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The phenotypic response of co-occurring *Banksia* species to warming and drying

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Abstract Projected warmer and drier climates are expected to impact heavily on plant diversity in Mediterranean-climate ecosystems, but experimental investigations of sensitivity and adaptive capacity are needed to better understand species responses. Here, we examine the effects of warming and drying on growth and allocation in seedlings from populations of two co-occurring *Banksia* shrub species from south-west Western Australia. We hypothesised that the species would show ecological divergence in functional traits reflecting niche differentiation. We expected to see tolerance to warming and drought correlated with position of the population on a climate gradient. We predicted that populations at the warmer/drier end of the gradient would show greater homeostasis of growth and allocation patterns in response to experimental treatment. Seedlings of the two species differed in leaf and allocation traits and in responses to experimental warming and drying. *B. coccinea* had smaller leaves with higher specific leaf area, and accumulated less overall biomass compared to *B. baxteri* when grown under cooler conditions. Under warmer conditions, *B. coccinea* could maintain growth, whereas *B. baxteri* suffered significant decline in biomass accumulation. Under water deficit conditions, both species showed significant reductions in leaf mass and area. Under combined warmer/drier conditions *B. baxteri* forfeited height growth and biomass and increased leaf allocation. The results support our hypothesis that seedlings of *B. baxteri* and *B. coccinea* are divergent in key functional traits and their sensitivity to warming and drying. However, we found no evidence for inter-population variation in traits being associated with position on a climate gradient.

Keywords Phenotypic plasticity · Global warming · *Banksia* · Drought

Introduction

How individual species respond to environmental stress is determined by the way in which species function in a particular environment, and how they

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interact with each other within their community. Consequently, measuring functional traits can indicate the environmental conditions (i.e. the niche parameters) required by a species to survive (Violle and Jiang 2009). Niche differences can play an important role in species co-existence (Chesson 2000; Silvertown 2004); however, the extent to which niche differences under current conditions will affect the interactions between species under future conditions is unclear.

Functional traits are a suite of morphological, physiological and phenological traits which impact on performance traits and are readily measurable at the level of an individual (Violle et al. 2007). These traits can be highly variable among populations of a species. Environmental gradients can induce ecological divergence among populations within a given species, and can be accompanied by differences in the interactions between co-occurring species. Among-population trait differences measured in the field may reflect plasticity or genetic differentiation, including both local adaptation and non-adaptive historical processes such as dispersal limitation and random genetic drift. The differences in traits may be correlated with each other or with the environment such that a change in one variable causes a change in another. These plastic responses are a key mechanism for rapid adjustment to environmental heterogeneity (Pigliucci 2001). Consequently, analysis of functional traits is increasingly being used to better understand physiological mechanisms underlying plant performance (e.g. drought tolerance within species), in the context of global climate change (Nicotra et al. 2010).

Mediterranean-climate ecosystems are characterised by warm, dry summers and cool, wet winters, with the water limitation associated with the normal dry season a major constraint to plant growth and survival (Allen 2003). Global forecasts of rising temperatures and increasing aridity are likely to exacerbate these limitations and impact severely on plant diversity in these disturbance-prone ecosystems (Klausmeyer and Shaw 2009). The seedling stage is expected to be more sensitive to changing climatic conditions than adult stages due to existing water limitations on small root systems (Walck et al. 2011). Current adaptive and plastic mechanisms involved in drought resistance are expected to underpin the distribution of populations along natural climate gradients of water availability in Mediterranean-climate regions (Richter et al. 2012). However, it is still unclear how forecasted environmental change will alter physiological, morphological and allocational responses, nor how variation in plant performance will affect species co-existence at both the species and population levels (Jump and Peñuelas 2005). The South Western Australian Floristic Region (SWAFR), a Mediterranean-climate biodiversity hotspot, provides an ideal focal point for investigating the effects of warming and drought as global climate forecasts for the region include a 1–5.5 °C increase in mean annual temperature and a 5–60 % decline in mean rainfall during winter and spring by 2070 (Bates et al. 2012). Although species in the SWAFR are projected to contract their range towards the cooler and wetter south west (Fitzpatrick et al. 2008), much of the region's flora is considered to be already at its physiological limits for survival with altered environmental conditions potentially impacting on recruitment (Walck et al. 2011). Climatic niche modelling has identified the genus *Banksia* as particularly vulnerable (Fitzpatrick et al. 2008; Yates et al. 2010), however, little is known of the physiological tolerances of these species, particularly in the seedling phase of the life cycle.

To better understand how forecast environmental change will affect seedling performance in a Mediterranean-climate, we tested the response of two *Banksia* R.Br. species (Proteaceae) from the SWAFR to drought and heat stress. A changing climate has important implications for species interactions and community dynamics therefore we investigated two closely related *Banksia* species that occur sympatrically across much of their range, generally in tall shrubland in deep white sands on plains and dunes. Closely related species are often divergent in many different kinds of traits and inter-specific variation may mediate species co-existence (Roff 1992). *Banksia coccinea* and *B. baxteri* differ in several morphological and functional traits, however, both are non-sprouting, obligate seeding, woody shrubs that are locally common where they occur but restricted to ca. 200 km linear distributional range along the southern coastline of the region. Seedlings recruit in the autumn–winter wet season, generally post-fire, with the dry summer conditions a main impediment to seedling survival. Previous research has revealed some basic differences between the species: seed mass, adult leaf nitrogen per unit area and above-ground seedling biomass are significantly higher in *B. baxteri* but adult and seedling specific leaf area (SLA), seedling leaf production rate and time to emergence are significantly lower in comparison to *B. coccinea* (Cochrane 2014; Cochrane et al. 2014a). Furthermore, *B. baxteri* has higher germination under conditions of moisture stress and higher than optimum temperatures for germination (Cochrane et al. 2014b). Lastly, seedlings of *B. baxteri* collected from seed at the higher rainfall end of their distribution appear to be more vulnerable to severe water withholding with increased mortality following drought compared with *B. coccinea* of a similar age (Holloway-Phillips per. comm.).

Building upon these findings, we investigated whether seedlings of *B. baxteri* and *B. coccinea* expressed species and population-level differentiation in leaf traits and seedling growth performance when grown under contrasting temperature and soil moisture conditions. Assessing growth, including leaf production and size, under contrasting conditions, can reveal features that ameliorate environmental constraints on plant growth, and studying the trade-offs that plants face in dealing with current habitat constraints provides the groundwork required for predicting future response to environmental change. Firstly, we hypothesised that the two species would show ecological divergence in functional traits reflecting niche differentiation. Secondly, we expected to see population divergence in traits with tolerance to warming and drought correlated with position of the population on a climate gradient. We predicted that the populations at the warmer/drier end of the gradient would show less plasticity in growth and allocation patterns in response to experimental warming and drying. We adopted this stance for a number of reasons. Firstly, plant populations at the rear edge of a species' geographic distribution are considered stable or eroding with evidence of strong local adaptation and therefore less likely to exhibit plastic responses to changing environmental conditions in comparison to expanding leading edge populations (Hampe and Petit 2005). Secondly, greater plasticity is expected to be found in plants experiencing greater environmental heterogeneity (Balaguer et al. 2001). In addition, plasticity in response to a given resource has been hypothesised to be lower in organisms from places where that resource is limited (Funk and Vitousek 2007). Finally, the Climate Variability Hypothesis (CVH) states that plasticity increases from warm to cold range in plants, albeit along a latitudinal gradient (Molina-Montenegro and Naya 2012). As part of our analysis, we assessed whether there were any significant associations between traits under 'current' or 'future' growth conditions and whether these relationships diverged depending on treatment.

Materials and methods

Study species and source of plant material

We selected three populations of two phylogenetically related *Banksia* species (series *Banksia*; subser. Cratistylis) from a longitudinal rainfall gradient in the South West Australian Floristic Region (SWAFR). These populations roughly cover the longitudinal range of the species and take into account the strong spatial and temporal heterogeneity in climate seen across the species' geographic distribution. Along the gradient, autumn–winter temperatures (May–October) increase from east to west only slightly, but precipitation doubles (Cochrane 2014).

Within each population we sampled mature fruits from 10 adult plants in order to obtain a broad sample of the local genetic diversity. Fruits from each adult individual were kept separate, as were populations. Seeds were extracted from fruits within 3 months of collection by (1) burning the fruits with a gas torch until follicles split, (2) soaking them in water for 6–8 h, and (3) drying them at 15 °C and 15 % relative humidity (rh) for 1–2 weeks until seeds were released. Fruits found to be infested with seed eating larvae were discarded. All potentially viable seeds were then stored at 15 °C and 15 % rh until use in this investigation.

Experimental design

We grew seedlings from each of the 10 maternal individuals from each of the three populations (low, medium and high rainfall zones) of each species ($n = 960$ seedlings) in 5 × 5 × 10 cm tube pots filled with a low phosphorous soil mix (2:1 sand:potting soil) in a controlled environment facility (constant 15 °C with a 12/12 photoperiod). Watering was as required for emergence and survival. At 5 months old, seedlings were transplanted into 1.6 l black plastic planter bags (300 mm deep × 70 mm wide), and moved into a glasshouse under non-limiting conditions (20 °C and daily water to saturation). For the following 2 months seedlings were watered as required to maintain steady growth. At 7 months of age, seedlings were given 5 gm of low phosphorous controlled release organic fertiliser (Osmocote®) and seedlings assigned to the experimental phase.

The experimental design included factorial combinations of temperature ('cool' and 'warm' glasshouses) and water availability ('wet' and 'dry'). To simulate 'current' and forecast 'future' seasonal conditions the glasshouse temperatures were scaled up over time commencing in early January 2013. The 'cool' glasshouse was set at 20/12 °C for 2 weeks, then 23/13 °C for a further 2 weeks then 25/15 °C for the remainder of the experiment until early March when harvest occurred. The 'warm' glasshouse was initially set at 25/17 °C for 2 weeks, then changed to 30/20 °C for another 2 weeks then to 35/25 °C until harvest. Half of the 960 seedlings were assigned to the 'cool' glasshouse, the other half to the 'warm' glasshouse. Within each glasshouse there were four blocks of 120 seedlings with half the seedlings assigned to the 'wet' treatment and half to 'dry', randomised within block. The spatial distribution of the seedlings within each block was a row/column design such that each block contained one seedling from each of the 10 maternal families from each of the three populations of the two species. Each seedling was watered by an individual dripper at soil level. Water regimes were controlled by a Water-Pro vapour pressure deficit (VPD) control system (MicroGrow Greenhouse Systems, Temecula, Calif.), and watering events were triggered by pre-set VPD targets (Nicotra et al. 2008). The 'dry' water VPD target was set twice as high (80,000 credits) as the 'wet' water target (at 40,000 credits); seedlings in the 'wet' treatment received water approximately every 2 days for 2 min, whereas the 'dry' seedlings received water for 1 min approximately every 4 days, depending on VPD in the glasshouse. The 'wet' treatment saturated seedlings, thereby provided highly favourable conditions for growth. The 'dry' conditions received approximately 75 % reduction in moisture from full saturation. We are confident that the 'dry' treatment would fall within the 5-60 % decline expected for the region (Bates et al. 2012). Drippers were calibrated at the beginning of the experiment to a standard flow rate and measured again at the end to assess the consistency in water delivery across seedlings within the treatments. The final water delivery rates (ca. 67 ± 28 ml/min) for each dripper were used as a covariate in the analysis.

Measured variables

Nine above-ground plant traits commonly used in ecological investigations were measured following standardised methods (Cornelissen et al. 2003). Seedling height (H_1 from base to growing tip), the number of leaves (LN) and the length of the longest leaf (LL, cm) were measured at the commencement of the experiment (t_1) when seedlings were 7 months old and again at 10 months old when above-ground biomass was harvested (t_2). Prior to harvest, a chlorophyll meter (Konica Minolta Spad-502) was used to provide an index of leaf chlorophyll concentration (CHL), an indicator of photosynthetic potential. Measurements were taken on one leaf from each seedling (mean of three readings). Harvest occurred over a 3 week period, one block at a time. One fresh leaf from each seedling was taken for scanning to allow analysis of leaf area (LA mm^2) (ImageJ, Rasband 1997–2012). Above-ground dry mass was determined by oven drying stems and leaves for 4 days at 60 °C. We did not measure root biomass as reliable estimates of root biomass are difficult to obtain due to the separation of live from dead roots, and the exhumation of cluster roots, a plant adaptation to low nutrient environments that occurs in *Banksia* (Poorter et al. 2012).

Leaf dry mass (LM, gm) was measured on the leaf taken for scanning; specific leaf area (SLA, $mm^2\ mg^{-1}$) was calculated by dividing the one-sided leaf area by the leaf dry mass. We calculated above-ground biomass as the total dry weight of stems and leaves (AB, gm) and calculated the ratio of leaf biomass to total above-ground biomass (LMR_{AB}, gm). We measured stem height at harvest (H_2) and calculated a relative height growth increment ($HGI = (\ln H_2 - \ln H_1)/(t_2 - t_1)$) as an indicator of growth rate.

Statistical analysis

For each population we calculated mean trait values for each of the contrasting treatment conditions to test for tolerance to warming, drought, and concurrent warming and drought. A restricted maximum likelihood (REML analysis) model was used to fit mixed models for all measured traits (leaf number, leaf area, leaf mass, leaf length, specific leaf area, growth height increment, above-ground biomass and leaf biomass ratio above-ground). These variates were examined for normality and transformed as needed to stabilise the variance. Species, populations, water and temperature were fitted as fixed terms (main and interacting effects) with populations nested within species. In addition, we included each individual seedling dripper delivery rate as a covariate in the fixed model The random error terms in the model were determined by the randomisation used in constructing the experimental design and included the position of plants in each block (i.e. replicate) and bench (by row and column) in the glasshouse environment. Maternal lines (mother) were also included in the random model. The full model used was: FIXED = (species/pop)*water*temp + drippers; RANDOM = (rep/row/col) + mother. We excluded data collected from three seedlings due to dripper malfunction leading to aberrant treatment effects (i.e. where drippers provided excessive water or were blocked). Least significant differences (lsd) at $P < 0.05$ were used to determine significant differences between treatments and populations.

We used simple correlations to evaluate trait covariance between the warm/dry and cool/wet growth conditions for each species; differences were considered significant at $P < 0.001$ unless otherwise stated.

To assess the impact of warming and/or drought on seedling performance, we calculated the magnitude of the effect of each treatment (warming, drought and warming plus drought) as the percentage change (i.e. divergence from optimum conditions) in mean trait values relative to mean trait values under 'current' conditions (i.e. cool/wet). Percentage values above zero indicate an increase in trait means, and values below zero represent a reduction in mean trait values. Significance is relative to trait means under current cool/wet conditions (i.e. zero).

All analyses were performed using GenStat 15th edition (Vsn 2011). Log transformed predicted means have been used in all figures.

Results

Impacts of warming and drought on seedling performance

There were strong differences between species for all traits (Table 1, Fig. 1). Seedlings of *B. baxteri* had significantly larger, but fewer, leaves and under well-

Table 1 REML summary of significant effects of species, population, water, temperature and their interactions on leaf number (LN), leaf mass (LM), leaf area (LA), leaf length (LL), specific leaf area (SLA), height growth increment (HGI), above-ground biomass (AB), above-ground leaf mass ratio (LMR_{AB}) and leaf chlorophyll concentration (CHL)

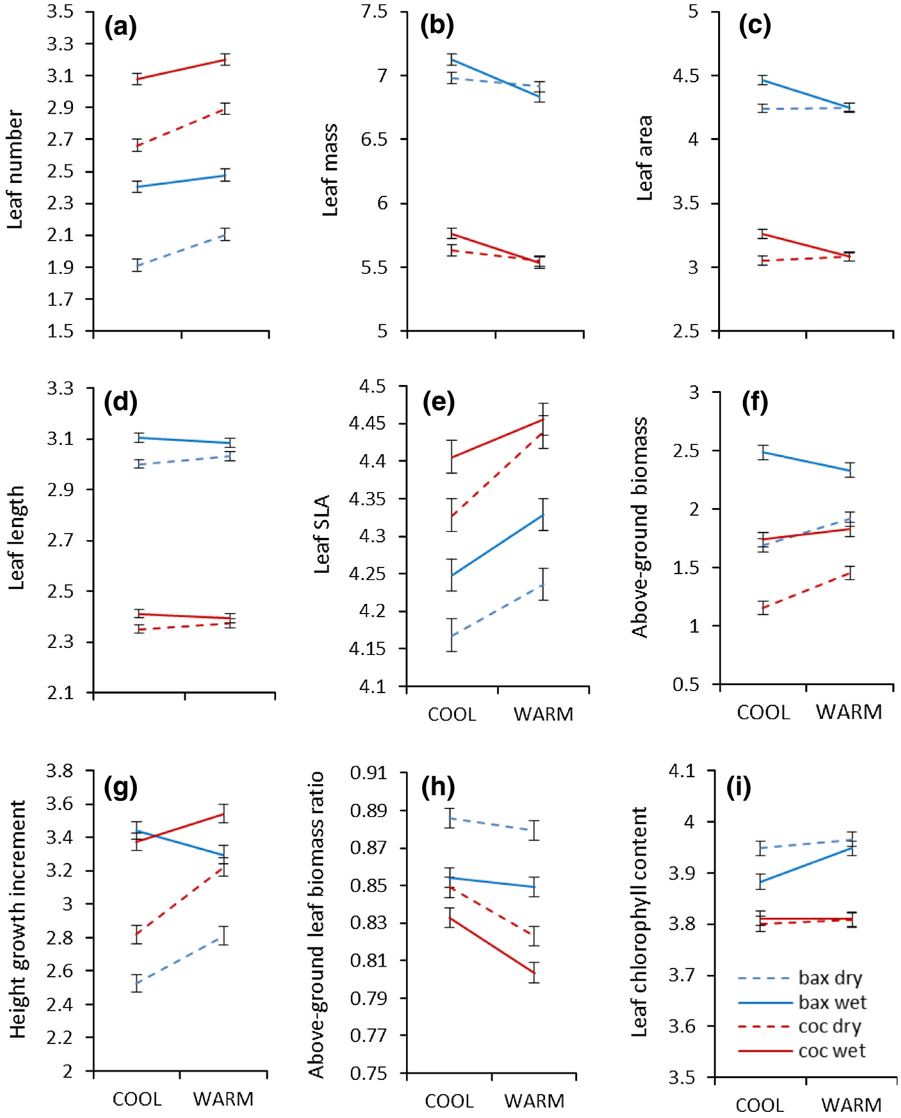
Fixed term	df	LN	LM	LA	LL	SLA	HGI	AB	LMR_{AB}	CHL
Species	1	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Water	1	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.026**
Temp	1	**<0.001**	**<0.001**	**<0.001**	0.950	**<0.001**	**<0.001**	**0.002**	**<0.001**	**0.002**
Species.pop	4	**<0.001**	**0.006**	**<0.001**	**<0.001**	0.095	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Species.water	1	0.103	0.397	0.888	**0.042**	0.072	**<0.001**	0.051	**0.013**	**0.002**
Species.temp	1	0.203	0.519	0.364	0.961	0.860	**<0.001**	**0.008**	**<0.001**	**0.012**
Water.temp	1	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.161	**<0.001**	**<0.001**	0.417	0.202
Species.pop.water	4	**<0.001**	0.591	0.534	0.335	0.347	**0.005**	0.390	**<0.001**	**0.004**
Species.pop.temp	4	0.391	0.298	0.178	0.667	0.220	0.265	0.624	0.055	0.742
Species.water.temp	1	0.889	0.32	0.911	0.708	0.096	**0.048**	0.168	0.572	0.060
Species.pop.water.temp	4	**0.037**	0.221	0.194	0.453	0.627	0.290	0.278	0.532	0.430
Dripper delivery rate	1	**<0.001**	**0.017**	**0.007**	**<0.001**	0.779	**<0.001**	**<0.001**	**0.004**	0.522

Results for model with fixed terms (species/pop)*water*temp + drippers; random terms: (rep/row/col) + mothers

Bold denotes significance at $P < 0.05$

df degrees of freedom

Fig. 1 Log transformed predicted means (±standard error of means) for species responses to drought and warming treatment in two temperature controlled glasshouses (Cool, warm). *Blue lines—B. baxteri*; *Red lines—B. coccinea*



watered conditions accumulated biomass faster (Fig. 1a–d). This is seen in the above-ground biomass (Fig. 1f) and leaf mass ratio (Fig. 1h), but not in height growth increment (Fig. 1g), as resource allocation is primarily to the large leaves, not the stems. However, this species has lower specific leaf area than *B. coccinea* under all conditions (Fig. 1e). Leaf chlorophyll content was higher in *B. baxteri* (Fig. 1i).

We used the cool/wet glasshouse conditions as an approximation of 'current' conditions in a good, wet year and plotted the percentage change in mean trait values under warming, drought and the combination of warming and drought (Fig. 2). These trait changes relate to a seedlings' potential response to the novel no analogue climatic conditions predicted for the future. As expected, water deficit had a significant effect on all measured traits i.e. seedlings of both species were generally smaller and had lower specific leaf area and relatively less allocation to leaves when exposed to water deficit conditions compared to well-watered ones (Fig. 2a). The notable divergence in response was an increase in leaf mass and higher leaf chlorophyll content for *B. baxteri,* whilst leaf mass declined in *B. coccinea* and chlorophyll levels remained stable. Warming resulted in a greater number of smaller leaves, with higher specific leaf area; however *B.*

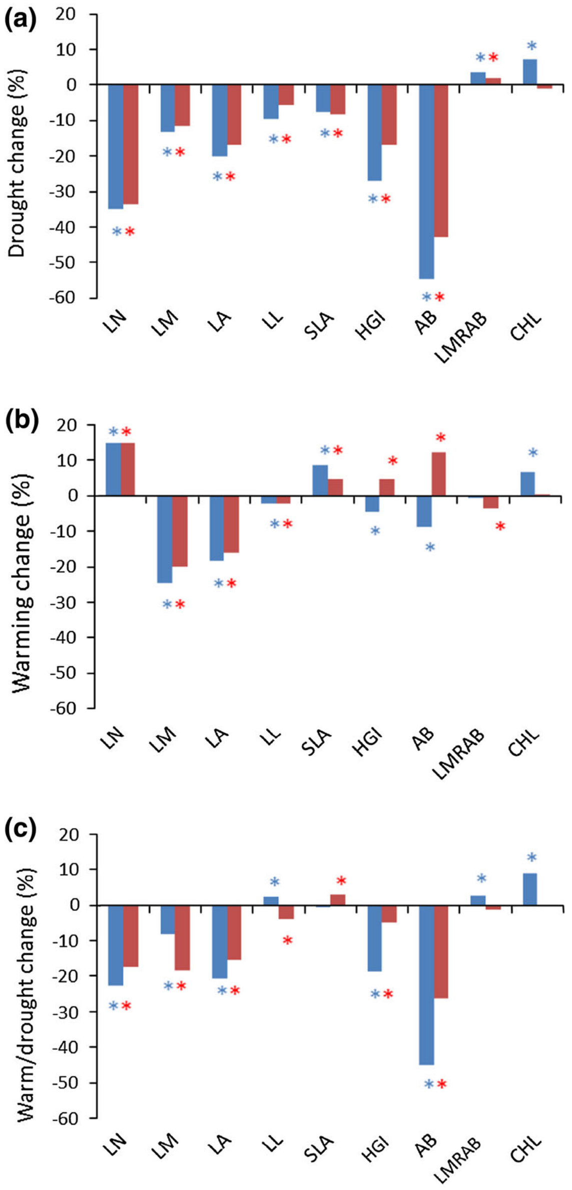


Fig. 2 Percentage changes in mean species values of leaf number (LN); leaf mass (LM); leaf area (LA); leaf length (LL); specific leaf area (SLA); height growth increment (HGI); above-ground biomass (AB); and above-ground leaf mass ratio (LMR_{AB}) produced by **a** warming; **b** drought; and **c** warming and drought. Changes expressed relative to the control (i.e. cool wet conditions). Predicted means of raw values used. *Asterisks* indicate a significant increase or decrease in trait value relative to the cool/wet conditions for a given species. *Blue bars*: *B. baxteri*; red bars: *B. coccinea*

coccinea produced more leaves with higher specific leaf area than *B. baxteri* under the warmer growth conditions (Fig. 2b). This was associated with faster height growth in *B. coccinea* and a greater allocation to leaves than stems compared to *B. baxteri,* and a relatively smaller decline in total above-ground biomass. The combination of water deficit and warmer temperatures interacted to reduce leaf size and number, biomass and height growth increment in both species, but lowered specific leaf area and caused leaf mass ratio to increase in *B. baxteri* (Fig. 2c).

Overall, the scale of decline was larger under drought than under warming, or warming and drought in combination. Drought alone caused significant declines in seven traits in both species, with increases in two traits in *B. baxteri* and one in *B. coccinea*. Warming led to fewer significant declines in trait means (five in *B. baxteri* and four in *B. coccinea*) and more increases (two in the former and four in the latter). Finally, the combined heat and drought stress moderated the drought effect and led to just four significant declines (three increases) in *B. baxteri*, but *B. coccinea* suffered declines in six out of the nine functional traits (Fig. 2c).

Co-variation in traits

At the species level, we found a suite of correlated traits that showed consistent positive and negative associations between plant characteristics (Supplementary Table S1). For both species, higher specific leaf area was correlated with higher growth increments, and lower leaf mass ratio, but not with increases in total biomass. Specific leaf area scaled positively with leaf number, but negatively with leaf mass. Leaf mass ratio scaled negatively with biomass accumulation. However, the relationship between specific leaf area and leaf area differed in direction between the species: in *B. baxteri* this relationship was positive ($F = 21.91$; $P < 0.001$); in *B. coccinea*, negative ($F = 10.98$, $P < 0.001$).

We assessed these correlations at the treatment level, investigating changes in trait associations under 'current' cool/wet growth conditions and those forecast for the 'future' (i.e. warm/dry). Some of the more notable relationships between traits included specific leaf area with leaf area, above-ground biomass and growth increment and growth increment with leaf mass ratio. The species frequently diverged in direction and extent of the relationships: the specific leaf area/leaf area relationship for both species was significant under warm/dry, not cool/wet conditions:

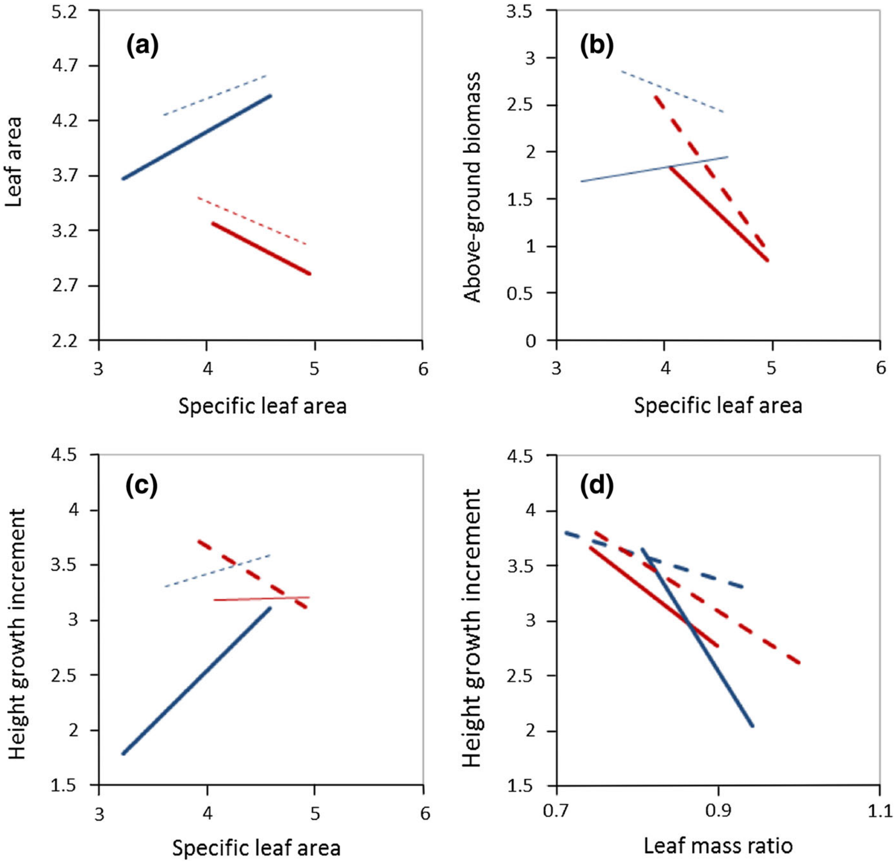


Fig. 3 The divergent pattern of trait associations under contrasting growth conditions (i.e. current cool/wet versus future warm/dry) in *B. baxteri* (*blue lines*) and *B. coccinea* (*red lines*). Regression lines based on log transformed predicted means. *Solid lines* denote warm/dry conditions; *broken lines* cool/wet. Significant relationships at $P < 0.001$ are marked with *thicker lines*

an increase in leaf area was associated with significantly larger values of specific leaf area in *B. baxteri* but not *B. coccinea* (Fig. 3a). Specific leaf area and above-ground biomass were negatively correlated in *B. coccinea* and under both 'current' and 'future' growth conditions, with no relationship between these traits in *B. baxteri* (Fig. 3b). Specific leaf area and growth increment were positively correlated in *B. baxteri* under warm/dry conditions, and negatively in *B. coccinea* under cool/wet conditions (Fig. 3c). Height growth increment was significantly and negatively correlated with leaf mass ratio in both species, and under both current and future conditions (Fig. 3d).

Plasticity in population responses to 'future' warm/dry conditions

The full REML model revealed significant species by population effects so we examined the population effects for each species individually (Table 2a, b). We predicted that the drier populations would show greater homeostasis of growth (less steep slopes of decline) underlain by differences in extent of plasticity in leaf and allocation traits (indicated by significant population by treatment interactions) (Fig. 4). The data reveal significant population by water interactions in *B. baxteri* for leaf number (Fig. 4a), and biomass accumulation (Fig. 4f, h); however, the drier population did not demonstrate greater homeostasis of growth nor were there consistent differences depending on rainfall at site origin. The drier population of *B. baxteri* suffered significant decline in specific leaf area relative to other populations under warm/dry conditions. While temperature had an overall impact on many traits, populations of *B. baxteri* did not differ in their response to temperature. Water deficit and warmer temperatures in combination delivered significant impacts on all traits with the exception of SLA (Fig. 4e) and leaf mass ratio (Fig. 4h), but again populations did not differ in their response. Overall,

Table 2 REML summary of significant effects of population, water, temperature and their interactions on leaf number (LN), leaf mass (LM), leaf area (LA), leaf length (LL), specific leaf area (SLA), height growth increment (HGI), above-ground biomass (AB), above-ground leaf mass ratio ($\mathrm{LMR}_{\mathrm{AB}}$) and leaf chlorophyll content (CHL) for two species of *Banksia*

Fixed term	df	LN	LM	LA	LL	SLA	HGI	AB	$\mathrm{LMR}_{\mathrm{AB}}$	CHL
(a) *Banksia baxteri*										
Pop	2	**<0.001**	**0.049**	**0.001**	**0.038**	0.094	**0.032**	**<0.001**	**<0.001**	**<0.001**
Water	1	**<0.001**	**0.013**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.649
Temp	1	**<0.001**	**<0.001**	**<0.001**	0.708	**<0.001**	0.201	0.829	0.256	0.681
Pop.water	2	**<0.001**	0.215	0.324	0.278	0.535	**0.024**	0.595	**0.037**	0.093
Pop.temp	2	0.752	0.264	0.146	0.668	0.747	0.184	0.403	0.665	0.935
Water.temp	1	**0.002**	**<0.001**	**<0.001**	**0.001**	0.855	**<0.001**	**<0.001**	0.354	0.700
Pop.water.temp	2	0.152	0.302	0.207	0.241	0.502	0.329	0.268	0.522	0.374
Dripper delivery rate	1	**0.011**	0.252	0.138	0.116	0.691	**0.006**	**0.003**	**0.700**	0.640
(b) *Banksia coccinea*										
Pop	2	**<0.001**	0.118	**0.013**	**<0.001**	0.308	**0.002**	**<0.001**	**<0.001**	**0.020**
Water	1	**<0.001**	**0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Temp	1	**<0.001**	**<0.001**	**<0.001**	0.904	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Pop.water	2	0.176	0.977	0.573	0.431	0.150	**0.016**	0.136	**<0.001**	**0.002**
Pop.temp	2	0.179	0.352	0.349	0.437	**0.047**	0.858	0.663	**0.012**	0.374
Water.temp	1	**0.004**	**0.003**	**<0.001**	**0.014**	**0.020**	**<0.001**	**<0.001**	0.846	**0.012**
Pop.water.temp	2	**0.042**	0.179	0.227	0.492	0.547	0.243	0.273	0.348	0.338
Dripper delivery rate	1	**0.043**	**0.027**	**0.001**	**<0.001**	0.535	**0.001**	**0.003**	**<0.001**	0.711

Results for model with fixed terms pop*water*temp + drippers; random terms: (rep/row/col) + mothers

Bold denotes significance at $P < 0.05$

df degrees of freedom

populations generally showed the same trends in direction of response to the treatment conditions.

Population by water interactions in *B. coccinea* were significant for growth increment (Fig. 4f), leaf mass ratio (Fig. 4h) and chlorophyll content (Fig. 4i). The dry range population of this species demonstrated a smaller decline in growth increment under warmer/drier conditions relative to other populations; however, seedlings from the dry population generally had smaller leaves, lower chlorophyll content and less above-ground biomass than other populations under both current and future growth conditions. Although populations were significantly different from each other, leaf mass ratio did not alter under warm/dry conditions.

In summary, no population of either species consistently demonstrated a superior ability to cope with warmer, drier conditions and population responses were largely independent of geographic origin contrary to our hypothesis.

Discussion

As predicted seedlings of *B. baxteri* and *B. coccinea* were found to be divergent in key functional traits and their sensitivity to warming and drying. *Banksia coccinea* had a greater number of small leaves with higher specific leaf area, but accumulated less overall biomass compared to *B. baxteri* when grown under favourable conditions. These differences in leaf size and specific leaf area are also supported by previous investigations on adult plants (Cochrane et al. 2014a) and seedlings of these same species (Cochrane et al. 2014b). Theory suggests that the greater the difference in functional traits between species pairs, the more likely there will be strong differences in response to limiting factors (Adler et al. 2013). Here, we found that when seedlings of the two species were grown under warmer conditions, the species diverged in their response: *B. coccinea* was able to maintain growth under warmer conditions, whereas the warmer

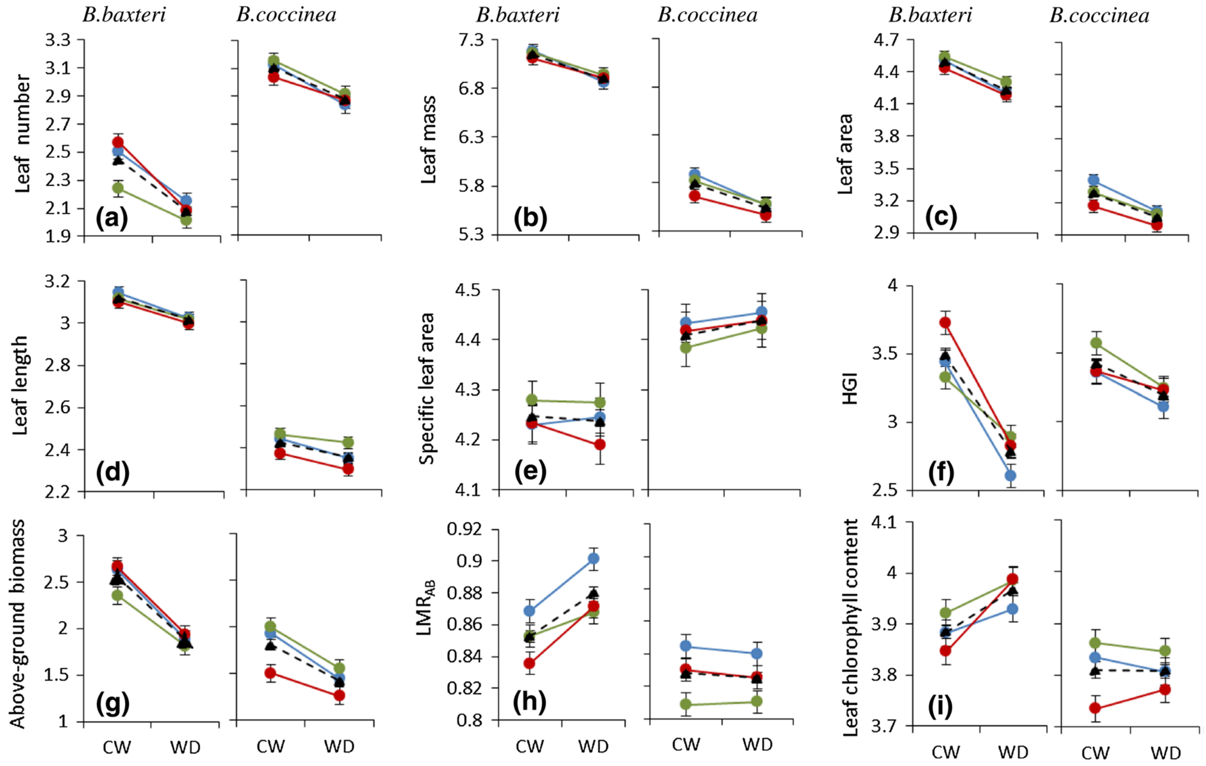


Fig. 4 Phenotypic differences in mean population trait values (log transformed predicted means) in response to contrasting treatments: (CD—cool/wet versus WD—warm/dry conditions. HGI—height growth increment; LMR$_{AB}$—above-ground leaf mass ratio. *Panel pairs*; *left*—*B. baxteri*; *right*—*B. coccinea*. *Blue lines*—high rainfall populations; *green lines*—medium rainfall populations; and *red lines*—low rainfall populations. *Black dashed lines*—species means

glasshouse conditions caused significant declines in biomass accumulation in *B. baxteri*. Higher temperatures commonly accelerate development and seedling growth and biomass production when water is available (Rustad 2008; Sardans et al. 2006), but obviously not in all species. However, the species tended to converge in their responses when grown under conditions of water deficit: there were consistent and significant reductions in leaf mass and area across both species relative to trait values expressed under ‘current’ cool, wet conditions. Smaller leaves reduce transpiration surface providing a means for plants to adapt to dry environments. The production of smaller leaves, including a reduction in leaf length and overall plant growth, resulted in a decline in biomass accumulation relative to the control in both species, though a far steeper decline in *B. baxteri*.

In contrast to the observed response to drought in isolation, the species showed phenotypic divergence when exposed to the combined impact of heat and drought stress: above-ground leaf mass ratio in *B. baxteri* seedlings increased with resource allocation primarily to the larger leaves rather than stems. Both stem and leaf mass decreased to effect this change in allocation; however, leaf mass showed the smaller decline. Biomass allocation is considered to be a strong driver of the capacity of plants to take up carbon, water and nutrients for future use. Higher leaf chlorophyll content in *B. baxteri* also suggests this species has both a higher photosynthetic rate and/or uptake of nitrogen than *B. coccinea*, at least on a mass basis, but greater leaf allocation in *B. baxteri* may render this species more susceptible to heat and drought. On the other hand, specific leaf area was lower in *B. baxteri* than *B. coccinea*, possibly due to the former’s triangular-lobed leaves that have greater allocation to veins and structure than apparent in *B. coccinea*. Low specific leaf area is associated with greater tissue density, higher resource conservation (Lamont et al. 2002; Wright et al. 2001), enhanced ability to withstand wilting (Cunningham et al. 1999; Reich et al. 1999) and greater allocation of biomass to structure rather than metabolic components, helping to promote lower rates of CO_2 gas exchange (Reich et al. 1999). Low specific leaf area usually comes with a trade-off of reduced growth rate. Although *B. baxteri* appears to be less competitive in terms of height under warm, dry conditions, the large leaves would shade out competitors at an early stage of growth. Under the 'current' favourable climatic conditions leaf number contributed significantly to biomass accumulation in *B. coccinea*; under warm/dry conditions this species showed less reduction in vegetative growth than *B. baxteri*: leaf mass ratio did not decline and specific leaf area increased as leaf mass and area declined. Whilst *B. coccinea* has lower overall germination under 'current' conditions, it suffered little decline in germination when exposed to warmer conditions (Cochrane et al. 2014b). This species can therefore maximise opportunities for recruitment and growth between fire intervals, as seed release is not fire-dependant (Lamont et al. 2007). These data support our hypothesis that seedlings of *B. baxteri* and *B. coccinea* are functionally divergent not only in key functional traits, but also in the response of these traits to drought and heat stress. This variation in physiological and morphological responses to temperature and water may facilitate co-existence of these species across their geographic range. The different strategies would appear to place *B. coccinea* as the more drought and heat tolerant species, at least at the seedling stage.

Surprisingly, drought in isolation appears to impact more heavily on seedling performance than the combined effects of drought and warming: declines in leaf and growth traits in response to water limitation were often more pronounced when plants were grown under cool conditions (i.e. cool temperatures exacerbated drought). Water deficit in Mediterranean ecosystems is normally associated with higher temperatures and we might surmise that cool, dry conditions signify a late start to cool season rains, such that plant development is delayed. The seemingly greater ability to maintain growth under warm, dry conditions seems to have placed these species at a disadvantage under cool, dry conditions. Our drought treatment, although large relative to full saturation, was not severe enough to cause major seedling death. Our warming treatment also did not result in substantial seedling losses and in particular, *B. coccinea* was able to demonstrate significant increases in biomass under the warmer conditions suggesting this species uses a strategy of tolerance to stress, rather than one of avoidance (Grime 1977). Alternatively, *B. coccinea* may have a higher optimal temperature for growth and under the warmer glasshouse conditions the species just was not sufficiently stressed. Mean temperature changes forecast for the future may not impact too heavily on these species; however, extreme events will likely pose greater and more complex threats to plant performance (Reyer et al. 2012; Thompson et al. 2013), and it is with some certainty that extreme climate events will become more prevalent both regionally and globally in the future (Bates et al. 2012).

Although trait-based analyses generally assume that differences among species will overwhelm variability among populations (McGill et al. 2006; Reich et al. 1999), variation in early seedling development traits under different stress regimes reinforces the importance of seed source when planning for ecological restoration (Vasques et al. 2013). However, the divergence in response of populations we saw was not consistently related to the observed species' distributions along the climate gradient from which they were sourced and therefore our hypothesis regarding population divergence based on geographic location is not supported. We are unable to single out any particular population that consistently showed superior performance under water limitation and/or high temperature. Indeed, case studies in the literature fail to show consistent geographic patterning of plant traits within species: in some cases clinal trends are evident (e.g. Molina-Montenegro and Naya 2012), and in others among-population differences have no consistent relationship to the climatic gradient (e.g. De Frenne et al. 2011; Cochrane et al. 2014b). However, lack of geographic patterning may be due to factors other than temperature and moisture and may include genetic and historical constraints (i.e. the presence of relict populations). In spite of the lack of geographic patterning in this current study, when populations perform in different ways to conditions of stress, there is evidence for taking a population perspective on species co-existence because species-specific responses can misrepresent the degree of variability and/or overlap in population response.

Conclusions

The effects of climate change may have a significant adverse impact on plant population persistence in the SWAFR, and in particular on long-lived *Banksia* species; undoubtedly rising temperatures and increasing incidence of drought will cause similar impacts in other Mediterranean-climate ecosystems of the world (Cabral et al. 2013; Talmon et al. 2011; Vicente-Serrano et al. 2012). More flexible species will respond to changes in the environment by exhibiting different morphology, behaviour and ecology making these species less vulnerable to extinction as a result of changing climates.

Here, we have demonstrated two sympatric species using alternative strategies for growth, perhaps in part as a result of selection for niche differentiation; however, the extent to which niche differences under current conditions will affect the interactions between these two species under future conditions remains unclear. The warm/dry conditions imposed reflect those forecast for the region in 2070 or beyond; the trait changes documented relate to potential responses to what can be considered no analogue climatic conditions. Seedlings of *B. coccinea* appear to be more flexible in mitigating fitness loss under warm, dry conditions relative to those of *B. baxteri*. We can speculate that some populations of *B. coccinea* may out-compete those of *B. baxteri* due to their greater ability to maintain steady growth under less favourable conditions.

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