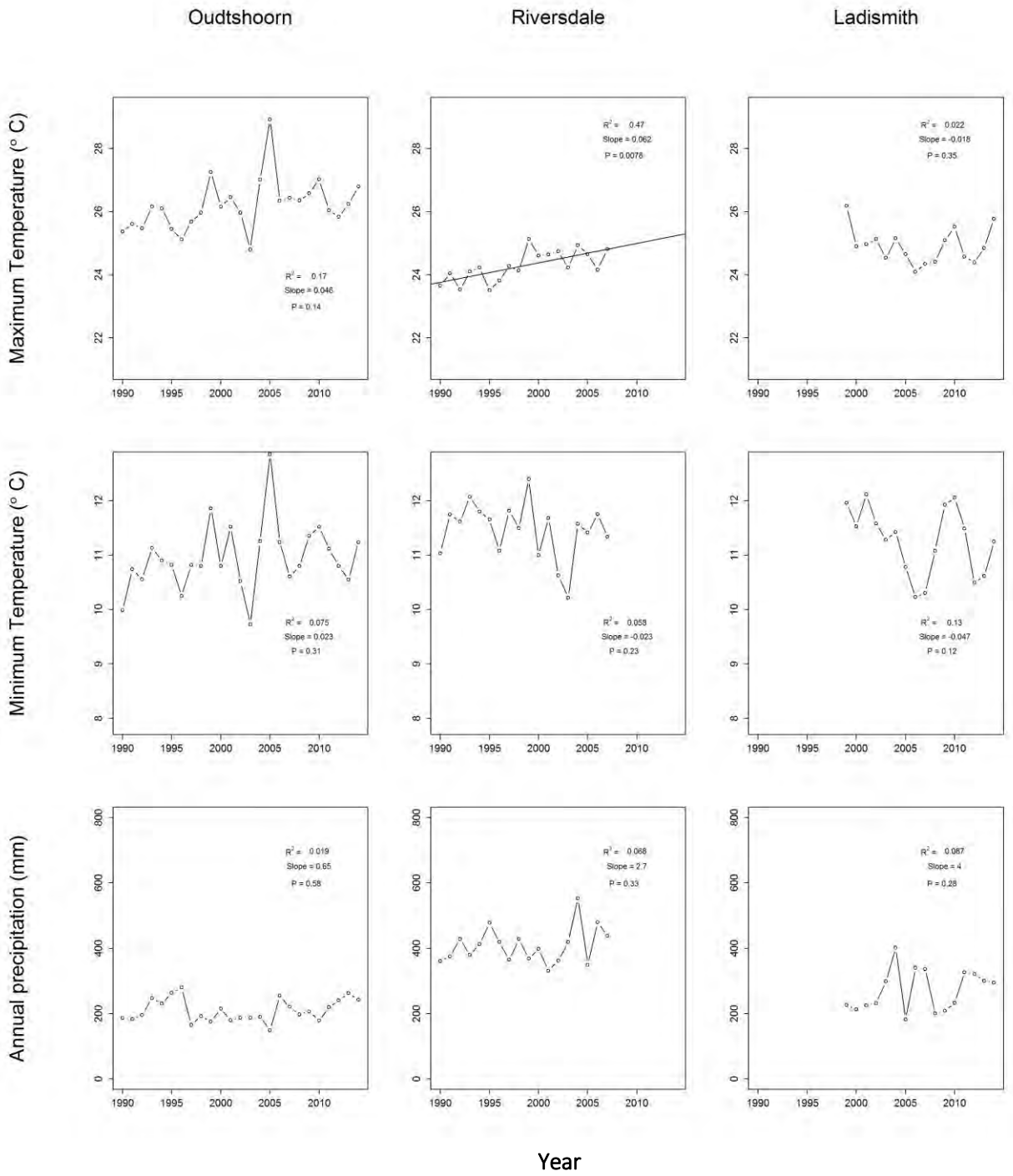

Figure 3.1 Observed change in mean annual maximum temperature (ºC), mean annual minimum temperature (ºC) and precipitation (mm) for the region. Data from the South African Weather Services (SAWS, 2015). Trend lines indicate significant change over time.

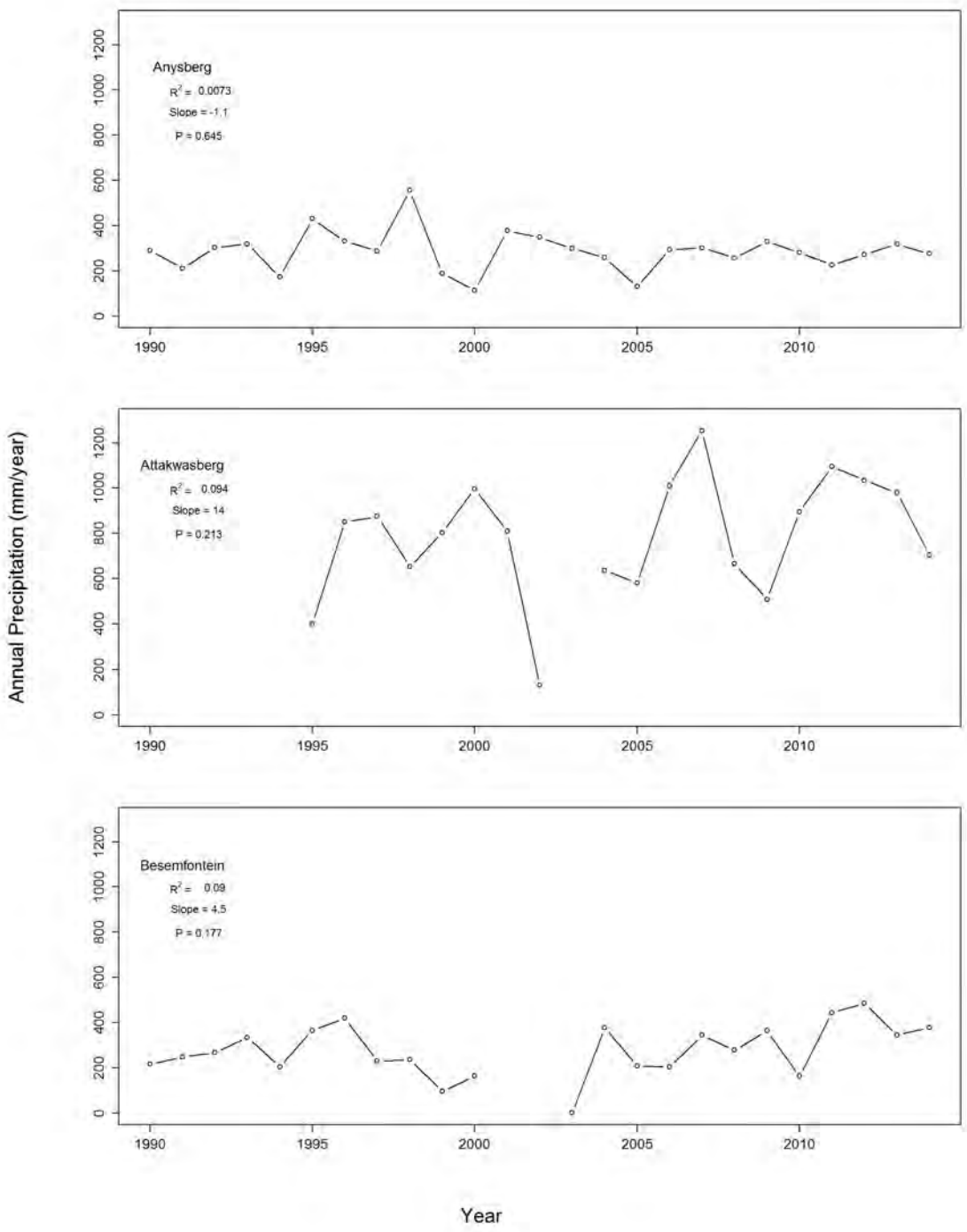

Figure 3.2 Observed annual precipitation provided by Cape Nature for each focal site. All three sites show no significant change in precipitation ($p > 0.1$) since 1990.

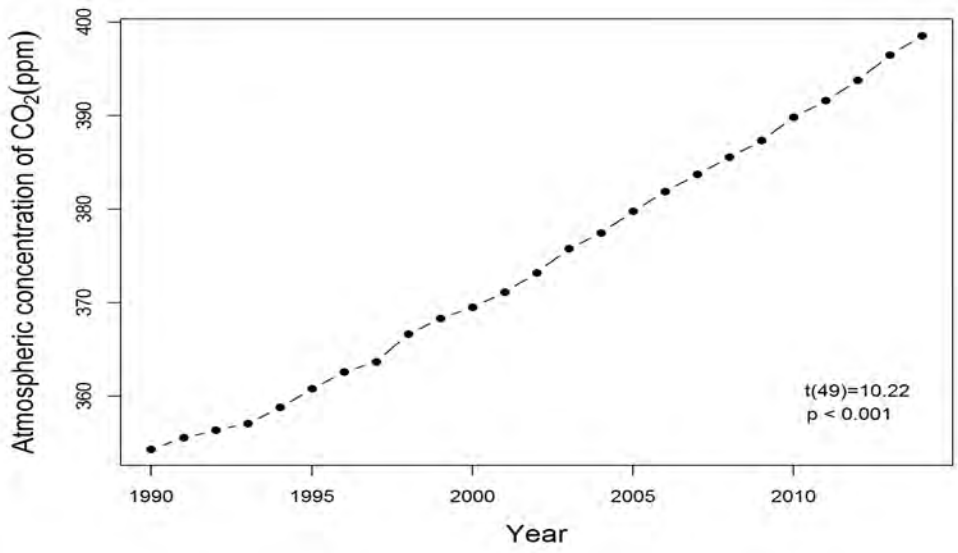

Figure 3.3 Linear regression showing the temporal change in Atmospheric CO_2 concentrations from 1990 until 2014, measured at the sea surface (NOAA, 2014).

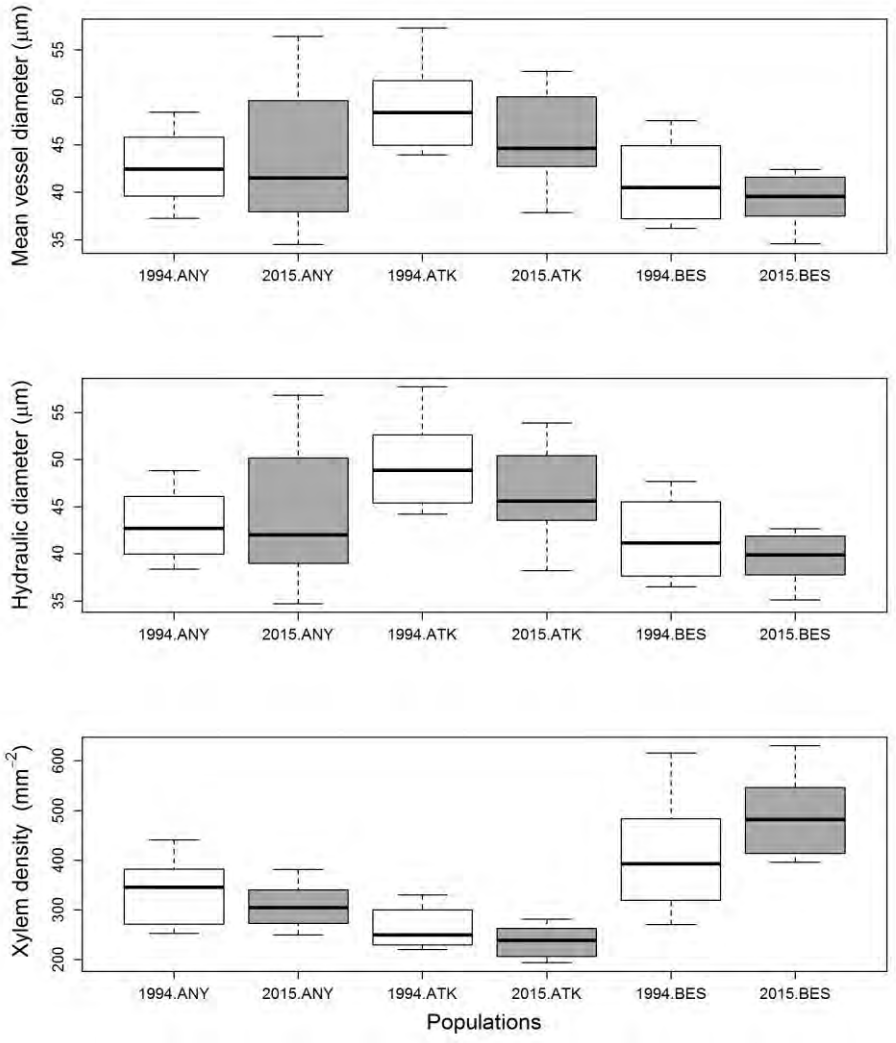

Figure 3.4 Differences in Hydraulic Diameter (D_H), mean vessel size and vessel density (mm^{-2}) between 1994 and 2014 for Anysberg (ANY), Attakwasberg (ATK) and Besemfontein (BES).

Discussion

I compared contemporary *P. repens* vessel morphology with a historical dataset taken from the same source populations 21 years before to determine whether there have been trait-level responses to increased temperature and CO_2. My results show that hydraulic diameter and densities in *P. repens* have not changed significantly over the last 21 years. This would suggest that *P. repens* individuals at the studied populations are either not experiencing noticeably higher plant water stress than individuals sampled 21 years ago, or they are not responding in the manner that we would expect. It is possible that the change in temperature over this period has not been sufficient to cause significant increases in plant water stress, or that elevated atmospheric CO_2 is compensating for any increases in temperature.

We observed little significant change in the weather datasets available for our region of interest over the period of study, with the only significant trend being an increase in mean annual maximum temperature in Riversdale since 1990. Unfortunately, weather station data for our study sites and region of interest were sparse especially for temperature. While the sparse weather station network and complex topography of the CFR make it difficult to generalize, recent regional analyses suggest the CFR in general has experienced significant increases in temperature, but little change in rainfall and some decline in wind run over the past few decades (Hoffman *et al*., 2011, MacKellar *et al*., 2014). Increased temperatures should have increased evaporative demand and, with little change in rainfall, should have increased plant water stress resulting in higher densities of smaller vessels (Tyree & Zimmermann, 2002). Conversely, reduced wind run would have reduced evaporative demand, potentially compensating for any increase in plant water stress caused by higher temperatures. Furthermore, increased CO_2 concentrations, are believed to have an alleviating effect on water stress in most C_3 photosynthetic plants (Midgley *et al.,* 2005; Bond & Midgley, 2011; Warren *et al.,* 2011), suggesting that xylem vessel size should increase if climate effects on plant water stress are unchanged (Maherali *et al.,* 2011). Unfortunately, little is known about the effects of elevated CO_2 on xylem vessel morphology in the CFR, and experiments are needed to test the extent to which this may be countering climatic impacts on plant water stress and vessel morphology of *P. repens* individuals.

Two traits of *P. repens* may also have limited the extent to which xylem vessel morphology has changed over the 21 year period. Firstly, Proteaceae species are known to be deep rooted, allowing the species to access deep water and thereby alleviating water stress during drought (Higgins *et al.,* 1987; Hawkins *et al.,* 2009, West *et al.,* 2012), potentially

buffering them from the need to alter their xylem anatomy. Secondly, *P. repens* is able to regulate stomatal conductance during dry periods (Skelton *et al.,* 2015). These traits allow *P. repens* to be dissociated from annual rainfall and temperature. While these two mechanisms enhance *P. repens'* resistance to drought in the short term, they may only buffer the plants to a point, beyond which the populations could show rapid decline. For example, it is anticipated that climate change in the CFR will lead increases in maximum temperature with high evaporative demand (Midgley *et al.,* 2005), likely causing a long term draw down of deep water resources. There may reach a point when *P. repens* individuals can no longer reach these deep layers, likely after fire when seedlings are still shallow-rooted, resulting in mass mortality. Similarly, reducing stomatal conductance may protect the plant from xylem dysfunction, but it also reduces rates of photosynthesis, and plants that shut down during prolonged periods of drought are susceptible to dying of carbon starvation (McDowell *et al.,* 2008; West *et al.,* 2012; Skelton *et al*. 2015). These aspects of the species' biology may thus prevent us from detecting trends in its response to climate change by examining xylem vessel morphology alone.

I found no significant changes in the xylem vessel morphology of *P. repens* over the 21 year period from 1994 to 2015. This may be for several reasons. Firstly, change in climate over this period may not have been sufficient to cause significant increases in plant water stress. Secondly, elevated atmospheric CO_2 may have compensated for any climate change induced increases in water stress. Lastly, *P. repens* response to increased water stress may be buffered in the short to medium term by its ability to access deep water and reduce stomatal conductance during periods of drought. Unravelling these alternative causes requires improved weather data, performing similar studies on species that maintain high stomatal conductance through periods of drought, and experiments investigating the effects of elevated CO_2 on the xylem vessel morphology of this and other species in the CFR. It also highlights the need for observations on the depth of the soil water table, population demography surveys and measures of photosynthesis and transpiration relative to these.

Chapter 4: Synthesis

Global changes in climate and atmospheric CO_2 concentrations are affecting all levels of biodiversity in a number of ways. To inform our expectations for future changes in fynbos composition and resultant ecosystem function, the extent to which hydraulic stress, expressed through leaf and wood anatomy, is affected by changing climate and atmospheric CO_2 were investigated in a woody shrub, *Protea repens,* that is widespread throughout the Cape Floristic Region (CFR). To this end, xylem vessel morphology and specific leaf area of 13 *Protea repens* populations were correlated with rainfall and temperature along a gradient between Cape Town and Port Elizabeth. I then use these results in comparison with that of a common garden experiment to determine if the observed changes in traits are plastic, or constrained by genotype. I finally conclude with a comparison of xylem anatomy between a contemporary sample and a sample collected in 1994 to determine the effects of climate change on vessel morphology.

I explored trait – climate relationships and tested for genetic constraint and phenotypic plasticity in *P.* repens by measuring the traits of individuals from multiple populations sampled across a climatic gradient, and by comparing these with individuals in a common garden experiment grown from seed collected from wild populations. Mean xylem vessel diameter, D_H and SLA of *P. repens* increased with greater numbers of soil moisture days in winter and in some cases with more soil moisture days in summer. My results show that xylem anatomy is genetically constrained, differing significantly among populations grown in the common garden, but somewhat plastic within populations, as evidenced by differences between the common garden and the source. Specific leaf area (SLA) of *P. repens* differed significantly between the common garden and corresponding source populations, but no significant differences were found among populations within the common garden, suggesting that this trait is highly plastic in response to environmental conditions irrespective of source population. This plasticity likely affords the species some resilience in the face of changing environmental conditions, but genetic constraint on xylem anatomy may limit its ability to respond beyond some threshold of change.

Comparison of contemporary samples with stem sections collected in 1994 showed that *P. repens* xylem vessel morphology has not changed significantly over the last 21 years. This is a rather surprising result given that temperatures have increased across the CFR over the last 30 years (Hoffman *et al.,* 2011). This increase in temperature should have increased evaporative

demand, increasing water stress in plants, and selected for smaller xylem vessels in *P. repens* (Tyree & Zimmermann, 2002). While, my results do show that maximum temperatures for the areas surrounding my sampled sites increased marginally, this had no significant effect on xylem vessel diameters over time. I hypothesise that the increase in atmospheric CO_2 should counter the increase in temperature by enhancing carbon assimilation efficiency (Midgley *et al.,* 2005; Kgope *et al.,* 2010; Warren *et al.,* 2011; Bond & Midgley, 2012). Several studies have also shown that the interactive effects of elevated CO_2 and higher temperatures on plant water transport suggest that xylem vessels size differences are less affected by CO_2 than temperature (Morison. & Lawlor, 1999; Maherali & Delucia, 2000; Kilpeläinen *et al.,* 2007). There are however no studies on the affect of increases in either temperature or CO_2 on xylem anatomy of species from the CFR. The poor spatial resolution of rainfall and especially temperature records for the region needs to be resolved with studies focussing on multi species comparisons to determine anatomical responses to environmental change.

The Proteaceae may be more resilient to drought stress through both stomatal control as well as access to deep moisture (Higgins *et al.,* 1987; Hawkins *et al.,* 2009; West *et al*., 2012). Notwithstanding, this safeguard to hydraulic stress likely comes at the cost of greater vulnerability to stress during extreme heat events, because there is less transpirative cooling, and/or carbon starvation when prolonged droughts occur, as the demand for carbohydrates outweighs their production due to stomatal closure and reduced carbon uptake via photosynthesis (McDowell *et al*., 2008).

My results suggest differences in the climatic thresholds at which the different *P. repens* populations begin to suffer extreme plant water stress. If these thresholds differ between populations, some populations would shut their stomata earlier than others when faced with stressful conditions, making these populations more vulnerable to carbon starvation or heat stress. *P. repens* populations with lower water stress thresholds should therefore be more vulnerable to carbon starvation than those with higher thresholds, typically adapted to more arid environments. This skewed response would result in certain populations succumbing to climate change sooner than others if there is no gene flow between populations as suggested by my study. Alternatively, and perhaps more concerning, is that given the inter-population differences and the high degree of specialization to localized conditions, relatively uniform changes in climate across the region may drive all populations beyond their stress thresholds at a similar rate, causing rapid decline in the species. Taken together, genetic structuring, low gene flow and differences in stress tolerance among populations make modelling and

predicting the responses of this species to anthropogenic change exceedingly difficult. If responses to change differ among populations then traditional species distribution modelling approaches that assume a common response across a species' range are not appropriate. More nuanced models that allow species response to vary, or treat populations as separate entities are required.

I conclude that the hydraulic consequences of a changing climate and increasing global CO_2 concentrations on plants in the CFR are yet to be fully established and does require further investigation. Growth chamber experiments should investigate the effects of increased CO_2 on xylem anatomy in combination with different soil moisture and nutrient treatments would provide insight into the degree to which plants can respond to elevated CO_2. These experiments should also include measures of transpiration, photosynthesis and xylem pressure potentials to investigate the interaction between xylem anatomy, stomatal conductance and the vulnerability of the plants to hydraulic failure or carbon starvation. What my study does however suggest is that conservation efforts should be directed toward maintaining the integrity of the different *P. repens* populations as these may be more vulnerable to climate change than what was at first expected.

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