

Local adaptation as a driver for intraspecific variation in climate vulnerability of *Stylidium*



THE UNIVERSITY OF
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Emily P. Tudor

The University of Western Australia
School of Biological Sciences

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This report is provided to the Australian Flora Foundation in fulfilment of the conditions of the grant awarded to the author in 2022. This report presents the results from a PhD conducted by author between 2021 and 2024 on *Stylidium hispidum*, a triggerplant endemic to the Jarrah Forest of Western Australia.

The study culminated in a dissertation submitted in March 2025 for the award of Doctor of Philosophy at the University of Western Australia.

The thesis and this report are my own work except where due reference has been made in the text or Authorship Declaration.

Reporting on the two funded projects is presented in the format of:

1. An author copy of the published manuscript:
 - Tudor EP, Lewandrowski W, Krauss S, Veneklaas EJ (2024) Local adaptation to climate inferred from intraspecific variation in plant functional traits along a latitudinal gradient. *Conservation Physiology* 12(00): coae018; doi:10.1093/conphys/coae018.
2. An author copy of PhD dissertation chapter in preparation for publication:
 - Chapter 3: Co-ordinated trait shifts in response to temperature and water availability reflect an intraspecific gradient of adaptive strategies in *Stylidium hispidum*

Signature:



Date: 17th July 2025

PhD RESEARCH SUMMARY

Global climate change is leading to more frequent heatwaves, extended severe droughts, and less predictable precipitation patterns. Such climatic events are anticipated to significantly impact biodiversity, posing risks of substantial geographical shifts and extinctions. The persistence of plant populations in response to climate change is defined by the capacity to express a suite of traits that ensure reproduction in their local environment. Understanding how adaptive traits are distributed within and among populations across a climate gradient allows us to predict population resilience to the combined effects of climate change. As such, there are two general objectives set out in my research; first, to define the extent to which intraspecific variation in trait expression is associated with local climates; and second, to contextualise this with respect to the sensitivities of demographic processes (i.e., germination, growth, flowering, and seed production) and populations that were distributed across a climate gradient.

To address these overarching aims, I conducted *in-situ* field studies and *ex-situ* growth trials to provide a mechanistic understanding into how population differentiation reflects local adaptation in a focal species, *Stylidium hispidum* Lindl. (White Butterfly Triggerplant). The first study assessed trait variation along a climate gradient spanning the latitudinal distribution of *S. hispidum* in the southwest of Western Australia to determine what proportion of intraspecific trait variation was accounted for by the independent and combined effects of climate and soils (Chapter Two). This field study revealed that climate factors accounted for a significantly greater portion of intraspecific trait variation than soil factors, with plants from hotter/drier sites in the northern populations presenting water conservation strategies that included reduced gas exchange, rosette size and floral investment compared to those from cooler/wetter sites in the southern populations. Though results from Chapter Two do not distinguish between the intraspecific trait variation that arises from heritable genetic differences or by phenotypic plasticity, the similarities in trait variability among populations may suggest that there is no differential selection for plasticity across the latitudinal gradient, or there is insufficient heritable variation to drive population differentiation in trait variability for *S. hispidum*. The second study further explored functional trait differentiation between focal populations under experimentally manipulated temperature and water conditions (Chapter Three). This study established that competitive traits were maximised under cooler, well-watered conditions, while stress avoidance and tolerance strategies were associated with elevated temperatures and water-deficit. Additionally, in this study, plants displayed

differences in their functional strategies and exhibited trait combinations that are associated with the specific conditions of their environments, suggesting that local adaptation can be a key driver of functional diversity. The third study examined how water-limitation impacted floral traits, pollinator attraction and reproduction by combining *in-situ* observations and *ex-situ* growth trials (Chapter Four). Multiple modifications to plant floral traits, pollinator attraction, seed production and germination were evident in response to water limitation. Specifically, plants originating from hotter/drier sites in the north had greater reproductive success under limited water availability compared to those originating from cooler/wetter sites. The final study assessed interspecific and intraspecific variation in moisture and temperature requirements for germination in *S. hispidum* and a wide-spread sympatric congener, *S. amoenum* (Chapter Five). Hydrothermal time-to-event modelling was used to characterise germination responses, producing further evidence to support local adaptations in germination strategies between populations of *S. hispidum*. Additionally, species-specific differences demonstrated that the comparatively range restricted *S. hispidum* may be more vulnerable to current and future climate scenarios, compared to the populations of the widespread *S. amoenum*.

The results of my research culminate to provide strong evidence to conclude that *S. hispidum* exhibits trait variation that reflects the specific conditions of their environments, and therefore, local adaptation to climatic conditions is a key driver of the species functional diversity and intraspecific variation. Although the current genotypes reflect local adaptation to their current environments, ongoing adaptations need to match the pace of environmental change to avoid detrimental effects to population performance and persistence. Given that this research demonstrates the significant role of local adaptation in supporting intraspecific variation and population performance, conservation and management initiatives could prioritise the use of locally adapted genotypes in assisted migration or climate-adjusted provenancing strategies to enhance population resilience in the warming and drying conditions of southwest Western Australia.

PhD Dissertation available through the University of Western Australia or upon request.

KEY PROJECTS FUNDED BY THE AUSTRALIAN FLORA FOUNDATION

Collectively, my research centred on linking the effects of climate to plant physiological performance across key developmental stages and understanding the cascading impacts to reproduction and distribution. Funding from the Australia Flora Foundation contributed primarily to two aims within my PhD research:

1. Assess the phenotypic variation of five provenances across a climate gradient spanning from warm dry conditions in the north (Julimar) to cool wet conditions in the south (Yarloop) to provide insight into the plasticity of physiological responses to differing edaphic and climatic factors and determine the strongest predictors of phenotypic variation *in situ* (Chapter 2 of PhD Dissertation)
2. Determine the impacts of physiological stress and resilience on floral signals, rewards, and phenology in *ex situ* glasshouse trials to establish the direct and indirect effects of temperature and moisture on flowering and reproduction and how these traits vary within and between populations across a climate gradient (Chapter 4 of PhD Dissertation)

The financial contributions from the Australian Flora Foundation covered fuel and vehicle hire costs, which allowed for extensive field work within the Jarrah Forest, enabling critical data collection in pursuit of the first research aim that is reported in Chapter 2 of my PhD dissertation, along with seed collection trips which facilitated the growth of experimental plants for the second objective. Additionally, funds also covered the costs of growth room facilities, allowing the use of climate-controlled growth rooms at the University of Western Australia (UWA) for controlled growth environments. High Performance Liquid Chromatography (HPLC) was also funded, allowing for the calibration and usage of HPLC equipment for detailed chemical analysis of nectar samples reported in Chapter Four. Soil nutrient analysis completed by CSBP was also supported. This support also extended to conference travel to the Ecological Society of Australia 2024 conference hosted in Melbourne, further enhancing dissemination of my research and networking opportunities.

1. LOCAL ADAPTATION TO CLIMATE INFERRED FROM INTRASPECIFIC VARIATION IN PLANT FUNCTIONAL TRAITS ALONG A LATITUDINAL GRADIENT

EMILY P. TUDOR^{1,2}, WOLFGANG LEWANDROWSKI^{2,1}, SIEGFRIED KRAUSS^{2,1}, ERIK J. VENEKLAAS¹

¹*School of Biological Sciences, University of Western Australia, Nedlands, WA 6009, Australia.* ²*Kings Park Science, Department of Biodiversity Conservation and Attractions, West Perth, WA 6005, Australia.*

1.1. Abstract

Ascertaining the traits important for acclimation and adaptation is a critical first step to predicting the fate of populations and species facing rapid environmental change. One of the primary challenges in trait-based ecology is understanding the patterns and processes underpinning functional trait variation in plants. Studying intraspecific variation of functional traits across latitudinal gradients offers an excellent *in-situ* approach to assess associations with environmental factors which naturally covary along these spatial scales such as the local climate and soil profiles. Therefore, we examined how climatic and edaphic conditions varied across a ~160 km latitudinal gradient to understand how these conditions were associated with the physiological performance and morphological expression within five spatially distinct populations spanning the entire distribution of a model species (*Stylidium hispidum* Lindl.). Northern populations had patterns of trait means reflecting water conservation strategies that included reduced gas exchange, rosette size, and floral investment compared to the southern populations. Redundancy analysis, together with variance partitioning, showed that climate factors accounted for a significantly greater portion of the weighted variance in plant trait data (22.1%; adjusted $R^2 = 0.192$) than edaphic factors (9.3%; adjusted $R^2 = 0.08$). Disentangling such independent and interactive abiotic drivers of functional trait variation will deliver key insights into the mechanisms underpinning local adaptation and population-level responses to current and future climates.

Keywords: Climate change, environment–trait relationships, intraspecific trait variation, morphology, physiology, soil, *Stylidium*

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1.2. Introduction

Anthropogenic activities are causing widespread and rapid changes to the earth's climate, with weather extremes, such as frequent heatwaves, prolonged severe droughts, and variability of precipitation events increasing (IPCC 2023). These climate events are expected to have major consequences for the fitness and persistence of numerous species, with risks of severe spatial displacements and extinctions (Fletcher *et al.* 2020; Miller *et al.* 2020). Impacts associated with climate change are some of the most challenging threats to manage, given the global scale and the uncertainties of the responses of species and ecosystems to multifaceted drivers of environmental change (Millar, Stephenson & Stephens 2007; Bellard *et al.* 2012; Dube *et al.* 2018). Additionally, there is growing need to understand how populations vary in their acclimation and adaptation to environmental change, and the risk of local populations not possessing the plasticity of phenotypic traits to effectively cope with changing conditions, or the genetic variability to adapt to them (Breed *et al.* 2018; Harrison 2021). Examining how populations vary in their responses to interacting environmental factors delivers key insights into the drivers of plant form and function, as well as the patterns and processes potentially underpinning species resilience or vulnerability to those environmental factors.

Trait based approaches provide an empirical foundation for explaining patterns of variation and forecasting consequences of environmental change to ecosystems (Heilmeier 2019; Liu *et al.* 2024). Functional traits can be considered as any morphological, physiological or phenological attribute of an organism which can ultimately impact a measure of performance or fitness (Lavorel *et al.* 2007; Violle *et al.* 2007). These attributes can include indicators of plant form or function, such as plant size and photosynthetic rate (Cornelissen *et al.* 2003; Perez-Harguindeguy *et al.* 2016). Plant functional ecology aims to establish generalisable predictions across biological, temporal, and spatial scales (Adler *et al.* 2014), but calls have been made to better understand relationships between functional traits and the environment with explanatory and predictive power (Shipley *et al.* 2016; Des Roches *et al.* 2018). Established trait-based approaches have historically focussed largely on trait variation between species (McGill *et al.* 2006). However, one of the primary challenges in trait-based ecology is accounting for, and identifying the factors driving intraspecific variation in functional traits (Violle *et al.* 2012). This is grounded in the knowledge that functional traits vary at the individual level and can have cascading effects across biological scales which ultimately drives the functioning and persistence of populations and communities (Bolnick *et al.* 2011).

Intraspecific trait variation emerges from ontogenetic, phenotypic, and genetic differentiation (Leimu & Fischer 2008; Mason, McGaughey & Donovan 2013; Henn *et al.* 2018). The ability to produce different morphological and physiological responses to mitigate against environmental stress can increase the capacity for populations to persist into the future (Nicotra *et al.* 2010; Araújo *et al.* 2021). Greater intraspecific trait variation is likely to allow species to cope better with environmental stress through phenotypic plasticity in the short term, and potentially promoting genotypic adaptation to future environmental norms in the long term (Nicotra *et al.* 2010). However, research across several biological scales suggests that intraspecific trait variation is often constrained under environmental stress, such as warming, drying or nutrient limitation (Valladares, Gianoli & Gómez 2007; Grassein, Till-Bottraud & Lavorel 2010; Gugger *et al.* 2015; Stotz *et al.* 2021). Reduced intraspecific variation may also be a result of reduced genetic diversity, environmental filtering and strong stabilising selection for certain traits, potentially leading to individuals being highly adapted to their local environment and improving their performance within a particular suite of conditions (Bradshaw 1965; Schlichting 1986; Schneider 2022). Therefore, measuring the environmental factors that may drive trait variation can provide insight into how individuals are responding to current conditions, and how they may perform in the face of environmental change (Arnold, Kruuk & Nicotra 2019).

Selection pressures along environmental gradients often give rise to morphophysiological traits that show marked and predictable responses that are functionally linked to climatic and edaphic conditions along the gradient (Reich *et al.* 2003; Dwyer & Laughlin 2017; Kandlikar, Kleinhesselink & Kraft 2022). Climate factors, such as temperature and precipitation, impact the distribution and persistence of species through effects on cellular processes, reproduction, and survival (Suzuki *et al.* 2014). Similarly, edaphic factors, such as nutrient availability, acidity, salinity, and metal toxicity can also present critical limitations to plant physiology and growth, via impaired metabolic processes, enzyme activity and membrane stability (Ahanger *et al.* 2016). Indeed, climatic and edaphic factors can also interact. For example, shifts in temperature and precipitation regimes can drive microbial activity and by extension, decomposition rates, soil nutrient turnover, organic carbon stocks and nutrient availability (Lloyd & Taylor 1994; Figueiredo *et al.* 2017; Jansson & Hofmockel 2020). More specifically, warming and drying can suppress microbial activity (Allison & Treseder 2008), with nutrient limitations often linked to drier, lower rainfall systems (Moreno-Jiménez *et al.* 2019). Despite their pervasive independent and interactive effects, there are few studies that

simultaneously consider both climate and soil on intraspecific variation in plant functional traits *in-situ* (Maire *et al.* 2015; Simpson, Richardson & Laughlin 2016; Joswig *et al.* 2022). Nevertheless, understanding plant responses to local environmental conditions is crucial for guiding the conservation of extant populations, as well as the restoration of those that are degraded which can maintain species functional diversity, and ultimately biodiversity in the face of rapid environmental change.

Increased temperatures and declining rainfall in south-west Australia risk the ‘transition or collapse’ of the vulnerable Northern Jarrah Forest (NJF) ecosystems (Lawrence *et al.* 2022). Therefore, this study assessed the physiological and morphological variation within and between five spatially distinct populations of the NJF endemic perennial, *Stylidium hispidum* (Lindl.), across a ~160km transect that spanned the latitudinal extent of the species distribution. We examined population differentiation in trait means and magnitude of trait variability along the latitudinal gradient and determined what proportion of morphophysiological variation was accounted for by the independent and combined effects of climate and soils. We set out to test whether northern populations would display reduced trait variability due to the hotter and drier conditions increasing the likelihood of abiotic stress (Figure 2.1), as intraspecific trait variation is often constrained under environmental stress (Valladares, Gianoli & Gómez 2007; Grassein, Till-Bottraud & Lavorel 2010; Gugger *et al.* 2015; Stotz *et al.* 2021). Moreover, we expected trait means to vary along the gradient (Figure 2.1), such that northern populations, exposed to warmer and drier conditions, would reflect water conservation strategies such as reduced stomatal conductance, greater water use efficiency, smaller plant sizes and fewer flowers (Descamps, Quinet & Jacquemart 2020; Valliere *et al.* 2021), in contrast to southern populations. Given the southern populations are exposed to higher rainfall and cooler climate conditions, we expected that they experience higher nutrient availabilities and reflect traits associated with higher productivity (i.e., higher gas exchange rates, larger plant size, increased number of flowers).

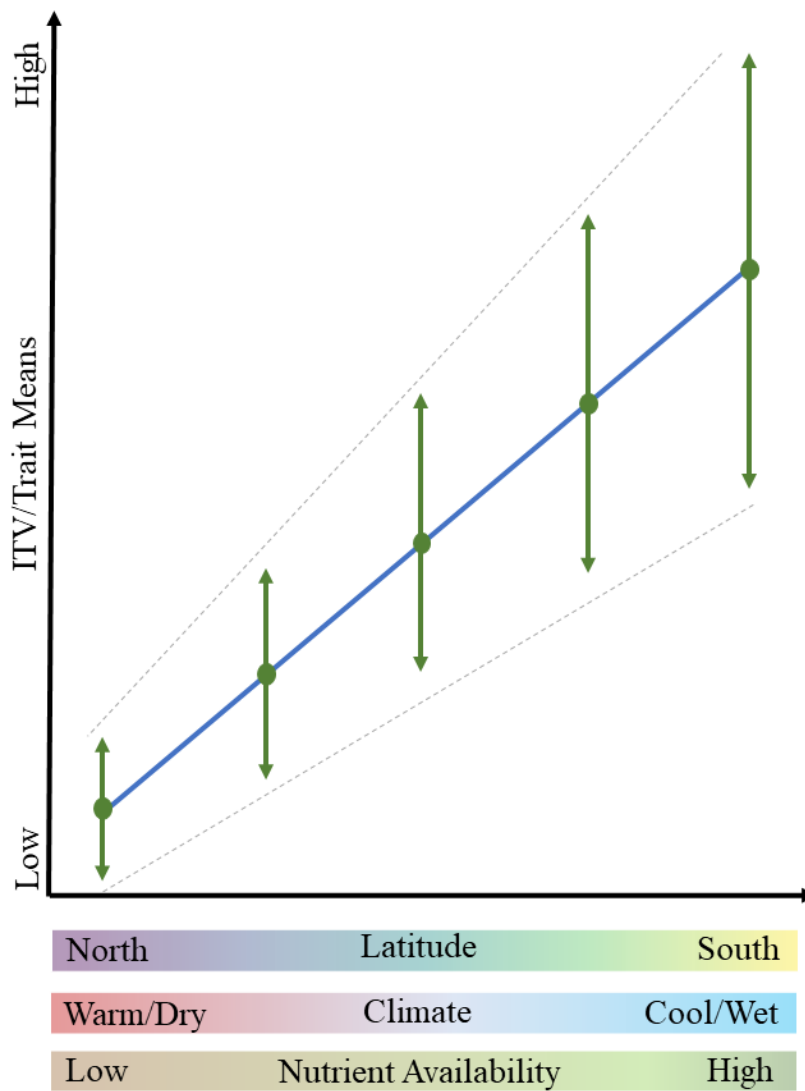


Figure 2.1: Conceptual diagram showcasing the hypotheses for change in intraspecific trait variation (ITV; calculated as the quartile coefficient of dispersion) and functional trait means for *Stylidium hispidum* populations along the local latitudinal gradient. We expected greater ITV (represented by green arrows) in southern populations and for trait means to vary along the gradient (represented by green dots), with northern populations having trait means reflective of water-conservation strategies (e.g., reduced stomatal conductance, smaller plant sizes and fewer flowers). Please note that not all functional traits were expected to exhibit the visualised positive trend across the latitudinal gradient (i.e., water-use efficiency was expected to decrease from north to south), the linear trend line is merely indicative of differences in means, irrespective of direction and slope.

1.3. Methods

1.3.1. Study species

Stylidium (Stylidiaceae) commonly known as triggerplants, is a large genus, comprising over 300 species distributed over a range of ecosystems and climate niches, mostly in Australia, with over 130 species occurring in the Jarrah Forest region of Western Australia (Western Australian Herbarium 1998-). The flowers of *Stylidium* are unique and feature a motile column comprised of fused staminate and pistillate tissues that can be ‘triggered’ by touch. The swift movement is a pollination mechanism to deposit or retrieve pollen from insect pollinators such as bee flies (Diptera: Bombyliidae), hoverflies (Diptera: Syrphidae) and bees (Hymenoptera: Apoidea; Armbruster, Edwards & Debevec 1994).

Stylidium hispidum Lindl., or the White Butterfly Triggerplant, is a small perennial herb, ~12-28 cm tall, endemic to the NJF of south-west Western Australia and represents the focal species for this study. The species is a member of the *Stylidium piliferum* R.Br. complex and is characterised by a basal rosette with one or more floral racemes that emerge and flower during austral spring. Young plants are comprised of a single rosette from an unbranched stem with green, linear, incurved leaves that may be tinged with pale maroon tones. Older plants typically feature up to eight tightly clustered rosettes which are raised above the ground on stilt roots (Lowrie & Kenneally 2017). The distribution of *S. hispidum* is restricted to a narrow transect parallel to the Darling Plateau with plants growing in highly weathered, lateritic soils. The genus has mycorrhizal associations (Brundrett & Abbott 1991), however, it is unknown whether these mycorrhizas play a significant role in nutrient acquisition (Lambers *et al.* 2018). Genetic analysis of spatially distinct populations of *S. hispidum* found significant differentiation among populations separated by at least 13–23 km along the latitudinal transect of distribution with 23% of the molecular variance partitioned among populations (Hufford *et al.* 2016). In addition to the intrinsic conservation value of understanding how trait diversity is distributed across the distribution of a local endemic species, *S. hispidum* serves as a tractable representative from which meaningful conservation insights may be derived, especially for the many *Stylidium* that are already listed as ‘priority’ and ‘threatened’ species under the Biodiversity Conservation Act 2016 (Department of Biodiversity Conservation and Attractions 2016).

1.3.2. Study area

Five study sites were selected along a ~160 km north-south gradient of the NJF along the Darling Range spanning Julimar (-31.484691, 116.166501) in the north, to Hoffman (-32.958298, 115.955832) in the south (Figure 2.2). Sites were selected based on populations included in genetic analysis conducted by Hufford *et al.* (2016). The forest vegetation is comprised of the dominant overstorey of *Eucalyptus marginata* Sm. and *Corymbia calophylla* (Lindl.) K.D.Hill & L.A.S.Johnson. and a midstorey dominated by *Allocasuarina fraseriana* (Miq.) L.A.S.Johnson, *Banksia grandis* Willd., *Bossiaea aquifolium* Benth., and *Acacia* Mill. species, and a biodiverse understorey dominated by Asteraceae, Fabaceae, Restionaceae and Proteaceae species (Gardner & Bell 2007; Koch & Samsa 2007). Broadly, the region is characterised by a dry Mediterranean climate that presents hot dry summers and cool wet winters. Sites in the north experience a mean annual rainfall of ~700 mm compared to ~1200 in the south (Figure 2.2), while mean maximum temperatures are approximately 1.5°C higher in the northern sites compared to the southern sites (Figure 2.2: Bureau of Meteorology, Perth, Western Australia). The NJF persists in an old, climatically buffered, infertile landscape (OCBIL; Hopper 2009; Hopper *et al.* 2021). The soils have developed on ~2.6-billion-year-old granite-gneiss metamorphic batholith (Nemchin & Pidgeon 1997; Anand & Paine 2002), and comprise of well drained, shallow to moderately deep gravelly sands overlaying lateritic duricrust. The nutrient concentrations in this ancient and deeply weathered landscape are typically very low, especially for phosphorus which limits plant growth despite several adaptations that maximise the efficiencies of phosphorus uptake and use (Lambers *et al.* 2011; Lambers, Martinoia & Renton 2015; Viscarra Rossel & Bui 2016). As much as practically possible, sites were selected to feature similar topographies and community compositions.

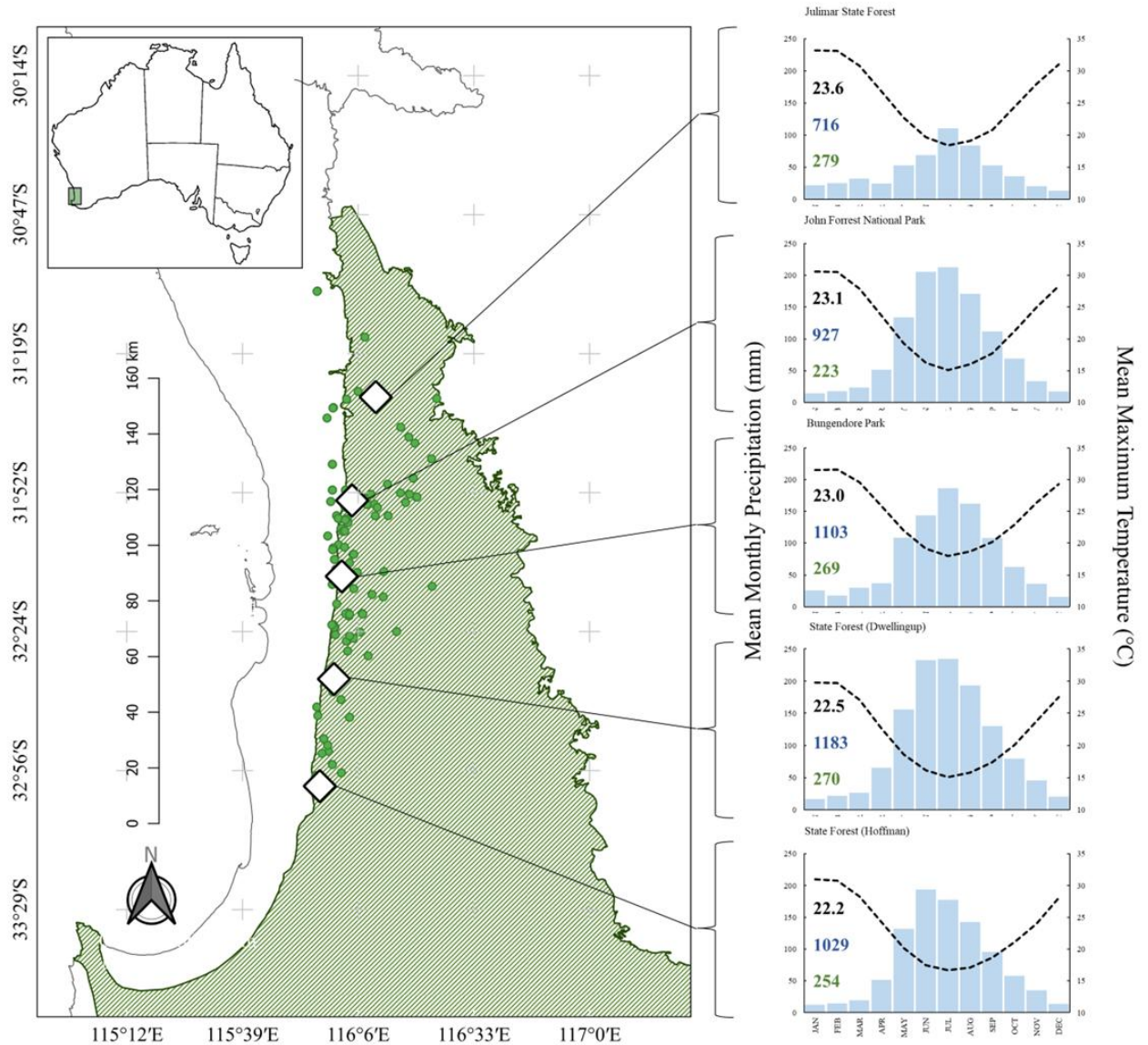


Figure 2.2: Location of study sites (◇) and climatologies distributed across ~160km latitudinal gradient along the Darling Scarp in the southwest corner of Western Australia (inset) with current distribution of *Stylidium hispidum* populations (●) overlaid on the Interim Biogeographic Regionalisation for Australia (IBRA) with the Northern Jarrah Forest (NJF) bioregion shaded in green. Monthly climate profiles comprised of mean monthly precipitation (blue bars) and mean maximum monthly temperature (black dashed line) are constructed from nearest available weather station data accessed from the Bureau of Meteorology, Perth, Western Australia. Mean annual precipitation (mm; blue text), mean temperature in the warmest quarter (°C; black text), and elevation (m; green text) are inset within each graph with precipitation and temperature data extracted from WorldClim.

1.3.3. *Climate and edaphic characterisation*

Climate conditions were quantified for soil microclimate, as well as local atmospheric climate conditions for each site. Hydrological (soil water content at 10 cm depth) and thermal conditions were measured through three TMS moisture and temperature loggers (TMS4, TOMST Ltd, Prague, Czech Republic) positioned in random locations at each site and active for the duration of the study, over October, November and December of 2021. Local climate data (e.g., cumulative rainfall) for each site was also obtained from weather station data available through the Bureau of Meteorology. We extracted mean annual temperature (BIO1, MAT) and mean annual precipitation (BIO12, MAP) data for each site (Figure 2.2) from WorldClim version 2.1 at a spatial resolution of 30 arc-seconds (Fick & Hijmans 2017). Atmospheric vapour pressure deficit (VPD_a ; kPa) and relative humidity (RH%), were captured using a LI-6400XT portable gas exchange system (LI-COR Biosciences, Lincoln, NE, USA) and averaged across months for each site, by taking the ‘reference’ measures of the atmospheric air that was taken in through the gas exchange system with desiccant column on ‘full bypass’.

Edaphic conditions were characterised by soil physicochemical properties of five samples from each site. Soil samples were collected at 0–10cm depth from areas within 1 m of where *S. hispidum* individuals were present, representing the active rooting zone of *S. hispidum* plants. Soil samples were dried and sieved to 2 mm particle size whereby larger rock fragments and woody debris > 2 mm were removed from samples. After sieving, chemical properties were determined by CSBP Soil and Plant Analysis Laboratory (Bibra Lake, WA) using methods presented in Rayment and Lyons (2011). The edaphic traits measured include: ammonium nitrate (NH_4NO_3 , mg kg⁻¹; Rayment and Lyons method 7C2b), Colwell phosphorus (P, mg kg⁻¹; Rayment and Lyons method 9B), Colwell potassium (K, mg kg⁻¹; Rayment and Lyons method 18A1), sulfur (S; mg kg⁻¹; Rayment and Lyons method 10D1), soil pH in H₂O (Rayment and Lyons method 4A1) and CaCl₂ (Hallmark, Wilding & Smeck 1983), electrical conductivity (dS m⁻¹; Rayment and Lyons method 3A1), trace elements including copper (Cu, mg kg⁻¹), zinc (Zn; mg kg⁻¹), manganese (Mn; mg kg⁻¹), iron (Fe; mg kg⁻¹) via DTPA extraction (Rayment and Lyons method 12A1), boron (B, mg kg⁻¹; Rayment and Lyons method 12C2), organic carbon (OC, %; modified Rayment and Lyons method 6B1), exchangeable aluminium (Al, meq/100g; Rayment and Lyons method 15G1), along with other exchangeable cations including calcium (Ca; meq/100g) magnesium (Mg, meq/100g) and sodium (Na, meq/100g) using a 1:5 soil:water extraction (modified Rayment and Lyons Method 5A4).

Calcium:Magnesium ratio, Effective cation exchange capacity (ECEC) and exchangeable sodium percentage (ESP) were also calculated.

1.3.4. Plant morphological and physiological traits

Field measurements were conducted in October, November and December 2021. Each month, morphological and physiological traits were measured across at least 10 reproductively mature plants at each site that had grown to a rosette diameter of at least 2 cm and separated from other sampled plants by at least 2 m. Morphological traits measured include rosette size (product of the perpendicular diameters; length, width and height; cm³), number of flowers, and plant height (cm). All morphological traits were paired with the physiological measurements from the same plant and no plant was repeatedly measured.

Physiological performance was characterised by photosynthetic carbon dioxide (CO₂) assimilation rate (A), stomatal conductance (g_s), transpiration (E) and leaf temperature using a LI-6400XT portable gas exchange system (LI-COR Biosciences, Lincoln, NE, USA) that was equipped with a 6400-15 Extended Reach 1 cm Chamber receiving ambient light and temperature conditions. The size and morphology of *Stylidium* rosettes limit the use of other, larger chambers. Measurements were taken with chamber conditions set to CO₂ concentrations of 400 $\mu\text{mol CO}_2 \text{ mol}^{-1}$, relative humidity and temperature were maintained at ambient conditions to reflect site seasonal conditions for climate. Rosette leaves were displayed as fans upon insertion into the leaf chamber, ensuring minimal overlap between leaves. Measurements were taken when stability was reached, determined via visual analysis of real-time response curves accompanied by system stability indicators. At least three measurements were taken per plant. All gas exchange measurements were adjusted by the leaf area, by capturing a bird's eye photograph of the leaves within the chamber and through pixel analysis using ImageJ. After gas exchange measurements were obtained, intrinsic water-use efficiency (WUE) was calculated as A/g_s .

Chlorophyll content was measured using a CCM-300 Chlorophyll Content Meter (Opti-Sciences, Hudson, NH, USA), by placing the sensor head directly on the leaf surface of each plant for three independent leaves per plant. Chlorophyll fluorescence (F_v/F_m) was captured using a Pocket PEA chlorophyll fluorometer (Hansatech Instruments Ltd, UK) with photosynthetically active radiation (PAR) set to 3500 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ following at least 10 minutes of dark adaptation on leaves. All physiological measurements were constrained to a sampling period between 9:00 am and 12:00 pm, representing the time when the plants were

most photosynthetically active, and conducted within the same sampling window (i.e., all sites were sampled within five consecutive days for each month of sampling).

1.3.5. *Statistical analysis*

1.3.5.1. *Patterns of latitudinal variation*

All statistical analyses and ordinations were performed in the R statistical environment (version 4.1.3) using RStudio Version 2022.02.0 (R Development Core Team 2022). Summary statistics are presented as means $\pm$ standard error for site-level differences.

Differences in morphological and physiological traits within (temporal) and between (spatial) sites were assessed using two-way ANOVA and post hoc Tukey tests using the *anova* and *TukeyHSD* functions in the ‘stats’ package, respectively. Prior to analysis, data were assessed for homogeneity of variance using the *leveneTest* function in the ‘car’ package, and data were log-transformed where appropriate to meet the assumptions for the ANOVA. Phenotypic variation was estimated using the quartile coefficient of dispersion (QCD; calculated as $((Q3 - Q1)/(Q1 + Q3))$ for each trait per population for each sampling month. This statistic is suggested to be a robust alternative to the coefficient of variation which has been found to underestimate intraspecific trait variability, or plasticity, in approximately 50% of cases (Rosas *et al.* 2019; Hurtado *et al.* 2020; Yang *et al.* 2020).

A Bray-Curtis dissimilarity matrix was used for a non-parametric permutational multivariate analysis of variance (PERMANOVA; Anderson 2005) to identify significant differences between trait assemblages along the latitudinal gradient using the *adonis* function in the ‘vegan’ statistical package. *Post hoc* pair-wise comparisons between trait assemblages were conducted to identify the multivariate differences between sites using the *pairwise.perm.manova* function in the ‘RVAideMemoire’ package (Anderson 2014; Hervé & Hervé 2020).

Principal Components Analysis (PCA) was used to identify the major dimensions of variation in the trait, climate and soil datasets and visualise multidimensional variation among populations and sites using the *PCA* function in the ‘FactoMineR’ package (Lê, Josse & Husson 2008). The PCA was carried out on log-transformed data, and trait correlations with the first two principal components were assessed using the *dimdesc* function.

1.3.5.2. Climatic and edaphic associations

Redundancy analysis (RDA) was used to construct an ordination of the variation in trait composition as constrained by the edaphic and climatic variables and identify dimensions of trait variability that correlate with environmental variables using the *rda* function in the ‘*vegan*’ statistical package (Oksanen *et al.* 2007). Beginning with the intercept-only model, we applied a step-wise selection procedure using the *ordistep* function to determine the most parsimonious subset of explanatory environmental variables for both the edaphic and climate model. Model choice was based on Akaike’s Information Criterion as a measure of model parsimony (Sakamoto, Ishiguro & Kitagawa 1986). Pearson correlation coefficients and variance inflation factors (VIF) were assessed to avoid unnecessary redundancies in the final suite of explanatory variables using the *vif.cca* function. Models and individual axes were tested for significance using the *anova.cca* function. Vectors were superimposed onto RDA biplots with significance established through 999 permutations using the *envfit* function, also in the ‘*vegan*’ statistical package (Oksanen *et al.* 2007). We log₁₀- transformed trait variables of stomatal conductance and rosette size to meet assumptions of homogeneity of variance and all variables were centred and standardised prior to RDA to provide a comparable unitless scale to compare the trait dataset with climatic and edaphic variables.

To partition the effects of climatic and edaphic factors on trait compositions we ran three different RDAs: a full model including both climatic and edaphic explanatory variables identified in the forward selection procedure, a partial model of climatic variables while controlling for edaphic effects and a partial model of edaphic variables while controlling for climatic effects. The unique and joint effects of climate and soil for explaining the variation in each morphological and physiological trait were then quantified using variation partitioning (Legendre & Legendre 2012). The shared variance accounted for by both climatic and edaphic factors was calculated as the difference between the sum of the adjusted R^2 for soil and climate and the adjusted R^2 of the full model encompassing all factors. Marginal significance of individual explanatory variables was established through 999 permutations using the *anova.cca* function in the ‘*vegan*’ statistical package (Oksanen *et al.* 2007). Partial RDAs were used to determine the proportion of variance that was individually constrained by the explanatory variables and the proportion that was ‘conditioned’ by all remaining variables from which we calculated the proportion of shared variance for each explanatory variable.

1.4. Results

1.4.1. *Patterns of latitudinal variation*

1.4.1.1. *Climate factors*

Sites along the latitudinal gradient differed significantly in all climatic traits both spatially and temporarily. Mean ambient temperature (Spatial: $F_{4,30} = 16.2$, $P < 0.001$; Temporal: $F_{2,30} = 711.9$, $P < 0.001$), temperature variability (Spatial: $F_{4,30} = 6.01$, $P = 0.001$; Temporal: $F_{2,30} = 39.4$, $P < 0.001$), maximum temperature (Spatial: $F_{4,30} = 4.63$, $P = 0.004$; Temporal: $F_{2,30} = 84.9$, $P < 0.001$) and minimum temperature (Spatial: $F_{4,30} = 5.0$, $P < 0.001$; Temporal: $F_{2,30} = 224.7$, $P < 0.001$) were all significantly higher in the northern sites (Table S2.1). Additionally, thermal factors also showed significant increases along the temporal gradient during the survey period, showcasing the expected warming moving into austral summer. Hygric factors including volumetric soil moisture (Spatial: $F_{4,30} = 2.99$, $P = 0.035$; Temporal: $F_{2,30} = 36.4$, $P < 0.001$) and relative humidity (Spatial: $F_{4,199} = 38.4$, $P < 0.001$; Temporal: $F_{2,199} = 45.9$, $P < 0.001$) both varied significantly spatially and temporarily, with southern sites typically reflecting wetter environments with greater soil moisture availability (Table S2.1). Soil moisture variability, however, only differed significantly between months (Spatial: $F_{4,30} = 1.17$, $P = 0.341$; Temporal: $F_{2,30} = 35.1$, $P < 0.001$). Atmospheric vapour pressure deficit (Spatial: $F_{4,199} = 27.3$, $P < 0.001$; Temporal: $F_{2,199} = 46.4$, $P < 0.001$) and photosynthetically active radiation (Spatial: $F_{4,199} = 6.75$, $P < 0.001$; Temporal: $F_{2,199} = 3.49$, $P = 0.032$) both showed significant spatial and temporal variation with northern sites generally reflecting higher VPD's which increase moving into austral summer and PAR values which are significantly higher in the northern sites, while presenting a slight decrease in the December measurements (Table S2.1).

1.4.1.2. *Edaphic factors*

Significant differences between sites were observed for all macronutrients: ammonium nitrate ($F_{4,20} = 8.79$, $P < 0.001$), phosphorus ($F_{4,20} = 5.25$, $P = 0.004$), potassium ($F_{4,20} = 13.9$, $P < 0.001$) along with sulfur ($F_{4,20} = 6.01$, $P = 0.002$), magnesium ($F_{4,20} = 3.43$, $P = 0.027$) and calcium ($F_{4,20} = 3.78$, $P = 0.019$). Of the trace elements, site level differences were only present for zinc ($F_{4,20} = 3.45$, $P = 0.027$), and boron ($F_{4,20} = 7.11$, $P < 0.001$). Significant differences between sites were also observed for the salinity metrics of sodium content ($F_{4,20}$

= 16.5, $P < 0.001$) and electrical conductivity ($F_{4,20} = 6.16$, $P = 0.002$). Post-hoc comparisons highlighted that these results were almost exclusively driven by a single site, John Forrest National Park, which presented significantly higher macronutrient availabilities, salinity, and the trace elements (Table S2.1). Soil acidity also showed spatial differences with the three most northern sites presenting significantly higher pH levels than the two most southern sites ($F_{4,20} = 3.09$, $P = 0.039$).

1.4.1.3. *Plant trait means and variability*

Overall, stomatal conductance differed between populations with the northern-most population presenting decreased stomatal conductance compared to the southern populations ($F_{4,167} = 7.89$, $P < 0.001$). Photosynthetic rate along the latitudinal gradient loosely followed a quadratic trend, though there were only marginal differences between populations ($F_{4,167} = 2.29$, $P = 0.061$). Additionally, there was a decreasing trend for both these traits across monthly sampling (Photosynthesis: $F_{2,171} = 17.1$, $P < 0.001$; Conductance: $F_{2,167} = 33.1$, $P < 0.001$; Figure 2.3a). There were no fixed effects of population or sampling month on WUE (Population: $F_{4,152} = 1.36$, $P = 0.247$; Month: $F_{2,152} = 0.57$, $P = 0.566$). Transpiration was marginally different between sites ($F_{4,154} = 2.42$, $P = 0.051$; Figure 2.3a), but trait means displayed significant decrease across monthly sampling ($F_{2,154} = 14.6$, $P < 0.001$; Figure 2.3a). Chlorophyll content and quantum yield (F_v/F_m) both differed significantly across populations (Content: $F_{4,196} = 15.1$, $P < 0.001$; F_v/F_m : $F_{4,196} = 8.08$, $P < 0.001$; Figure 2.3a), with chlorophyll content peaking in the second most northern site, followed by a decrease into the southern populations. Though F_v/F_m responses differed between sites there were no distinct latitudinal pattern. Chlorophyll content displayed significant decreases across monthly sampling (Content: $F_{2,196} = 12.3$, $P < 0.001$) but F_v/F_m was statistically indistinguishable between sampling months (F_v/F_m : $F_{2,196} = 1.31$, $P = 0.273$; Figure 2.3a).

Morphological traits, including rosette size ($F_{4,196} = 6.72$, $P < 0.001$), plant height ($F_{4,196} = 3.08$, $P = 0.017$) and floral production ($F_{4,196} = 10.1$, $P < 0.001$) all increased with latitude, with plants being, on average, larger and taller with greater floral displays in southern populations (Figure 2.3a). However, temporal trends generally showed a decrease in rosette size moving into the warmer months ($F_{2,196} = 20.0$, $P < 0.001$; Figure 2.3a), consistent with rosette contraction and curling. Floral production reflected population flowering phenology,

with the greatest number of flowers present in warmer months ($F_{2,196} = 34.3$, $P < 0.001$; Figure 2.3), following the full development of all panicles.

Significant interaction effects of population and sampling month were only observed for water-use efficiency ($F_{8,152} = 2.04$, $P = 0.044$), stomatal conductance ($F_{8,167} = 4.67$, $P < 0.001$), chlorophyll content ($F_{8,196} = 5.92$, $P < 0.001$), rosette size ($F_{8,196} = 2.02$, $P = 0.044$) and floral production ($F_{8,196} = 5.39$, $P < 0.001$). Despite the significant population differentiation in most of the functional trait means, trait variability (quartile coefficient of dispersion) was remarkably similar across the latitudinal gradient ($F_{4,130} = 0.48$, $P = 0.751$; Figure 2.3b).

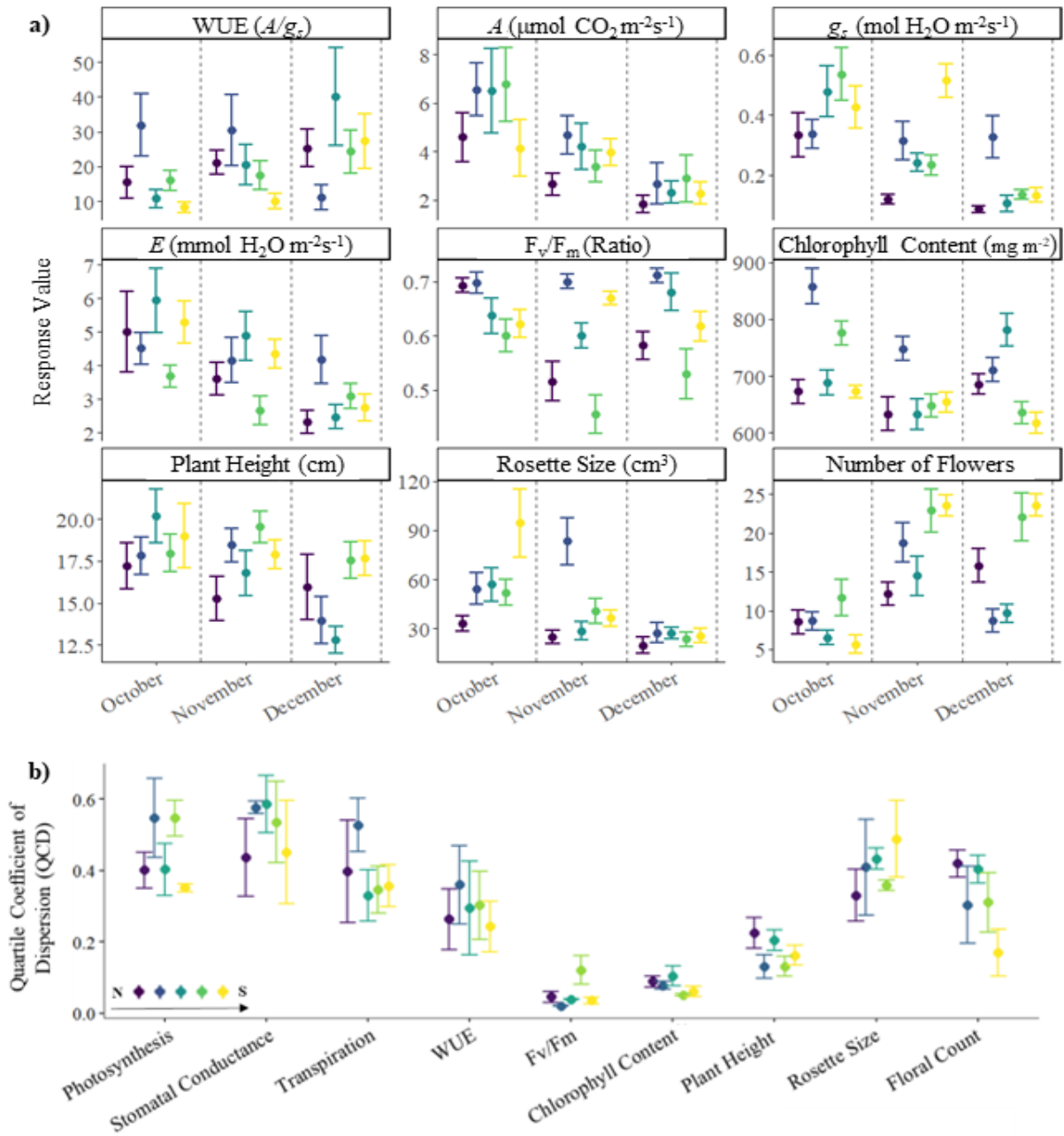


Figure 2.3: a) Spatiotemporal variation in functional traits across at least 10 *Styliidium hispidum* plants (mean $\pm$ standard error) for each site along the surveyed latitudinal gradient by sampling month. Error bars, from left to right, correspond to sites from north to south—Julimar Conservation Reserve (●), John Forrest National Park (●), Bungendore Park (●), State Forest (Dwellingup; ●), State Forest (Hoffman; ●); and b) Intraspecific trait variation as measured by the quartile coefficient of dispersion (QCD) across the latitudinal gradient. Error bars (mean QCD $\pm$ standard error), from left to right, correspond to sites from north to south—Julimar Conservation Reserve (●), John Forrest National Park (●), Bungendore Park (●), State Forest (Dwellingup; ●), State Forest (Hoffman; ●)

1.4.1.4. Multivariate analysis

Leaf traits varied significantly both spatially and temporally (Latitude: $Pseudo-F_{1,136} = 8.00$, $P < 0.001$; Month: $Pseudo-F_{1,136} = 26.99$, $P < 0.001$; Figure 2.4a). Similarly, climate factors also showed significant spatiotemporal variation (Latitude: $Pseudo-F_{1,136} = 44.8$, $P < 0.001$; Month: $Pseudo-F_{2,44} = 352.7$, $P < 0.001$; Figure 2.4b). Edaphic conditions varied spatially ($Pseudo-F_{1,138} = 23.3$, $P < 0.001$; Figure 2.4c).

The first two components of the PCA describing variation in functional traits accounted for 46.1% of variation across the latitudinal gradient (Figure 2.4a). The first axis, which was characterised primarily by a gas exchange gradient, accounted for 26.1% of this variation. The leaf traits which displayed the strongest positive correlations to the first dimension were stomatal conductance ($R^2 = 0.91$, $P < 0.001$) and transpiration ($R^2 = 0.85$, $P < 0.001$), respectively, while water use efficiency (A/g_s ; $R^2 = 0.46$, $P < 0.001$) presented the strongest negative correlation to the first axis. The second axis accounted for 20.0% of the trait variation. However, this axis was characterised by a morphological gradient with the floral counts ($R^2 = 0.76$, $P < 0.001$), plant height ($R^2 = 0.75$, $P < 0.001$) and rosette size ($R^2 = 0.42$, $P < 0.001$) presenting the strongest positive correlations. Chlorophyll content ($R^2 = 0.55$, $P < 0.001$) and F_v/F_m ($R^2 = 0.42$, $P < 0.001$) presented the strongest negative correlations with the second axis.

The PCA describing climate cumulatively described 69.2% of variation with the first two principal component axes (Figure 2.4b). The first axis, representing local microclimate, accounted for 50.6% of this variation while the second accounted for 18.6%, representing long term climate averages. The first axis was positively associated with the mean ambient temperature as constrained by the survey period ($R^2 = 0.96$, $P < 0.001$), followed closely by local maximum ($R^2 = 0.92$, $P < 0.001$) and negatively associated with soil moisture variability ($R^2 = 0.92$, $P = 0.001$) and rainfall events as constrained by the survey period ($R^2 = 0.91$, $P = 0.001$). The second axis was positively associated with mean annual temperatures ($R^2 = 0.83$, $P < 0.001$) and negatively associated with mean annual precipitation ($R^2 = 0.81$, $P < 0.001$).

The first two components of the PCA describing variation in the edaphic environment across the gradient accounted for a cumulative 72.3% of variation (Figure 2.4c). The first axis component, which reflects a gradient of macro- and micronutrient availability, accounted for 53.5% of this variation. Sulfur ($R^2 = 0.94$, $P < 0.001$) presented the strongest positive association with the first component, closely followed by boron, ammonium nitrate, potassium, sodium, and phosphorus, respectively (R^2 range = 0.82–0.90, $P < 0.001$). The second principal

component axis accounted for 18.8% of the variation. However, this axis corresponded most strongly to a trace metal and acidity gradient, which was significantly positively associated with soil pH ($R^2 = 0.91$, $P < 0.001$), and negatively with aluminium ($R^2 = 0.88$, $P < 0.001$) and iron ($R^2 = 0.71$, $P < 0.001$) concentrations.

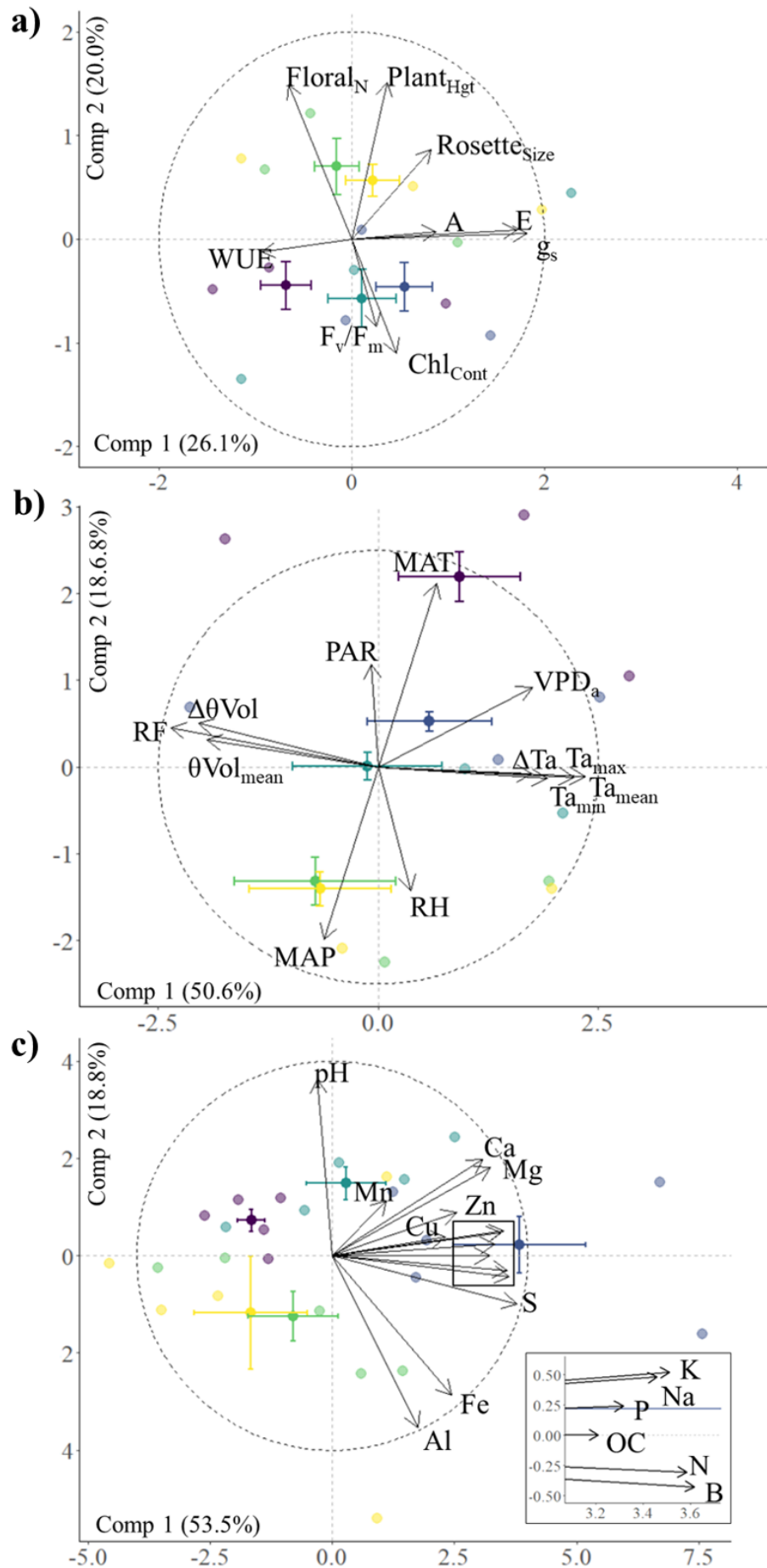


Figure 2.4 [Previous page]: Principal component analyses (PCA) biplots showing the multidimensional differences in **a)** functional traits of *Stylidium hispidum*, **b)** climate proxies; and **c)** soil chemical profiles across a local latitudinal gradient. Data are presented as centroids and standard errors. The proportion of variance explained by each component axis appears in parentheses within each biplot while axes labels correspond to the dominant gradient of variation. In c) the inset provides ease of visualisation for clustered vectors. Survey sites, from north to south—Julimar Conservation Reserve (●), John Forrest National Park (●), Bungendore Park (●), State Forest (Dwellingup; ●), State Forest (Hoffman; ●). Refer to methods for units for each trait. Vector loadings for functional traits correspond to; photosynthesis (A), transpiration (E), stomatal conductance (g_s), water-use efficiency (defined as A/g_s ; WUE), maximum quantum yield of PSII (F_v/F_m), chlorophyll content (Chl_{cont}), number of flowers (Floral_N), plant height (cm; $\text{Plant}_{\text{Hgt}}$) and rosette size (cm^3 ; $\text{Rosette}_{\text{Size}}$). Vector loadings for climate factors correspond to; Mean annual temperature ($^{\circ}\text{C}$; MAT), mean annual precipitation (mm; MAP), mean relative humidity (%; RH), mean atmospheric vapour pressure deficit (kPa; VPD_a), mean volumetric soil moisture (%; $\theta\text{Vol}_{\text{mean}}$), volumetric soil moisture variability ($\Delta\theta\text{Vol}_{\text{mean}}$), mean ambient temperature ($^{\circ}\text{C}$, T_{mean}), minimum temperature ($^{\circ}\text{C}$, T_{min}), maximum temperature ($^{\circ}\text{C}$, T_{max}), temperature variability ($^{\circ}\text{C}$, ΔT_a) and mean photosynthetically active radiation (PAR) and cumulative rainfall during the survey period (mm; RF). Vector loadings for edaphic factors are based on; soil pH (pH), ammonium nitrate (N; NH_4NO_3), phosphorus (P), sodium (Na), potassium (K), sulfur (S), magnesium (Mg), and calcium (Ca), copper (Cu), zinc (Zn), manganese (Mn), iron (Fe), aluminium (Al), boron (B) and organic carbon (OC).

1.4.2. *Climatic and edaphic associations*

1.4.2.1. *Climate factors*

We used redundancy analysis (RDA) to understand how the multivariate structure of leaf traits can be explained by the climate characteristics at each site. Following the forward selection procedure, only five factors were identified for inclusion in the final model: mean ambient temperature ($^{\circ}\text{C}$), mean maximum temperature ($^{\circ}\text{C}$), relative humidity (%), mean atmospheric vapour pressure deficit (kPa), and mean annual precipitation (mm). The RDA model of climatic effects explained a significant portion (22.1%) of the weighted variance (inertia) in plant traits ($F_{5,136} = 7.71$, $P = 0.001$), and a cumulative 85.6% of variance in the

weighted averages and class totals was accounted for by the first two axes (RDA1: *Pseudo- $F_{1,136}$* = 21.7, P = 0.001; RDA2: *Pseudo- $F_{1,136}$* = 8.35, P = 0.001; Figure 2.5a; Table 2.1a). Analysis of individual terms indicated that the strongest climatic association with leaf trait data was mean maximum temperature (adjusted R^2 = 0.126, $F_{1,140}$ = 23.8, P = 0.001, Table 1b), followed by mean ambient temperature (adjusted R^2 = 0.118, $F_{1,140}$ = 22.4, P = 0.001; Table 1b), atmospheric vapour pressure deficit (adjusted R^2 = 0.077, $F_{1,140}$ = 15.0, P = 0.001; Table 1b), mean annual precipitation (adjusted R^2 = 0.018, $F_{1,140}$ = 4.53, P = 0.002; Table 2.1b) and relative humidity (adjusted R^2 = 0.011, $F_{1,140}$ = 3.16, P = 0.01; Table 2.1b).

1.4.2.2. *Soil factors*

In the redundancy analysis (RDA) for leaf traits and soil characteristics, only two factors, soil pH and phosphorus content, remained in the model after the forward selection procedure. The RDA model constructed for the edaphic drivers explained 9.3% (R^2 = 0.093, adjusted R^2 = 0.080) of the weighted variance (inertia) in the leaf trait data, as constrained by phosphorus and soil pH (RDA1: *Pseudo- $F_{1,139}$* = 7.89, P = 0.001; RDA2: *Pseudo- $F_{1,139}$* = 6.44, P = 0.001; Figure 2.5b; Table 1a). Analysis of individual terms indicated that both phosphorus (adjusted R^2 = 0.042, $F_{1,140}$ = 8.69, P = 0.001; Table 1b) and soil pH (adjusted R^2 = 0.041, $F_{1,140}$ = 8.49, P = 0.001; Table 1b) accounted for a similar amount of variation in the leaf trait data.

1.4.2.3. *Variance partitioning*

Variance partitioning showed that overall, climate factors, as determined by the forward selection procedure, explained more variation (adjusted R^2 = 0.192; Table 2.1a) in the collective matrix of leaf traits than did soil (adjusted R^2 = 0.080; Table 1a). A partial model of climatic variables while controlling for edaphic effects captured more variation (adjusted R^2 = 0.134; Table 2.1a) in the plant trait dataset, than did the partial model of edaphic variables while controlling for climatic effects (adjusted R^2 = 0.022; Table 2.1a). Climatic and edaphic factors had a shared explained variance of 5.8% (adjusted R^2 = 0.058; Figure 2.6a, Table 2.1a). Partitioning out the effects of climate, soils and their joint effect for each independent plant trait showcased between 15.1 and 97.6% of the explained variation observed within each trait was captured by the independent effects of climate and up to 38.5% of the variance was captured by the independent effects of soil (Figure 2.6b). Joint contributions ranged between

2.02 and 84.9% (Figure 2.6b). Collectively, climate captured the most variance in all traits except for water use efficiency (WUE), whereby climate effects were captured by joint contributions with edaphic factors.

Table 2.1: a) Results of redundancy analyses (RDA) on trait compositions of *Stylidium hispidum* Lindl. Models partitioned inertia (variance) into that encompassed by climate and soil factors, as well as a partial model of climatic variables while controlling for edaphic effects (Climatic | Edaphic), a partial model of edaphic variables while controlling for climatic effects (Edaphic | Climatic) and a joint model capturing the shared inertia between both climatic and edaphic models (Climatic $\cap$ Edaphic). The full model included all climatic and edaphic explanatory variables that were identified in a forward selection procedure. Asterisks denote model significance (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). **b)** Partitioned inertia by simple RDAs of independent climatic and edaphic factors. Proportion constrained corresponds to the variance constrained by the sub-models (in a) and independent factors (in b) relative to the proportion constrained by the full model. Asterisks denote marginal (Type III) effects of individual explanatory variables in a model with all other terms accounted for.

a) Summary of RDA model results for plant trait composition variation					
Model	Partitioned Inertia	Prop. Constrained	% relative	adjR²	F(p)
Climatic	1.988	0.221	87.155	0.192	7.71***
Edaphic	0.841	0.093	36.870	0.080	7.16***
Climatic Edaphic	1.440	0.160	63.130	0.134	5.74***
Edaphic Climatic	0.293	0.033	12.845	0.022	2.92***
Climatic $\cap$ Edaphic	0.548	0.061	24.025	0.058	NA
Full	2.281	0.253	100%	0.214	6.49***
Total Inertia	9.000				
b) Independent factor contributions to full RDA model and marginal effects					
Variable	Partitioned Inertia	Prop. Constrained	% relative	adjR²	F(p)
T _{max} (°C)	1.193	0.133	52.307	0.126	2.996 **
T _{mean} (°C)	1.121	0.125	49.159	0.118	2.818 **
VPD _a (kPa)	0.752	0.084	32.962	0.077	1.647
MAP (mm)	0.227	0.025	9.963	0.018	1.551
RH (%)	0.159	0.018	6.968	0.011	2.894 *
pH	0.426	0.047	18.671	0.041	1.061
Phosphorus	0.436	0.048	19.109	0.042	1.520

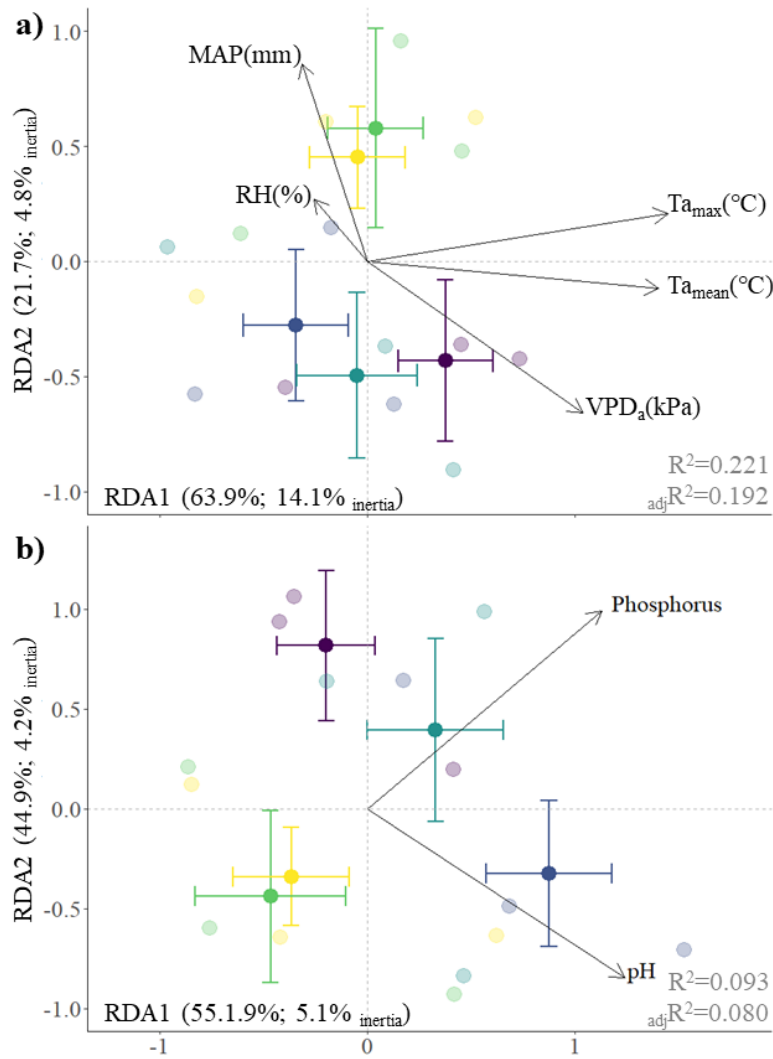


Figure 2.5: Redundancy analysis (RDA) biplots showing plant functional trait composition as constrained by **a)** climatic factors and **b)** edaphic factors. Opaque points ($\pm$ 95% confidence intervals) in panel a) and b) represent site centroids, showcasing the latitudinal variation of survey sites, from north to south—Julimar Conservation Reserve (●), John Forrest National Park (●), Bungendore Park (●), State Forest (Dwellingup; ●), State Forest (Hoffman; ●). The proportion of variance explained by each axis appears in parentheses, followed by the proportion of inertia explained. R^2 and adjusted R^2 ($adjR^2$) are overlaid in grey text. The climatic and edaphic factors used in these analyses were the ones revealed to be the most relevant following a forward selection procedure using the *ordistep* function in the R statistical package ‘*vegan*’. Vector labels correspond to mean atmospheric vapour pressure deficit (kPa; VPD_a), mean maximum temperatures ($^{\circ}C$, $T_{a_{max}}$), mean ambient temperature ($^{\circ}C$, $T_{a_{mean}}$), mean annual precipitation (mm; MAP), relative humidity (%; RH), soil pH (pH) and phosphorus content.

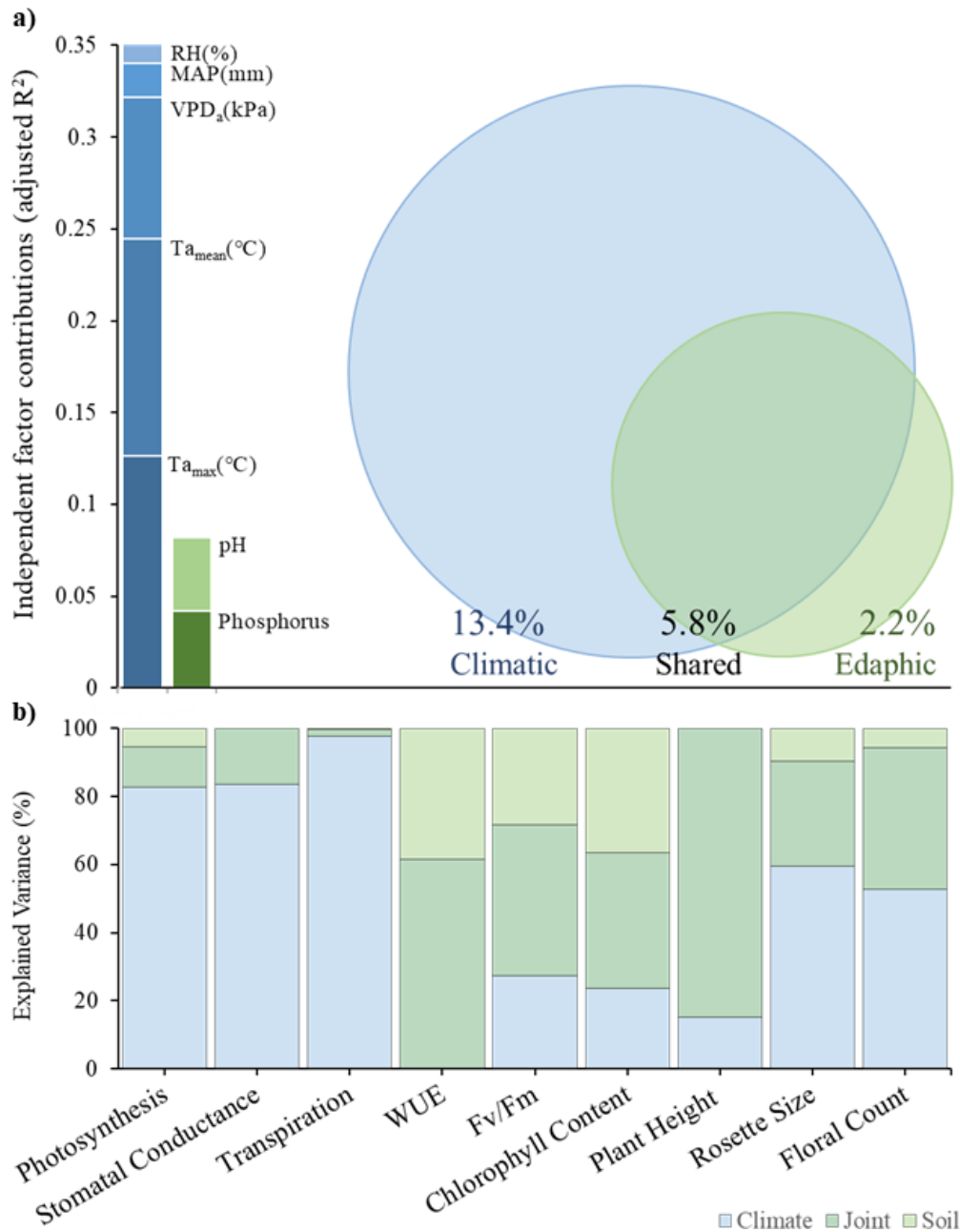


Figure 2.6: a) Variance partitioning of plant functional traits between the independent effect of climatic factors, the independent effect of edaphic factors, and the effect of climatic and edaphic factors in combination. Independent contributions of climatic and edaphic factors are presented in the stacked bar graph and reflect adjusted R^2 contributions. The climatic and edaphic factors used in these analyses were the ones revealed to be the most relevant following a forward selection procedure using the *ordistep* function in the R statistical package ‘*vegan*’; and **b)** results of partitioning variation driven independently by climate and soil factors as well as their joint effects for all functional traits. Refer to methods for unit measures for each trait.

1.5. Discussion

Examining where morphological and physiological trait diversity exists, and the environmental factors associated with intraspecific trait variation, can provide insight into how plants are performing in current conditions, and their resilience to environmental change. Our research details the physiological and morphological variation within and among five spatially distinct *S. hispidum* populations and the associations with local climatic and edaphic conditions. There were marked differences in the thermal and hygric conditions along the latitudinal gradient, confirming that northern sites were hotter and drier than southern sites. We found that northern populations presented significantly reduced gas exchange, rosette size, and floral investment compared to the southern populations, despite the similar patterns of trait variability (QCD) across populations. Trait expression in the northern populations is likely a result of morphological and physiological strategies that work to cope with heat and drought stress. Overall, climate factors, and in particular, temperature, accounted for the greatest proportion of variation in independent morphophysiological traits, as well as multivariate trait composition.

1.5.1. *Patterns of trait variation*

Conforming to our hypothesis, that trait means would vary along the gradient, we found northern populations to present trait means reflective of water conservation strategies such as reduced stomatal conductance, smaller rosettes, and limited floral investment compared to the southern populations. Generally, these patterns of form and function across the latitudinal gradient were captured by two main axes of variation (Figure 2.4a) with the first axis broadly reflecting a gas-exchange gradient and the second reflecting a productivity and morphological gradient. Such axes have emerged in various forms across several previous studies. For example, both Díaz *et al.* (2016) and Joswig *et al.* (2022) conclude that two main axes of variation include size and leaf economics across global plant trait datasets. Along a local elevational gradient, Visakorpi *et al.* (2022) define a physiological gradient, based on water-use efficiency and gas exchange, and a morphological gradient, based on measures such as plant width and leaf area. Collectively, these findings support the notion that axes of variation relating to physiology, economics and morphology persist at local intraspecific, and at global interspecific scales (Joswig *et al.* 2022). Therefore, morphophysiological trait data may be used

to generate predictions of how species may respond to changing environments to gain insights into the mechanisms driving trait–environment relationships.

Intraspecific variation is a key component of functional trait diversity and adaptive plasticity is a major mechanism by which plants respond to abiotic and biotic stress (Gratani 2014). High intraspecific trait variation is considered to promote species acclimation, increase competitive capacity and population persistence in dynamic environments and may be a key index for predicting population and species resilience in the face of a changing climate or increasingly degraded landscapes (Valladares, Gianoli & Gómez 2007; Valladares *et al.* 2014). However, we found that trait variability was evenly distributed along the latitudinal gradient with all populations presenting statistically indistinguishable differences in the quartile coefficient of dispersion. Consequently, our results do not support the prediction that northern populations would reflect reduced trait variability, although this finding is consistent with previous studies that report no evidence to suggest that there is population differentiation in intraspecific trait variability (Matesanz, Horgan-Kobelski & Sultan 2012; Kühn *et al.* 2021). While we do not distinguish between the trait variation that arises from heritable genetic differences or by phenotypic plasticity, the similarities in trait variability among populations may suggest that there is no differential selection for plasticity across the latitudinal gradient, or there is insufficient heritable variation to drive population differentiation in trait variability for *S. hispidum* (Goldstein & Ehrenreich 2021). It could also be suggested that the similar levels of population level variability are the result of similar degrees of heterogeneity, despite different means, in abiotic conditions at different sites (De Kort *et al.* 2020). However, these conclusions are context dependent, and depend largely on the species, environmental heterogeneity, and the extent of a species distribution.

1.5.2. Drivers of latitudinal variation

Generally, climatic and edaphic gradients are associated with significant differences in functional traits, relating to the morphology, physiology and reproduction of plants, and they are recognised as important predictors of population persistence in the face of environmental change (Violle *et al.* 2007). We found marked differences in the thermal and hygric conditions along the latitudinal gradient, such that northern sites on average, were 1.6°C warmer than southern sites with maximum temperatures reaching 3.3°C higher, while there was a 99% increase in mean rainfall from the driest northern site to the wettest southern site during the

survey period alone. Moreover, we observed significantly higher macronutrient availability in the second most northern site, John Forrest. While the effects of climate on edaphic factors were not examined here, the patterns observed in soil factors loosely correspond to Albrecht's conceptual model (Huston 2012), such that soil pH, exchangeable cations, and nutrient availability track a unimodal pattern from being low at the extremes for dry and wet conditions (Huston 2012; Maire *et al.* 2015).

Redundancy analysis, together with variance partitioning, allowed the determination that climate factors consistently accounted for a significantly greater portion of the weighted variance in plant traits than edaphic factors. Of the climate factors defined within this study, only ambient temperature, maximum temperature, atmospheric vapour pressure deficit, moisture availability and rainfall presented significant associations with variance in plant functional traits along the latitudinal gradient, while only soil pH and phosphorus emerged as significant predictors out of the available soil factors. Separately, the climate factors alone constrained 22.1% (0.192 adjusted R^2) of the total inertia in plant traits, while the soil factors constrained 9.3% (0.080 adjusted R^2). This result contrasts with findings recorded at global scales, whereby soil predictors were found explain more variance in individual leaf traits than climate (Ordoñez *et al.* 2009; Maire *et al.* 2015). However, this finding highlights the dominant role that climate can play in structuring trait expression in *S. hispidum* and suggests that future climate change scenarios may lead to trait shifts across populations in response to the effects of warming and drying. Moreover, there is uncertainty surrounding the extent of abiotic stress that this species can tolerate, highlighting the importance of characterising physiological thresholds and resilience at biologically meaningful scales.

Despite edaphic factors being less correlated with plant traits than climate in our model species, we found that soil pH and phosphorus content explained 4.1 and 4.2% of total inertia, respectively. These results conform, in part, to those observed in a global, interspecific analysis conducted by Maire *et al.* (2015), finding the same two soil variables to have the strongest explanatory power over the variation in plant traits. While we found the explanatory power of soil pH and phosphorus availability to be substantially less than climatic variables, both soil pH and phosphorus content play key roles in shaping plant growth, resource acquisition and performance (Malhotra *et al.* 2018; Neina 2019). When considered across a broader gradient of soil ecotypes, increased alkalinity will often be associated with a higher availability of nutrients and reduce the acquisition costs of key macronutrients, such as nitrogen (Maire *et al.* 2015). Increased acidity, however, can compromise root form and function and, as a

consequence, inhibit CO₂ assimilation (Long *et al.* 2017), reduce water uptake (Bian *et al.* 2013) and induce oxidative stress (Martins *et al.* 2013). We observed a unimodal distribution of phosphorus content along the latitudinal gradient with intermediate populations presenting significantly greater phosphorus availability than marginal populations. Photosynthesis is generally reduced in phosphorus deficient soils, although there is some degree of adaptation to be expected for plants growing in environments with reduced phosphorus availability (Lambers, Martinoia & Renton 2015). Nevertheless, the unimodal patterns in photosynthesis do correspond to those of phosphorus availability, likely contributing to spatial variance observed in photosynthesis in this study.

The tendency for plants to exhibit morphological and physiological shifts along climate gradients is well known and in accordance with theory to predict shifts in gas exchange and water relations as strategies to minimise water loss and maximise water uptake whilst maintaining physiological functioning (Reich *et al.* 2003; Westoby & Wright 2006). We found that the strongest climate driver of variation in the leaf trait data was temperature, with mean ambient and maximum temperatures independently explaining 11.8% and 12.6% of inertia, respectively. This conforms to global-scale trends in plants traits as they relate to temperature and precipitation, with Moles *et al.* (2014) finding that temperature is significantly correlated with 15 out of 21 plant traits while precipitation correlated to a lesser extent, with only six of these traits. Above-optimal temperatures and drying environments can impair plant growth, development, form, and function (Bykova *et al.* 2012), often exerting greater influences in combination than either abiotic stress does independently (Zandalinas *et al.* 2018). Collectively, the patterns observed in plant traits observed in the northern populations can be seen as strategies to manage the economics of gas exchange and be under stronger selection pressures from climatic factors to mitigate or cope with warming and drying conditions. However, we are limited in our capacity to conclude whether the patterns of trait means are truly reflective of genetic differentiation supporting local adaptation. Therefore, future studies should seek to disentangle the relative contribution of environmental, maternal, and genetic effects on plant responses (e.g., growth rates and physiological function) to combined heat and drought stress under controlled ‘common garden’ scenarios.

1.5.3. *Implications for conservation*

While our results show short-term responses to interacting environmental drivers, there may be long-term implications for local extinction if trait modifications that impact plant fitness fail to keep up with rapidly changing environments. In the south-west region of Western Australia, daily maximum temperatures are expected to increase up to 2.4°C by mid-century and 4.2°C by the end of the century (Jakob *et al.* 2012; CSIRO & BOM 2015). Similarly, mean annual precipitation is expected to decline by 17% by mid-century, and 46% by the end of the century under an intermediate-emission scenario (Jakob *et al.* 2012; Sudmeyer *et al.* 2016). In this regard, it is possible that by the end of the century, the southern populations may present a phenotype more reflective of those currently observed in northern populations, as the latitudinal gradient represents a thermal gradient difference of 1.4°C in the warmest quarter, and 39.4% decrease in mean annual precipitation. The consequences for ongoing warming and drying in the northern populations are less clear, though they may be detrimental if the adaptive capacity of the species is exceeded by the rate of change, or if tolerance thresholds for abiotic stress are surpassed. In this instance, hotter and drier conditions may result in range contractions, seeing the local extinction of northern-most populations, or potentially range shifts or expansions further south to habitats that foster milder conditions (Fitzpatrick *et al.* 2008), especially considering that climate related local extinctions are already widespread across the globe (Wiens 2016). However, to date, there are no studies to report on the intergenerational effects of abiotic stress on *Stylidium*, nor any specific parameterisation of drought or thermal tolerance. Therefore, it is difficult to predict with greater certainty as to how this species will perform in future climates, highlighting a major gap in our scientific knowledge for not only the focal species of this study, but also the exceptionally diverse flora of south-west Australia.

Unless appropriate conservation and management initiatives are implemented to address the potential threat of climate change, the future remains unclear for northern marginal populations of *S. hispidum* in a warming and drying south-west Western Australia. From a conservation perspective, *ex-situ* storage of seed material may temporarily safeguard population genotypes and serve as a repository source for *in-situ* conservation, restoration, or translocation efforts (Merritt & Dixon 2011). Additionally, it is important to monitor changes in population abundance and trait compositions as climate-associated changes unfold. If vulnerabilities are exacerbated in the future, targeted gene flow (Hufford, Krauss & Veneklaas

2012; Hufford *et al.* 2016), managed relocation (Butt *et al.* 2021; Onley, Moseby & Austin 2021), and microclimatic amelioration (Soliveres *et al.* 2011; Brigham & N. Suding 2023), may be considered to facilitate adaptation or open migration pathways, especially for priority species. Nevertheless, it is important to note that the findings, interpretations, and speculations posed in this study do not necessarily translate to other species, as the results are largely species-specific and context dependent, with other trends potentially emerging at different spatial and temporal scales than those observed here. As such, ongoing conservation of at-risk species calls for investment into evidence-based insight into the intraspecific variation in tolerance to interactive stressors associated with environmental change.

1.5.4. Conclusions

Here, we have demonstrated that while intraspecific trait variability may be consistent among populations spanning a latitudinal gradient, patterns of trait means are strongly associated with climate, and to a lesser extent, edaphic factors. While the trait–environment correlations identified in our study should not be assumed to be direct causal relationships, the dominant impact of climatic factors shaping functional trait expression along the gradient suggest that trait shifts will occur with future climate change. Disentangling the abiotic drivers of functional trait variation will be key to understanding the mechanisms underpinning local adaptation and population-level responses to ongoing environmental change. Understanding these dynamics is of global relevance; however, it is particularly important within the context of our study system, where increased temperatures and declining rainfall risk the ‘transition or collapse’ of the vulnerable jarrah forest ecosystems (Lawrence *et al.* 2022)

1.6. References

- Adler, P.B., Salguero-Gómez, R., Compagnoni, A., Hsu, J.S., Ray-Mukherjee, J., Mbeau-Ache, C. & Franco, M. (2014) Functional traits explain variation in plant life history strategies. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, 740-745.
- Ahanger, M.A., Morad-Talab, N., Abd-Allah, E.F., Ahmad, P. & Hajiboland, R. (2016) Plant growth under drought stress: Significance of mineral nutrients. *Water stress and crop plants: a sustainable approach*, **2**, 649-668.
- Allison, S.D. & Treseder, K.K. (2008) Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. *Global Change Biology*, **14**, 2898-2909.
- Anand, R.R. & Paine, M. (2002) Regolith geology of the Yilgarn Craton, Western Australia: implications for exploration. *Australian Journal of Earth Sciences*, **49**, 3-162.
- Anderson, M.J. (2005) Permutational multivariate analysis of variance. *Department of Statistics, University of Auckland, Auckland*, **26**, 32-46.
- Anderson, M.J. (2014) Permutational multivariate analysis of variance (PERMANOVA). *Wiley StatsRef: Statistics Reference Online* (eds N. Balakrishnan, T. Colton, B. Everitt, W. Piegorisch, F. Ruggero & J. Teugels), pp. 1-15.
- Araújo, I., Marimon, B.S., Scalon, M.C., Cruz, W.J., Fauset, S., Vieira, T.C., Galbraith, D.R. & Gloor, M.U. (2021) Intraspecific variation in leaf traits facilitates the occurrence of trees at the Amazonia–Cerrado transition. *Flora*, **279**, 151829.
- Armbruster, W.S., Edwards, M.E. & Debevec, E.M. (1994) Floral character displacement generates assemblage structure of Western Australian triggerplants (*Stylidium*). *Ecology*, **75**, 315-329.
- Arnold, P.A., Kruuk, L.E. & Nicotra, A.B. (2019) How to analyse plant phenotypic plasticity in response to a changing climate. *New Phytologist*, **222**, 1235-1241.
- Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W. & Courchamp, F. (2012) Impacts of climate change on the future of biodiversity. *Ecology Letters*, **15**, 365-377.
- Bian, M., Zhou, M., Sun, D. & Li, C. (2013) Molecular approaches unravel the mechanism of acid soil tolerance in plants. *The Crop journal*, **1**, 91-104.
- Bolnick, D.I., Amarasekare, P., Araújo, M.S., Bürger, R., Levine, J.M., Novak, M., Rudolf, V.H.W., Schreiber, S.J., Urban, M.C. & Vasseur, D.A. (2011) Why intraspecific trait variation matters in community ecology. *Trends in Ecology & Evolution*, **26**, 183-192.

- Bradshaw, A.D. (1965) Evolutionary significance of phenotypic plasticity in plants. *Advances in genetics*, **13**, 115-155.
- Breed, M.F., Harrison, P.A., Bischoff, A., Durruty, P., Gellie, N.J., Gonzales, E.K., Havens, K., Karmann, M., Kilkenny, F.F. & Krauss, S.L. (2018) Priority actions to improve provenance decision-making. *Bioscience*, **68**, 510-516.
- Brigham, L.M. & N. Suding, K. (2023) Cross-scale facilitation: a framework for microclimate moderation of climate change. *Oikos*, e10241.
- Brundrett, M.C. & Abbott, L. (1991) Roots of jarrah forest plants. I. Mycorrhizal associations of shrubs and herbaceous plants. *Australian journal of botany*, **39**, 445-457.
- Butt, N., Chauvenet, A.L., Adams, V.M., Beger, M., Gallagher, R.V., Shanahan, D.F., Ward, M., Watson, J.E. & Possingham, H.P. (2021) Importance of species translocations under rapid climate change. *Conservation Biology*, **35**, 775-783.
- Bykova, O., Chuine, I., Morin, X. & Higgins, S.I. (2012) Temperature dependence of the reproduction niche and its relevance for plant species distributions. *Journal of Biogeography*, **39**, 2191-2200.
- Cornelissen, J., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D., Reich, P., Ter Steege, H., Morgan, H. & Van Der Heijden, M. (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian journal of botany*, **51**, 335-380.
- CSIRO & BOM (2015) Climate change in Australia information for Australia's natural resource management regions: technical report. CSIRO and Bureau of Meteorology Australia,
- De Kort, H., Panis, B., Helsen, K., Douzet, R., Janssens, S.B. & Honnay, O. (2020) Pre-adaptation to climate change through topography-driven phenotypic plasticity. *Journal of ecology*, **108**, 1465-1474.
- Department of Biodiversity Conservation and Attractions (2016) Biodiversity Conservation Act 2016. Government of Western Australia,,
- Des Roches, S., Post, D.M., Turley, N.E., Bailey, J.K., Hendry, A.P., Kinnison, M.T., Schweitzer, J.A. & Palkovacs, E.P. (2018) The ecological importance of intraspecific variation. *Nature Ecology & Evolution*, **2**, 57-64.
- Descamps, C., Quinet, M. & Jacquemart, A.-L. (2020) The effects of drought on plant–pollinator interactions: What to expect? *Environmental and experimental botany*, **182**, 104297.

- Díaz, S., Kattge, J., Cornelissen, J.H., Wright, I.J., Lavorel, S., Dray, S., Reu, B., Kleyer, M., Wirth, C. & Colin Prentice, I. (2016) The global spectrum of plant form and function. *Nature*, **529**, 167-171.
- Dube, O., Allen, M., Solecki, W., Aragón-Durand, F., Cramer, W., Humphreys, S., Kainuma, M., Kala, J., Mahowald, N. & Mulugetta, Y. (2018) *Chapter 1: Framing and Context In Global Warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty*. Cambridge University Press, Cambridge, UK and New York, NY, USA.
- Dwyer, J.M. & Laughlin, D.C. (2017) Selection on trait combinations along environmental gradients. *Journal of Vegetation Science*, **28**, 672-673.
- Fick, S.E. & Hijmans, R.J. (2017) WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, **37**, 4302-4315.
- Figueiredo, F.O.G., Zuquim, G., Tuomisto, H., Moulatlet, G.M., Balslev, H. & Costa, F.R.C. (2017) Beyond climate control on species range: The importance of soil data to predict distribution of Amazonian plant species. *Journal of Biogeography*, **45**, 190-200.
- Fitzpatrick, M.C., Gove, A.D., Sanders, N.J. & Dunn, R.R. (2008) Climate change, plant migration, and range collapse in a global biodiversity hotspot: the *Banksia* (Proteaceae) of Western Australia. *Global Change Biology*, **14**, 1337-1352.
- Fletcher, A.L., Chen, C., Ota, N., Lawes, R.A. & Oliver, Y.M. (2020) Has historic climate change affected the spatial distribution of water-limited wheat yield across Western Australia? *Climatic Change*, **159**, 347-364.
- Gardner, J.H. & Bell, D.T. (2007) Bauxite mining restoration by Alcoa World Alumina Australia in Western Australia: social, political, historical, and environmental contexts. *Restoration Ecology*, **15**, 3-10.
- Goldstein, I. & Ehrenreich, I.M. (2021) Genetic variation in phenotypic plasticity. *Phenotypic Plasticity & Evolution*, 91.
- Grassein, F., Till-Bottraud, I. & Lavorel, S. (2010) Plant resource-use strategies: the importance of phenotypic plasticity in response to a productivity gradient for two subalpine species. *Annals of Botany*, **106**, 637-645.
- Gratani, L. (2014) Plant phenotypic plasticity in response to environmental factors. *Advances in botany*, **2014**

- Gugger, S., Kesselring, H., Stöcklin, J. & Hamann, E. (2015) Lower plasticity exhibited by high- versus mid-elevation species in their phenological responses to manipulated temperature and drought. *Annals of Botany*, **116**, 953-962.
- Hallmark, C.T., Wilding, L.P. & Smeck, N.E. (1983) Silicon. *Methods of Soil Analysis*, pp. 263-273.
- Harrison, P.A. (2021) Climate change and the suitability of local and non-local species for ecosystem restoration. *Ecological Management & Restoration*, **22**, 75-91.
- Heilmeyer, H. (2019) Functional traits explaining plant responses to past and future climate changes. *Flora*, **254**, 1-11.
- Henn, J.J., Buzzard, V., Enquist, B.J., Halbritter, A.H., Klanderud, K., Maitner, B.S., Michaletz, S.T., Pötsch, C., Seltzer, L., Telford, R.J., Yang, Y., Zhang, L. & Vandvik, V. (2018) Intraspecific trait variation and phenotypic plasticity mediate alpine plant species response to climate change. *Frontiers in Plant Science*, **9**, 1548.
- Hervé, M. & Hervé, M.M. (2020) Package 'RVAideMemoire'. See <https://CRAN.R-project.org/package=RVAideMemoire>.
- Hopper, S.D. (2009) OCBIL theory: towards an integrated understanding of the evolution, ecology and conservation of biodiversity on old, climatically buffered, infertile landscapes. *Plant and soil*, **322**, 49-86.
- Hopper, S.D., Lambers, H., Silveira, F.A.O. & Fiedler, P.L. (2021) OCBIL theory examined: reassessing evolution, ecology and conservation in the world's ancient, climatically buffered and infertile landscapes. *Biological Journal of the Linnean Society*, **133**, 266-296.
- Hufford, K.M., Krauss, S.L. & Veneklaas, E.J. (2012) Inbreeding and outbreeding depression in *Stylidium hispidum*: implications for mixing seed sources for ecological restoration. *Ecology and evolution*, **2**, 2262-2273.
- Hufford, K.M., Veneklaas, E.J., Lambers, H. & Krauss, S.L. (2016) Genetic delineation of local provenance defines seed collection zones along a climate gradient. *AoB Plants*, **8**, 1-12.
- Hurtado, P., Prieto, M., Martínez-Vilalta, J., Giordani, P., Aragón, G., López-Angulo, J., Košuthová, A., Merinero, S., Díaz-Peña, E. & Rosas, T. (2020) Disentangling functional trait variation and covariation in epiphytic lichens along a continent-wide latitudinal gradient. *Proceedings of the Royal Society B*, **287**, 20192862.
- Huston, M.A. (2012) Precipitation, soils, NPP, and biodiversity: resurrection of Albrecht's curve. *Ecological Monographs*, **82**, 277-296.

- IPCC (2023) *Summary for Policymakers. In: Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]*. IPCC c/o World Meteorological Organization (WMO), Geneva, Switzerland.
- Jakob, D., Imielska, A., Charles, S., Fu, G., Frederiksen, C., Frederiksen, J., Zidikheri, M., Hope, P., Keay, K., Ganter, C., Li, Y., Phatak, A., Palmer, M., Wormworth, J., McBride, J., Dare, R., Lavender, S., Abbs, D., Rotstayn, L. & Rafter, T. (2012) *Western Australia's Weather and Climate: A Synthesis of Indian Ocean Climate Initiative Stage 3 Research*. CSIRO and BoM, Australia.
- Jansson, J.K. & Hofmockel, K.S. (2020) Soil microbiomes and climate change. *Nature Reviews Microbiology*, **18**, 35-46.
- Joswig, J.S., Wirth, C., Schuman, M.C., Kattge, J., Reu, B., Wright, I.J., Sippel, S.D., Rüger, N., Richter, R. & Schaepman, M.E. (2022) Climatic and soil factors explain the two-dimensional spectrum of global plant trait variation. *Nature Ecology & Evolution*, **6**, 36-50.
- Kandlikar, G.S., Kleinhesselink, A.R. & Kraft, N.J.B. (2022) Functional traits predict species responses to environmental variation in a California grassland annual plant community. *Journal of Ecology*, **110**, 833-844.
- Koch, J.M. & Samsa, G.P. (2007) Restoring Jarrah forest trees after bauxite mining in Western Australia. *Restoration Ecology*, **15**, S17-S25.
- Kühn, P., Ratier Backes, A., Römermann, C., Bruelheide, H. & Haider, S. (2021) Contrasting patterns of intraspecific trait variability in native and non-native plant species along an elevational gradient on Tenerife, Canary Islands. *Annals of Botany*, **127**, 565-576.
- Lambers, H., Albornoz, F., Kotula, L., Laliberté, E., Ranathunge, K., Teste, F.P. & Zemunik, G. (2018) How belowground interactions contribute to the coexistence of mycorrhizal and non-mycorrhizal species in severely phosphorus-impooverished hyperdiverse ecosystems. *Plant and soil*, **424**, 11-33.
- Lambers, H., Brundrett, M.C., Raven, J.A. & Hopper, S.D. (2011) Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant and soil*, **348**, 7-27.
- Lambers, H., Martinoia, E. & Renton, M. (2015) Plant adaptations to severely phosphorus-impooverished soils. *Current Opinion in Plant Biology*, **25**, 23-31.

- Lavorel, S., Díaz, S., Cornelissen, J.H.C., Garnier, E., Harrison, S.P., McIntyre, S., Pausas, J.G., Pérez-Harguindeguy, N., Roumet, C. & Urcelay, C. (2007) Plant functional types: are we getting any closer to the Holy Grail? *Terrestrial ecosystems in a changing world* (eds J. Canadell, D. Pataki & L. Pitelka), pp. 149-164. Springer,
- Lawrence, J., Mackey, B., Chiew, F., Costello, M., Hennessy, K., Lansbury, N., Nidumolu, U., Pecl, G., Rickards, L. & Tapper, N. (2022) Australasia. Climate Change 2022: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (eds.)]. pp. 1581-1688. Cambridge University Press, Cambridge, UK and New York, NY, USA.
- Lê, S., Josse, J. & Husson, F. (2008) FactoMineR: an R package for multivariate analysis. *Journal of statistical software*, **25**, 1-18.
- Legendre, P. & Legendre, L. (2012) *Numerical ecology*. Elsevier, Oxford, UK.
- Leimu, R. & Fischer, M. (2008) A meta-analysis of local adaptation in plants. *Plos One*, **3**, e4010.
- Liu, H., Yin, D., He, P., Cadotte, M.W. & Ye, Q. (2024) Linking plant functional traits to biodiversity under environmental change. *Biological Diversity*, **1**, 22-28.
- Lloyd, J. & Taylor, J.A. (1994) On the Temperature Dependence of Soil Respiration. *Functional Ecology*, **8**, 315.
- Long, A., Zhang, J., Yang, L.-T., Ye, X., Lai, N.-W., Tan, L.-L., Lin, D. & Chen, L.-S. (2017) Effects of low pH on photosynthesis, related physiological parameters, and nutrient profiles of citrus. *Frontiers in Plant Science*, **8**, 185.
- Lowrie, A. & Kenneally, K.F. (2017) A taxonomic revision of the basin-like rosetted triggerplants of the *Stylidium piliferum* complex (Stylidiaceae) from south-western Australia. *Nuytsia*, **28**, 339-382.
- Maire, V., Wright, I.J., Prentice, I.C., Batjes, N.H., Bhaskar, R., van Bodegom, P.M., Cornwell, W.K., Ellsworth, D., Niinemets, Ü., Ordóñez, A., Reich, P.B. & Santiago, L.S. (2015) Global effects of soil and climate on leaf photosynthetic traits and rates. *Global ecology and biogeography*, **24**, 706-717.
- Malhotra, H., Vandana, Sharma, S. & Pandey, R. (2018) Phosphorus nutrition: plant growth in response to deficiency and excess. *Plant nutrients and abiotic stress tolerance*, 171-190.

- Martins, N., Osório, M.L., Gonçalves, S., Osório, J. & Romano, A. (2013) Differences in Al tolerance between *Plantago algarbiensis* and *P. almogravensis* reflect their ability to respond to oxidative stress. *Biometals*, **26**, 427-437.
- Mason, C.M., McGaughey, S.E. & Donovan, L.A. (2013) Ontogeny strongly and differentially alters leaf economic and other key traits in three diverse *Helianthus* species. *Journal of experimental botany*, **64**, 4089-4099.
- Matesanz, S., Horgan-Kobelski, T. & Sultan, S.E. (2012) Phenotypic plasticity and population differentiation in an ongoing species invasion. *Plos One*, **7**, e44955.
- McGill, B.J., Enquist, B.J., Weiher, E. & Westoby, M. (2006) Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution*, **21**, 178-185.
- Merritt, D.J. & Dixon, K.W. (2011) Restoration seed banks—a matter of scale. *Science*, **332**, 424-425.
- Millar, C.I., Stephenson, N.L. & Stephens, S.L. (2007) Climate change and forests of the future: managing in the face of uncertainty. *Ecological Applications*, **17**, 2145-2151.
- Miller, A.D., Nitschke, C., Weeks, A.R., Weatherly, W.L., Heyes, S.D., Sinclair, S.J., Holland, O.J., Stevenson, A., Broadhurst, L. & Hoebee, S.E. (2020) Genetic data and climate niche suitability models highlight the vulnerability of a functionally important plant species from south-eastern Australia. *Evolutionary applications*, **13**, 2014-2029.
- Moles, A.T., Perkins, S.E., Laffan, S.W., Flores-Moreno, H., Awasthy, M., Tindall, M.L., Sack, L., Pitman, A., Kattge, J. & Aarssen, L.W. (2014) Which is a better predictor of plant traits: temperature or precipitation? *Journal of Vegetation Science*, **25**, 1167-1180.
- Moreno-Jiménez, E., Plaza, C., Saiz, H., Manzano, R., Flagmeier, M. & Maestre, F.T. (2019) Aridity and reduced soil micronutrient availability in global drylands. *Nature Sustainability*, **2**, 371-377.
- Neina, D. (2019) The role of soil pH in plant nutrition and soil remediation. *Applied and Environmental Soil Science*, **2019**, 1-9.
- Nemchin, A. & Pidgeon, R. (1997) Evolution of the Darling range batholith, Yilgarn craton, western Australia: a SHRIMP zircon study. *Journal of Petrology*, **38**, 625-649.
- Nicotra, A.B., Atkin, O.K., Bonser, S.P., Davidson, A.M., Finnegan, E.J., Mathesius, U., Poot, P., Purugganan, M.D., Richards, C.L., Valladares, F. & van Kleunen, M. (2010) Plant phenotypic plasticity in a changing climate. *Trends in Plant Science*, **15**, 684-692.
- Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M.H.H., Oksanen, M.J. & Suggests, M. (2007) The vegan package. *Community ecology package*, **10**, 631-637.

- Onley, I.R., Moseby, K.E. & Austin, J.J. (2021) Genomic approaches for conservation management in Australia under climate change. *Life*, **11**, 653.
- Ordoñez, J.C., Van Bodegom, P.M., Witte, J.P.M., Wright, I.J., Reich, P.B. & Aerts, R. (2009) A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. *Global Ecology and Biogeography*, **18**, 137-149.
- Perez-Harguindeguy, N., Diaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M.S., Cornwell, W.K., Craine, J.M. & Gurvich, D.E. (2016) Corrigendum to: new handbook for standardised measurement of plant functional traits worldwide. *Australian journal of botany*, **64**, 715-716.
- R Development Core Team, R. (2022) RStudio: Integrated Development Environment for R. Boston, MA: RStudio, Inc; 2016.
- Rayment, G.E. & Lyons, D.J. (2011) *Soil chemical methods: Australasia*. CSIRO publishing,
- Reich, P.B., Wright, I.J., Cavender-Bares, J., Craine, J., Oleksyn, J., Westoby, M. & Walters, M. (2003) The evolution of plant functional variation: traits, spectra, and strategies. *International Journal of Plant Sciences*, **164**, S143-S164.
- Rosas, T., Mencuccini, M., Barba, J., Cochard, H., Saura-Mas, S. & Martínez-Vilalta, J. (2019) Adjustments and coordination of hydraulic, leaf and stem traits along a water availability gradient. *New Phytologist*, **223**, 632-646.
- Sakamoto, Y., Ishiguro, M. & Kitagawa, G. (1986) Akaike information criterion statistics. *Dordrecht, The Netherlands: D. Reidel*, **81**, 26853.
- Schlichting, C.D. (1986) The evolution of phenotypic plasticity in plants. *Annual Review of Ecology and Systematics*, **17**, 667-693.
- Schneider, H.M. (2022) Characterization, costs, cues, and future perspectives of phenotypic plasticity. *Annals of Botany*, **130**, 131-148.
- Shipley, B., De Bello, F., Cornelissen, J.H.C., Laliberté, E., Laughlin, D.C. & Reich, P.B. (2016) Reinforcing loose foundation stones in trait-based plant ecology. *Oecologia*, **180**, 923-931.
- Simpson, A.H., Richardson, S.J. & Laughlin, D.C. (2016) Soil–climate interactions explain variation in foliar, stem, root and reproductive traits across temperate forests. *Global Ecology and Biogeography*, **25**, 964-978.
- Soliveres, S., Eldridge, D.J., Maestre, F.T., Bowker, M.A., Tighe, M. & Escudero, A. (2011) Microhabitat amelioration and reduced competition among understorey plants as drivers of facilitation across environmental gradients: towards a unifying framework. *Perspectives in Plant Ecology, Evolution and Systematics*, **13**, 247-258.

- Stotz, G.C., Salgado-Luarte, C., Escobedo, V.M., Valladares, F. & Gianoli, E. (2021) Global trends in phenotypic plasticity of plants. *Ecology Letters*, **24**, 2267-2281.
- Sudmeyer, R., Edward, A., Fazakerley, V., Simpkin, L. & Foster, I. (2016) Climate change: Impacts and adaptation for agriculture in Western Australia. Department of Agriculture and Food, Western Australia, Perth.
- Suzuki, N., Rivero, R.M., Shulaev, V., Blumwald, E. & Mittler, R. (2014) Abiotic and biotic stress combinations. *New Phytologist*, **203**, 32-43.
- Valladares, F., Gianoli, E. & Gómez, J.M. (2007) Ecological limits to plant phenotypic plasticity. *The New Phytologist*, **176**, 749-763.
- Valladares, F., Matesanz, S., Guilhaumon, F., Araújo, M.B., Balaguer, L., Benito-Garzón, M., Cornwell, W., Gianoli, E., van Kleunen, M., Naya, D.E., Nicotra, A.B., Poorter, H. & Zavala, M.A. (2014) The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecology Letters*, **17**, 1351-1364.
- Valliere, J.M., Ruscalleda Alvarez, J., Cross, A.T., Lewandrowski, W., Riviera, F., Stevens, J.C., Tomlinson, S., Tudor, E.P., Wong, W.S. & Yong, J.W. (2021) Restoration ecophysiology: an ecophysiological approach to improve restoration strategies and outcomes in severely disturbed landscapes. *Restoration Ecology*, **30**, e13571.
- Violle, C., Enquist, B.J., McGill, B.J., Jiang, L., Albert, C.H., Hulshof, C., Jung, V. & Messier, J. (2012) The return of the variance: intraspecific variability in community ecology. *Trends in Ecology & Evolution*, **27**, 244-252.
- Violle, C., Navas, M.L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I. & Garnier, E. (2007) Let the concept of trait be functional! *Oikos*, **116**, 882-892.
- Visakorpi, K., Block, S., Pellissier, L., Levine, J.M. & Alexander, J. (2022) Eco-physiological and morphological traits explain alpine plant species' response to warming. *Functional Ecology*, **37**, 287-301.
- Viscarra Rossel, R.A. & Bui, E.N. (2016) A new detailed map of total phosphorus stocks in Australian soil. *The Science of the Total Environment*, **542**, 1040-1049.
- Western Australian Herbarium (1998-) Florabase—the Western Australian Flora. Department of Biodiversity, Conservation and Attractions. Available from <https://florabase.dpaw.wa.gov.au/>.
- Westoby, M. & Wright, I.J. (2006) Land-plant ecology on the basis of functional traits. *Trends in Ecology & Evolution*, **21**, 261-268.
- Wiens, J.J. (2016) Climate-related local extinctions are already widespread among plant and animal species. *PLoS biology*, **14**, e2001104.

- Yang, J., Lu, J., Chen, Y., Yan, E., Hu, J., Wang, X. & Shen, G. (2020) Large underestimation of intraspecific trait variation and its improvements. *Frontiers in Plant Science*, **11**, 1-9.
- Zandalinas, S.I., Mittler, R., Balfagón, D., Arbona, V. & Gómez-Cadenas, A. (2018) Plant adaptations to the combination of drought and high temperatures. *Physiologia Plantarum*, **162**, 2-12.

1.7. Supplementary information for Chapter Two

Table S2.1: Summary statistics (mean $\pm$ standard error) for climate and soil factors across five sampling locations in the Southwest of Western Australia. JU: Julimar Conservation Reserve; JF: John Forrest National Park; BU: Bungendore Park; DP: State Forest [Dwellingup]; HO: State Forest [Hoffman]).

Factor	Abv.	JU	JF	BU	DP	HO
Ammonium Nitrate (NH ₄ NO ₃ ; mg kg ⁻¹)	N	6.80 $\pm$ 0.66	26.2 $\pm$ 4.75	11.8 $\pm$ 1.24	11.0 $\pm$ 2.28	8.40 $\pm$ 5.66
Colwell Phosphorus (mg kg ⁻¹)	P	4.00 $\pm$ 0.31	8.80 $\pm$ 0.86	6.20 $\pm$ 0.80	5.60 $\pm$ 0.51	5.60 $\pm$ 1.07
Colwell Potassium (mg kg ⁻¹)	K	0.08 $\pm$ 0.01	0.35 $\pm$ 0.06	0.11 $\pm$ 0.02	0.10 $\pm$ 0.02	0.08 $\pm$ 0.02
Sulphate Sulfur (mg kg ⁻¹)	S	5.48 $\pm$ 0.44	18.0 $\pm$ 3.06	8.48 $\pm$ 1.21	8.64 $\pm$ 1.67	7.54 $\pm$ 2.36
Organic Carbon (%)	OC	2.29 $\pm$ 0.19	3.95 $\pm$ 0.33	3.85 $\pm$ 0.38	3.22 $\pm$ 0.54	2.21 $\pm$ 0.66
Soil pH (in H ₂ O)	pH	5.74 $\pm$ 0.06	5.76 $\pm$ 0.12	5.82 $\pm$ 0.02	5.40 $\pm$ 0.11	5.46 $\pm$ 0.15
Copper (mg kg ⁻¹)	Cu	0.30 $\pm$ 0.02	0.29 $\pm$ 0.04	0.31 $\pm$ 0.02	0.27 $\pm$ 0.02	0.24 $\pm$ 0.03
Iron (mg kg ⁻¹)	Fe	27.1 $\pm$ 3.68	47.5 $\pm$ 5.92	30.8 $\pm$ 2.82	47.1 $\pm$ 10.4	40.1 $\pm$ 13.6
Manganese (mg kg ⁻¹)	Mn	11.2 $\pm$ 0.86	5.99 $\pm$ 1.85	15.9 $\pm$ 3.25	9.62 $\pm$ 2.07	10.9 $\pm$ 5.06
Zinc (mg kg ⁻¹)	Zn	0.21 $\pm$ 0.03	0.64 $\pm$ 0.19	0.63 $\pm$ 0.22	0.22 $\pm$ 0.04	0.14 $\pm$ 0.01
Boron (mg kg ⁻¹)	B	0.30 $\pm$ 0.02	0.74 $\pm$ 0.12	0.33 $\pm$ 0.06	0.28 $\pm$ 0.04	0.30 $\pm$ 0.08
Aluminium (meq/100g)	Al	0.32 $\pm$ 0.05	0.80 $\pm$ 0.22	0.26 $\pm$ 0.04	0.82 $\pm$ 0.23	0.79 $\pm$ 0.38
Calcium (meq/100g)	Ca	2.93 $\pm$ 0.33	5.01 $\pm$ 0.85	5.20 $\pm$ 1.46	2.14 $\pm$ 0.53	1.33 $\pm$ 0.82
Magnesium (meq/100g)	Mg	0.94 $\pm$ 0.11	1.51 $\pm$ 0.27	1.18 $\pm$ 0.34	0.66 $\pm$ 0.17	0.39 $\pm$ 0.23
Sodium (meq/100g)	Na	0.08 $\pm$ 0.01	0.45 $\pm$ 0.08	0.12 $\pm$ 0.02	0.11 $\pm$ 0.03	0.08 $\pm$ 0.03
Mean annual temperature (°C)	MAT	17.5 $\pm$ 0.00	17.3 $\pm$ 0.00	17.1 $\pm$ 0.00	16.9 $\pm$ 0.00	16.6 $\pm$ 0.00
Mean annual precipitation (mm)	MAP	716 $\pm$ 0.00	927 $\pm$ 0.00	1103 $\pm$ 0.00	1183 $\pm$ 0.00	1029 $\pm$ 0.00
Mean ambient temperature (monthly; °C)	T_a_{mean}	19.3 $\pm$ 0.94	19.5 $\pm$ 1.29	18.5 $\pm$ 0.99	18.1 $\pm$ 0.94	17.9 $\pm$ 1.49
Temperature variability (monthly; °C)	ΔT_a	37.6 $\pm$ 1.00	32.3 $\pm$ 1.35	34.7 $\pm$ 1.60	34.6 $\pm$ 1.24	34.8 $\pm$ 1.35
Minimum temperature (monthly; °C)	T_a_{min}	7.00 $\pm$ 0.45	8.66 $\pm$ 0.79	6.30 $\pm$ 0.54	6.64 $\pm$ 0.36	6.98 $\pm$ 0.59
Maximum temperature (monthly; °C)	T_a_{max}	44.6 $\pm$ 1.25	41.0 $\pm$ 1.52	41.0 $\pm$ 1.95	41.3 $\pm$ 1.48	41.7 $\pm$ 1.78
Cumulative rainfall (monthly; mm)	RF	1014 $\pm$ 332	670 $\pm$ 224	1209 $\pm$ 470	1332 $\pm$ 566	1172 $\pm$ 429
Relative humidity (monthly; %)	RH	43.3 $\pm$ 1.38	48.7 $\pm$ 1.10	49.6 $\pm$ 1.44	54.1 $\pm$ 1.53	48.4 $\pm$ 5.37
Mean volumetric soil moisture content (θ)	θVol_{mean}	0.23 $\pm$ 0.02	0.19 $\pm$ 0.02	0.20 $\pm$ 0.02	0.23 $\pm$ 0.02	0.25 $\pm$ 0.02
Volumetric soil moisture variability (Δθ)	ΔθVol	0.17 $\pm$ 0.05	0.11 $\pm$ 0.03	0.15 $\pm$ 0.04	0.20 $\pm$ 0.07	0.15 $\pm$ 0.04
Mean photosynthetically active radiation (mmol m ⁻² s ⁻¹)	PAR	773 $\pm$ 109	807 $\pm$ 32.5	945 $\pm$ 69.9	528 $\pm$ 137	468 $\pm$ 39.5
Atmospheric vapour pressure deficit (kPa)	VPD_a	2.74 $\pm$ 0.12	1.97 $\pm$ 0.13	2.63 $\pm$ 0.19	1.91 $\pm$ 0.35	1.71 $\pm$ 0.13

Table S2.2: Summary statistics (mean $\pm$ standard error) for climate factors across sampling months.

Factor	Abv.	October	November	December
Mean ambient temperature ($^{\circ}\text{C}$)	T_a_{mean}	15.0 $\pm$ 0.06	18.8 $\pm$ 0.07	22.1 $\pm$ 0.10
Temperature variability ($^{\circ}\text{C}$)	ΔT_a	30.5 $\pm$ 0.21	37.5 $\pm$ 0.16	36.5 $\pm$ 0.28
Minimum temperature ($^{\circ}\text{C}$)	T_a_{min}	5.51 $\pm$ 0.09	6.91 $\pm$ 0.06	8.87 $\pm$ 0.15
Maximum temperature ($^{\circ}\text{C}$)	T_a_{max}	36.0 $\pm$ 0.23	44.4 $\pm$ 0.15	45.3 $\pm$ 0.16
Mean cumulative rainfall (mm)	RF	217 $\pm$ 8.65	28.3 $\pm$ 1.16	6.79 $\pm$ 0.96
Mean volumetric soil moisture content (θ)	$\theta\text{Vol}_{\text{mean}}$	0.28 $\pm$ 0.01	0.21 $\pm$ 0.01	0.16 $\pm$ 0.01
Relative humidity (%)	RH	46.8 $\pm$ 0.97	50.4 $\pm$ 0.79	43.2 $\pm$ 0.68
Volumetric soil moisture variability ($\Delta\theta$)	$\Delta\theta\text{Vol}$	0.28 $\pm$ 0.01	0.13 $\pm$ 0.01	0.03 $\pm$ 0.01
Mean photosynthetically active radiation ($\text{mmol m}^{-2}\text{s}^{-1}$)	PAR	725 $\pm$ 34.2	769 $\pm$ 39.2	580 $\pm$ 24.7
Atmospheric vapour pressure deficit (kPa)	VPD_a	1.63 $\pm$ 0.06	2.05 $\pm$ 0.09	2.66 $\pm$ 0.04

2. CO-ORDINATED TRAIT SHIFTS IN RESPONSE TO TEMPERATURE AND WATER AVAILABILITY REFLECT AN INTRASPECIFIC GRADIENT OF ADAPTIVE STRATEGIES IN *STYLIDIUM HISPIDUM*

EMILY P. TUDOR^{1,2}, WOLFGANG LEWANDROWSKI^{2,1}, SIEGFRIED KRAUSS^{2,1}, ERIK J. VENEKLAAS¹

¹*School of Biological Sciences, University of Western Australia, Nedlands, WA 6009, Australia.* ²*Kings Park Science, Department of Biodiversity Conservation and Attractions, West Perth, WA 6005, Australia.*

2.1. Abstract

Addressing the drivers of intraspecific variation in plant functional traits is essential for predicting the capacity of a species to adapt to environmental change. Here, we sought to identify intraspecific variation in plant functional traits and how they align with various functional strategies (i.e., competitive/tolerant/avoidant) in response to interacting effects of warming and water deficit. To do so, we examined plants from four spatially distinct populations of a perennial herb (*Stylidium hispidum* Lindl.) spread across a suite of climates in southwest Western Australia. We assessed 19 morphological, physiological, phenological and floral traits under manipulated temperature (20/10, 25/15 and 30/20°C) and watering (well-watered, water-deficit) regimes. Our results demonstrated competitive strategies, facilitated by traits that maximise growth, were dominant under cooler, well-watered conditions, whereas avoidance and tolerance strategies, that mitigate against abiotic stress, were both associated with the elevated temperatures and water-deficit, and expressed more so in the northern-most population sourced from a hotter and drier climate. The southernmost population, originating from a cooler and wetter climate, had reduced functional richness compared to more northern populations. Our findings established that *S. hispidum* populations are not homogenous in their functional strategies but rather exhibit trait combinations that are associated with climate of origin suggesting that local adaptation is a key driver of functional diversity. Moreover, the southern population may be disproportionately vulnerable to climate change due to the limited functional richness constraining the populations' ability to cope with environmental stressors and increasing the likelihood of local extinctions if populations cannot adapt quickly enough to changing conditions.

Keywords: Climate Change, Drought, Functional Traits, Population Biology, Temperature, *Stylidium*.

2.2. Introduction

The capacity of species to persist across their distribution, as well as to adapt to future conditions, is contingent, at least in part, upon functional strategies that enable them to cope with their local environment (Anderson, Panetta & Mitchell-Olds 2012; Anderson & Song 2020). Contemporary rates of climate change can cause morphological, physiological and phenological changes to plants, which can have cascading effects to population persistence and community compositions (Heilmeier 2019; Hassan *et al.* 2023; Sato *et al.* 2024). Climate change is one of the most complex and difficult anthropogenic threats to manage due to the uncertainty of species capacities to adapt, avoid or tolerate change (Hoffmann & Sgrò 2011; Merilä & Hoffmann 2016; Hoffmann *et al.* 2019). Trait-based approaches offer a robust empirical framework for explaining patterns of variation and predicting the impacts of environmental changes across the suite of biological scales (Bjorkman *et al.* 2018; Heilmeier 2019).

As functional traits are closely tied to environmental conditions and ecological contexts, they can serve as meaningful indicators of the environmental conditions, or ‘niche breadths,’ which species persist in (Violle & Jiang 2009; Schellenberger Costa *et al.* 2018). However, trait-based ecological research has primarily focused on interspecific variation, with comparative studies often seeking to examine trait variation across ecological gradients for several species (Westerband, Funk & Barton 2021). Assessing the patterns and processes driving trait variation among populations across an environmental gradient helps to understand the mechanisms underpinning higher order processes such as population persistence and species distribution. Recently, calls have been made for future work to unpack how intraspecific trait variation (ITV) influences such higher order processes and shapes species resilience to environmental change (Westerband, Funk & Barton 2021). Therefore, achieving this aim can provide critical insights into how species’ respond to environmental stressors and shed light on the adaptability and evolution of species under changing conditions. This knowledge can simultaneously inform management strategies that can increase population resilience to climate change (Ahrens, Rymer & Tissue 2021).

Since individuals within a population exhibit many traits, selection acting on one trait inevitably induces concomitant shifts in others (Carmona *et al.* 2016; Funk *et al.* 2017). Such trait shifts can happen through direct changes in plant phenotypes in response to environmental change (phenotypic plasticity) and can also arise from genetic variation among different

populations (Nicotra *et al.* 2010). Therefore, determining how populations vary in their functional trait space (Puglielli *et al.* 2024), and the potential for these traits to change through plasticity or evolutionary processes (Nicotra *et al.* 2010), is fundamental to predicting responses to global change (Andrew *et al.* 2022). In this context, it is essential to establish quantitative links between phenotypes and performance in various environmental settings. The primary experimental approaches to achieve this include sampling across environmental gradients to observe trait responses, reciprocal transplant experiments to test local adaptation by comparing individuals between different environments, and common garden experiments to grow different genotypes in standardised environments (De Frenne *et al.* 2013; Schwinning *et al.* 2022).

Existing trait-based research is dominated by univariate approaches, and multidimensional trait diversity patterns have been comparatively overlooked due to the challenges associated with modelling and comparing complex representations of the functional trait space (Blonder 2018). However, a growing body of research advocates for trait relationships to be evaluated using multivariate approaches as they can provide valuable insights that move beyond univariate interpretations to reveal the emergent patterns of trait combinations across populations (Laughlin *et al.* 2020; Westerland, Funk & Barton 2021). In addition, an understanding of trait variance (i.e., functional diversity) is also important as individuals vary in their responses to current conditions, which influences the trajectory of a population's adaptive capacity over time (Anderegg *et al.* 2021). This functional diversity is crucial for understanding the multifaceted ways plants adapt and cope with their local environmental challenges, as these often involve inherent trade-offs between different physiological, morphological, and phenological attributes.

Plants have evolved various traits to cope with environmental stressors through physiological, morphological and phenological mechanisms (Hemantaranjan *et al.* 2014; Basu *et al.* 2016). Such complex trait combinations often coalesce into distinct ecological strategies, a concept formally captured by frameworks such as the Competitive–Stress Tolerator–Ruderal (CSR) theory (Grime 1979; Grime 1988). This general approach has also been extended to convey trade-offs that correspond to a spectrum of competitive, tolerance or avoidance strategies to cope with stressors (Grime 1979; Adler *et al.* 2014; Ravenscroft, Fridley & Grime 2014). Traits that define a competitive strategy can include high specific leaf area (SLA), photosynthetic capacity, and vegetative allocation (Grime 1979; Reich *et al.* 2003). Collectively, these traits can facilitate rapid growth, leading to high respiration and leaf and

root turnover rates, at the cost of retaining resources (Grime 1979; Reich *et al.* 2003; Ravenscroft, Fridley & Grime 2014). Tolerance strategies are characterised by slow growth rates, coupled with high water and resource-use efficiency to tolerate periods of abiotic stress (Osmond *et al.* 1987). This often occurs through a combination of conservative stomatal regulation, reduced transpiration and lower maximal photosynthetic rates, along with smaller specific leaf areas (SLA) and increased leaf thickness amounting to reductions in total biomass (Grime 1979; Reich *et al.* 2003; Díaz *et al.* 2016). Avoidance strategies, on the other hand, are often characterised by phenological shifts with reproductive allocation that is associated with earlier growth and flowering periods, prior to the onset of stress (Geber & Dawson 1990; Heschel & Riginos 2005; Chandler & Bartels 2008). Physiological and morphological adaptations such as reduced vegetative allocation and increased root-to-shoot ratios, along with physiological offsets to minimise water-loss and maximise resource retention, are also often prevalent among avoidance strategies (Osmond *et al.* 1987; Kooyers 2015). While these strategies have been extensively documented between species, how they might vary among populations that occur across an environmental gradient across a species distribution has been rarely assessed (but see Estarague *et al.* 2022).

Our recent results showed the presence of clinal variation in morphological and physiological traits for five spatially distinct populations of *Stylidium hispidum* across an environmental gradient (Tudor *et al.* 2024). This gradient was defined by northern sites that were 1.6°C warmer than southern sites, on average, with maximum temperatures reaching 3.3°C higher, while the southern sites received almost double the rainfall (Tudor *et al.* 2024). Climatic factors accounted for a greater proportion the observed phenotypic divergence in the study, with temperature presenting the strongest association with trait data (Tudor *et al.* 2024). Here, we complement this existing work with controlled *ex-situ* approaches to isolate the specific effects of temperature and water availability. By controlling for environmental variation, ‘common garden’ studies can more accurately identify the genetic basis of phenotypic divergence and provide deeper insights into the adaptive mechanisms underlying clinal variation (Lortie & Hierro 2021; Schwinning *et al.* 2022). Therefore, our objective in this study was to characterise the multivariate trait space of a subset of the previously sampled populations within a controlled environment. More specifically, we sought to identify differences in trait expression and functional diversity and how they align with various ecological strategies in response to interacting effects of warming and water deficit. To do so, we assessed 17 morphological, physiological, phenological and floral traits that collectively

represent the primary axes of intraspecific functional variation. To characterise tolerance, avoidance, and competitive strategies within *S. hispidum* populations, we employed a modified CSR approach that quantified a subset of key functional traits and integrated them into indices reflecting established ecological trade-offs (Osmond *et al.* 1987; Ravenscroft, Fridley & Grime 2014). This approach was designed to align with established principles of trait-based ecology and CSR theory, while allowing flexibility to incorporate multiple traits and draw intraspecific comparisons. Due to the genetic differences and the latitudinal cline in the climate of origin for each *S. hispidum* population (Hufford *et al.* 2016; Tudor *et al.* 2024), it was hypothesised that the populations would exhibit responses associated with local climate. We expected populations originating from cooler and wetter climates to be more sensitive to combined water limitation and warmer conditions, therefore reflecting restricted functional diversity. We also hypothesised that avoidance or tolerance strategies would be expressed under hotter and drier conditions, while competitive strategies would be more prominent under cooler and wetter conditions.

2.3. Methods

2.3.1. Study species and propagation

Stylidium hispidum seeds were collected in December 2021 at dehiscence from four spatially distinct populations spanning a ~160 km latitudinal transect in southwest Western Australia (Figure 3.1; Refer to Tudor *et al.* (2024) for edaphic and climatic characterisations of these sites). At each site, core sampling occurring in a 200 m x 200 m plot with opportunistic collections from surrounding habitat to ensure a broad spectrum of population genetic diversity was captured. After raceme removal and shaking mature seeds into a collection bag, seeds were stored in a dry room conditioned to 15% relative humidity and 15°C before use. To stimulate germination, seeds were placed on filter paper (Advantec, Dublin, CA, USA: 84 mm seed-germination papers) in 90 mm Petri dishes that were irrigated with 8 ml of distilled water or PEG solution to different concentrations that contained 1% (v/v) PPM (Plant Preservation Mixture, Austratec Pty Ltd, Bayswater, Vic) and 1% (v/v) karrikinolide (KAR1; Flematti *et al.* 2004; Chiwocha *et al.* 2009) solutions to minimise microbial contamination and stimulate germination, respectively. Petri dishes were wrapped air-tight to further minimise contamination and reduce evaporation. Germinated seeds from each population were then transplanted into a gridded seedling tray and maintained in a 15°C-growth environment for four

weeks to allow for root establishment. Seedlings were then transplanted from the seedling tray into 950 ml forestry pots (filled with 65% native potting mix soil, 32% sand and 3% perlite) and grown under standardised glasshouse conditions maintained between 25/20°C (day/night conditions) at Kings Park Biodiversity and Conservation Centre and watered daily through irrigation drippers at a rate of 4 L/h for one minute every day until transfer into the climate-controlled growth chambers.

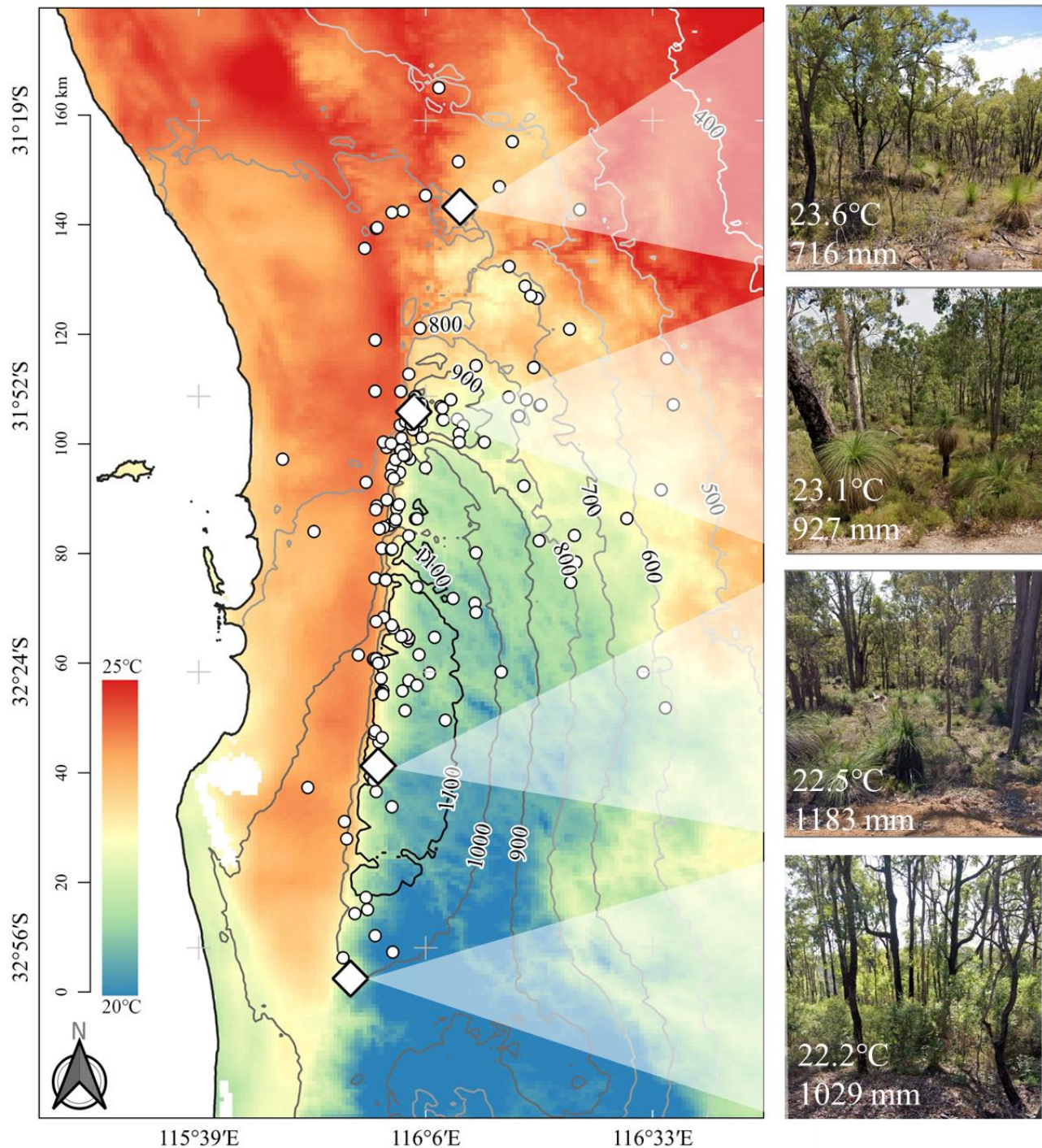


Figure 3.1 [Previous page]: Location of *Stylidium hispidum* source populations (◇; from north to south: Julimar Conservation Reserve, John Forrest National Park; State Forest [Dwellingup]; State Forest [Hoffman]) distributed across ~160 km latitudinal gradient along the Darling Scarp in the southwest corner of Western Australia. White points represent other known populations of *S. hispidum*. Grey-black lines are isohyets representing mean annual precipitation (mm; values overlayed) and spectral shading corresponds to mean temperatures (°C) in the warmest quarter (austral summer) with local values inset in adjacent images of each source population. Climate data reflect historical averages (1970-2000) sourced from Fick and Hijmans (2017).

All established plants were transferred into one of three growth chambers (Conviron Ltd, Winnipeg, Canada) in July 2023 and maintained in this environment for four months (n= 96 plants; three chambers comprised of four plants per population across each watering treatment, i.e., 32 plants per chamber). The experimental treatments consisted of a ‘well-watered’ and ‘water-limited’ treatment within three temperature conditions (20/10, 25/15 and 30/20°C) under an alternating 12-hour light/dark photoperiod between 9:00 am and 9:00 pm (photosynthetically active radiation; PAR: 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Relative humidity of the chambers was set to 50% and carbon dioxide concentrations maintained at 400 ppm. At the beginning of the experiment, all pots were watered to field capacity and weighed to obtain weight at pot capacity (1.1 kg; 42.86% gravimetric water content). Water was then withheld for pots under the water-limited treatments until pot weight reached 0.8 kg and then watered to maintain pot weight at 0.8 kg (3.90% gravimetric water content). Pots were weighed and watered every Monday, Wednesday and Friday and only watered if weight was below the threshold set (i.e., 1.1 kg or 0.8 kg). To validate experimental watering treatments, predawn leaf water potential measurements were undertaken during the dark period (i.e., before 9.00 am), using a Scholander Pressure Chamber (Model 1000, PMS Instruments Co, USA), whereby a single leaf was sampled from each experimental plant replicate and measured in the dark. Plants from the well-watered treatment had water potentials of -0.75 ± 0.03 MPa while those from water-limited treatments were -2.3 ± 0.08 MPa.

2.3.2. *Morphological and physiological parameters*

Morpho-physiological assessments were conducted every two weeks between the 26th of July and 6th of October. Each plant was characterised by rosette size (diameter and height; mm), raceme height (mm), raceme diameter (mm), number of racemes and number of flowers.

Simultaneous to the morphological measurements, physiological performance was characterised by photosynthetic assimilation rate (A) and stomatal conductance (g_s) using a LI-6400XT portable gas exchange system (LI-COR Biosciences, Lincoln, NE, USA) that was equipped with the standard 6400-07 (6 cm²) clear top chamber illuminated with an external 6400-18 RGB light source fixed at 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The internal leaf chamber conditions set to carbon dioxide (CO_2) concentrations of 400 $\mu\text{mol CO}_2 \text{mol}^{-1}$, relative humidity was maintained at 50% and temperature was set to match diurnal growth chamber conditions (i.e., 20, 25 or 30°C). Rosette leaves were displayed as fans upon insertion into the leaf chamber, ensuring minimal overlap between leaves. Measurements were taken when stability was reached, determined via visual analysis of real-time response curves accompanied by system stability indicators. At least three measurements were taken per plant, per sampling round. All gas exchange measurements were adjusted by the leaf area, by capturing a bird's eye photograph of the leaves within the chamber and through pixel analysis using ImageJ. Intrinsic water-use efficiency (WUE) was calculated as A/g_s . Chlorophyll content was measured using a CCM-300 Chlorophyll Content Meter (Opti-Sciences, Hudson, NH, USA), by placing the sensor head directly on the leaf surface of each plant and chlorophyll fluorescence (F_v/F_m) was captured using a Pocket PEA chlorophyll fluorometer (Hansatech Instruments Ltd, UK) with a saturation pulse of 3500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ following a 10 minute dark adaptation period on leaves. All physiological measurements were constrained to a sampling period between 09:00 am and 14:00 pm, representing the time when the plants were most photosynthetically active given the controlled photoperiod and conducted on either a Tuesday or Thursday to allow stabilisation following watering days.

2.3.3. *Nectar analysis*

Nectar was sampled from 83 of the 96 plants as some plants did not flower at all during the survey period ($n=11$) and an additional two plants were unable to be sampled due to an insufficient amount of nectar produced. We collected nectar from an average of five flowers per plant (range: 2–10) between 9:00 am and 12:00 pm by sampling directly using a 0.5 μL microcapillary tube (Drummond Scientific Co., USA). Upon collection, nectar volume was measured by recording the height of the liquid column and calculating the cross-sectional area using the microcapillary internal diameter. All microcapillaries were stored in Eppendorf vials on ice to reduce nectar evaporation. Nectar samples were then transferred to a -80°C freezer until further analysis for sugar content. Thawed microcapillary tubes were subject to a series

of ‘rinses’ with 60 μ L of Milli-Q water via centrifugation at 5000 rpm for three two-minute cycles following a 30 second stand period between cycles. The solution was then transferred into 0.05 ml micro-insert tubes suitable for high-pressure liquid chromatography (HPLC; 600E pump, 717 plus autoinjector, 2996 Photodiode array detector Waters Corporation, Milford, USA; Alltech Evaporative Light Scattering Detector [ELSD 500], Grace Materials Technologies, Deerfield, IL, USA) analysis for sucrose, glucose and fructose (Nicolson, Nepi & Pacini 2007). HPLC analysis methods were adapted from (Slimestad & Vågen 2006). Data acquisition and processing were carried out with Empower™ Chromatography Data Software (Waters Corporation, Milford, USA).

2.3.4. *Biomass and allocation patterns*

Following the experimental period, pots were carefully emptied to harvest plants and wash the growing media from roots, ensuring the root systems remained intact. Dry weights were derived from the racemes, roots and rosettes following oven drying at 60°C for at least 96 h. Total biomass was the sum of all constituent parts, leaf allocation was calculated as leaf (rosette) mass/total biomass, while reproductive allocation was calculated as raceme mass/total biomass and root allocation as root mass/total biomass. Specific leaf area (SLA) was calculated from dry weight and area of leaves plucked from the rosette (Perez-Harguindeguy *et al.* 2016).

2.3.5. *Statistical analysis*

All statistical analyses and ordinations were performed in the R statistical environment (version 4.4.2) using RStudio Version 2024.09.1 (R Development Core Team 2023). Summary statistics are presented as means $\pm$ standard error for site, treatment and population level differences. Prior to analysis, plant trait data were assessed for homogeneity of variance using the *leveneTest* function in the ‘car’ package (version 3.1-2; Fox *et al.* 2012), and normality using the ‘*shapiro.test*’ function in the ‘stats’ package (version 4.2.3; R Core Team 2018).

Linear mixed-effect models assuming Gaussian error distribution through the *lmer* function in the R statistical package ‘lme4’ (version 1.1-32; Bates *et al.* 2007) were used to assess the interactive effect of temperature and water availability on gas exchange physiology, plant morphology and floral traits. Source population, temperature treatment and watering treatment were fixed effects with size cohort (to account for the variation in plant size) classified as a random effect in the model. Count data (i.e., total number of flowers and

racemes) were analysed using generalised linear mixed-effect models (GLMM) with Poisson distribution. Zero-inflation was also assessed for both GLMMs and identified for the total number of flowers using the '*check_zeroinflation*' function in the '*performance*' package (version 0.11.0; Lüdecke *et al.* 2021). As such, the model for the total number of flowers was fit with the zero-inflation formula reflective of the prescribed fixed and random effects using the *glmmTMB* function in the '*glmmTMB*' package (version 1.1.7; Magnusson *et al.* 2017). Variance inflation factors (VIF) were also assessed using the '*check_collinearity*' function in the '*performance*' package (version 0.11.0; Lüdecke *et al.* 2021), with VIF >5 considered a strong indicator of multicollinearity and a threshold for variable exclusion in model construction.

A Bray-Curtis dissimilarity matrix was used for a non-parametric permutational multivariate analysis of variance (PERMANOVA; Anderson 2005) to identify significant differences between trait assemblages along the latitudinal gradient using the *adonis* function in the '*vegan*' statistical package (version 2.6-4; Oksanen *et al.* 2007). *Post hoc* pair-wise comparisons between trait assemblages were conducted to identify the multivariate differences between sites using the *pairwise.perm.manova* function in the '*RVAideMemoire*' package (version 0.9-83-7; Anderson 2014; Hervé & Hervé 2020). Principal Components Analysis (PCA) followed to identify the major dimensions of variation in the multivariate plant trait data and visualise multidimensional variation among treatments and populations using the *princomp* function in the '*stats*' package (version 4.2.3; R Core Team 2018). The PCA was carried out on log-transformed data, and trait correlations with the first two principal components were assessed using the *dimdesc* function in the '*FactoMineR*' package (version 2.8.0; Lê, Josse & Husson 2008).

To understand the functional structure of the trait space occupied by each population and treatment, we compared a set of functional diversity metrics including functional richness (the 2-dimensional area occupied by the expressed trait space; Carmona, Pavanetto & Puglielli 2024), functional divergence (a metric of deviation from the centre of a trait space; Carmona, Pavanetto & Puglielli 2024), and trait hypervolume (n-dimensional functional trait volume, encompassing all possible combinations of traits; Blonder *et al.* 2014; Blonder 2018). We estimated the probability of distribution of trait values in the functional trait space using kernel density estimation with unconstrained bandwidth, employing the '*funspace*' package (version 0.2.2; Carmona, Pavanetto & Puglielli 2024). Functional trait dissimilarity between treatments within populations was then compared with estimates of functional richness and divergence

under a 99.9% probability threshold. *Kruskal-Wallis* tests were selected for the analysis of diversity indices in response to source population, water deficit and temperature, as these data did not meet the assumption of normality for parametric analysis and were conducted using the *kruskal.test* function in the ‘stats’ package.

Using Gaussian kernel density estimation, trait hypervolumes were created based on the first three PCA axes using the *hypervolume_gaussian* function from the ‘hypervolume’ package (version 3.1.4) for each population with a quantile threshold of 5%, and the Silverman bandwidth estimator (Silverman 1992, Blonder et al., 2014). The overlap between each pair of hypervolumes using the *hypervolume_overlap_statistics* function in the ‘hypervolume’ package by calculating the Euclidean distance between the centroids of the hypervolumes in the multidimensional trait space, identifying the unique volume fraction of each hypervolume, and calculating the Jaccard similarity, representing the proportion of the intersection volume to the combined volume of the hypervolume.

We selected 11 morphological, phenological and physiological traits to be included in the defining of functional strategies based on their presence in existing literature (Ravenscroft, Fridley & Grime 2014; Matos *et al.* 2021; Genty *et al.* 2023); flowering phenology, specific leaf area, total biomass, vegetative (rosette) allocation, reproductive (raceme and floral organs) allocation, root allocation, nectar volume per flower, photosynthetic rate (A), stomatal conductance (g), water-use efficiency (WUE), maximum quantum yield (F_v/F_m). Missing values were imputed into the data set and all traits were standardised using the ‘scale’ function. This standardisation ensures all traits are on a comparable scale, preventing traits with larger absolute values from disproportionately influencing the strategy indices. Three functional strategy indices were constrained to reflect competitive, avoidance and tolerance traits for each individual (Table S3.1). In principle, the calculation of these indices follows methods akin to those of CSR analysis (Grime 1988; Negreiros *et al.* 2014; Pierce *et al.* 2017). Traits contributing to competitive dominance (SLA, total biomass, vegetative and reproductive allocation, gas exchange rates, maximum quantum yield and nectar content) were summed as positive components. Traits related to below-ground investment and water use efficiency were subtracted to form a competitive index. The avoidance index focused on traits associated with phenological shifts, root allocation, and physiological traits favouring a fast life cycle. Positive contributors included root allocation, WUE, and reproductive allocation and early flowering, while negative contributors included traits linked to competitive strategies, such as increased biomass and nectar production, maximum quantum yield and gas exchange rates. Traits linked

to maintenance of plant form and function under stress were included in the tolerance index. Positive contributors included root allocation, WUE, maximum quantum yield and gas exchange rates, while traits aligned with growth and reproductive investment (e.g., SLA, biomass, reproductive allocation, nectar production) were subtracted. Each index was calculated as the difference between the sums of positive and negative trait contributors. The calculated indices were then normalised to range between zero and 100. The highest index value was assigned as the dominant strategy and visualised using the *ggtern* function in the *ggtern* package (version 3.5.0; Hamilton & Ferry 2018). All calculations of indices were performed at the individual level, and the effect of source population, watering treatment and temperature on the relative index of each strategy was assessed using a three-way analysis of variance (ANOVA) following normalisation of the avoidance index to meet the assumptions for ANOVA.

2.4. Results

Generally, greater water-deficit reduced above ground growth, reproductive investment and stomatal conductance while maximising root allocation whereas higher temperature increased leaf allocation while reducing reproductive allocation and photosynthetic rates (Figure S3.1). The mixed-effects models revealed significant effects of source population, temperature, water availability, and their interactions on univariate response of plant traits (Table 3.1, Figure S3.2). Source population by latitude had a significant effect on multiple traits, indicating population-level variation in response to environmental conditions. The effect of population on flowering phenology was significant ($\chi^2 = 14.3$, $P < 0.001$), with the southern population flowering approximately three weeks later than the northern populations. Source population also had a significant effect on the number of racemes ($\chi^2 = 21.1$, $P < 0.01$), with fewer produced in the southernmost population (1.54 ± 0.28 racemes) compared to northern populations ($4.33\text{--}6.80 \pm 1.15\text{--}1.55$ racemes). Similar trends were observed for the effect of source population on number of flowers per plant ($\chi^2 = 37.9$; $P < 0.001$), reproductive allocation ($\chi^2 = 5.64$; $P = 0.017$), plant height ($\chi^2 = 5.97$; $P = 0.014$) and rosette size ($\chi^2 = 15.8$; $P < 0.001$), with lower reproductive investment and smaller plants observed in southern populations. Leaf allocation, however, increased in southern populations ($\chi^2 = 15.8$; $P < 0.001$).

Temperature had a significant positive association with leaf allocation ($\chi^2 = 11.0$; $P < 0.001$), as well as nectar sugar content ($\chi^2 = 81.7$; $P < 0.001$). Warmer temperatures also

resulted in earlier flowering phenology ($\chi^2 = 14.3$; $P < 0.001$), with the onset of flowering occurring 1.5 weeks earlier in the highest temperatures (4.25 ± 0.28 weeks) compared to the lowest (5.75 ± 0.38 weeks). Temperature had significant negative associations with decreases seen in the total number of flowers ($\chi^2 = 507$; $P < 0.001$), total biomass ($\chi^2 = 17.5$; $P < 0.001$), reproductive allocation ($\chi^2 = 13.2$; $P < 0.001$), photosynthetic rate ($\chi^2 = 5.58$; $P = 0.018$), water-use efficiency ($\chi^2 = 6.39$; $P = 0.011$), chlorophyll content ($\chi^2 = 5.25$; $P = 0.022$).

Decreased water availability had a negative association with the total number of flowers ($\chi^2 = 396$; $P < 0.001$) and racemes ($\chi^2 = 29.6$; $P < 0.001$), overall biomass ($\chi^2 = 5.51$; $P = 0.018$), stomatal conductance ($\chi^2 = 4.26$; $P = 0.039$), maximum quantum yield ($\chi^2 = 13.3$; $P < 0.001$) and chlorophyll content ($\chi^2 = 15.7$; $P < 0.001$). Additionally, root allocation was significantly increased under water-deficit ($\chi^2 = 6.18$; $P = 0.012$), as was sugar content ($\chi^2 = 3.99$; $P = 0.045$).

Significant interactions between source population and water treatment were observed for nectar volume per flower ($\chi^2 = 6.31$; $P = 0.012$), nectar sugar content ($\chi^2 = 5.97$; $P = 0.014$), total number of flowers ($\chi^2 = 73.0$; $P < 0.001$) and the number of racemes per plant ($\chi^2 = 11.7$; $P < 0.001$), indicating the effect of water-deficit varies between populations (Figure S3.2). Additionally, significant non-additive interactive effects were observed between temperature and water-deficit for flowering phenology ($\chi^2 = 5.64$; $P = 0.017$), nectar volume per flower ($\chi^2 = 4.37$; $P = 0.036$), nectar sugar content ($\chi^2 = 5.21$; $P = 0.022$) and the total number of flowers per plant ($\chi^2 = 24.5$; $P < 0.001$). Interactions between population and temperature was only significant for the number of flowers per plant ($\chi^2 = 88.4$; $P < 0.001$).

Table 3.1: Impact of source population (P), temperature (T) and water-deficit (W) on the floral, morphological and physiological traits of *S. hispidum* plants grown under combined temperature (10-20, 15-25, 20-30°C) and watering regimes (well-watered, water-deficit). Models were constructed as linear mixed effects or generalised linear mixed effects models with reported χ^2 values and positive (↑), negative (↓) or unimodal associations (∩). Asterisks indicate significance level as follows: ‘.’ P < 0.100; ‘*’ P < 0.05; ‘**’ P < 0.01; ‘***’ P < 0.001. Blank cells reflect no significant effects



Trait	P	T	W	P:T	P:W	T:W	P:T:W
Phenology (week to first flower)	14.3 *** ↓	14.4 *** ↓	3.83 . ↑			5.64 *	
Plant Height (cm)	5.97 * ↓						
Nectar Volume per Flower (μl)		263 *** ↓	3.66 . ↑		6.31 *	4.37 *	
Sugar Content (g/L)		81.7 *** ↑	3.99 * ↓	3.00 .	4.07 *	5.20 *	
Total Number of Flowers	37.9 *** ↓	507 *** ↓	396 *** ↑	88.4 ***	73.0 ***	24.6 ***	
Number of Racemes	21.1 *** ↓		29.7 *** ↑		11.7 ***		
Reproductive Allocation (Re: Re/Bm)	5.64 * ↓	13.2 *** ↓					
Photosynthetic Rate (A : $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)		5.58 * ↓					
Stomatal Conductance (g : $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)			4.26 * ↑				
Water Use Efficiency (WUE: A/g)		6.39 * ↓					
Maximum Quantum Yield (F_v/F_m)			13.3 *** ↑				
Chlorophyll Content (mg m^{-2})		5.25 * ↓	15.7 *** ↑			5.31 *	
Rosette Size (mm^3)	15.8 *** ↓						
Specific Leaf Area (SLA)		3.09 .					
Vegetative Allocation (Ve: Ve/Bm)	17.3 *** ↑	11.0 *** ↑			3.82 .		
Root Allocation (Ro: Ro/Bm)			6.18 * ↓				
Total Biomass (Bm: g)	3.83 . ↓	17.5 *** ↓	5.51 * ↑	3.03 .			

2.4.1. Functional trait dimensions

Multivariate plant trait data varied significantly both between treatments and across source populations (Treatment: $Pseudo-F_{5,73} = 2.81$, $P = 0.004$, Population: $Pseudo-F_{3,73} = 3.91$, $P = 0.002$; Figure 3.2a). Principal Component Analysis (PCA) was performed on scaled and centred data to assess the relationships among all plant traits. The analysis revealed that the first two principal components explained 41.4% of the total variance. The first axis, which was characterised primarily by a gradient of above-ground versus below ground investment, accounted for 27.0% of this variation.

The traits which displayed the strongest positive correlations to the first dimension were total number of flowers ($R^2 = 0.87$, $P < 0.001$), total biomass ($R^2 = 0.87$, $P < 0.001$) and number of racemes ($R^2 = 0.69$, $P < 0.001$). Root allocation ($R^2 = 0.50$, $P < 0.001$) presented the strongest negative correlation with the first axis. The second axis accounted for 14.4% of the total trait variation. This axis was characterised by a reproductive allocation and productivity gradient with sugar content ($R^2 = 0.58$, $P < 0.001$), maximum quantum yield ($R^2 = 0.58$, $P < 0.001$) and stomatal conductance ($R^2 = 0.63$, $P < 0.001$) presenting the strongest positive correlations, while reproductive allocation ($R^2 = 0.59$, $P < 0.001$) presented the strongest negative correlation with the second axis.

Collectively, the PCA results indicate trait such as total biomass, number of flowers, and plant height play significant roles in shaping the overall variability within the data, while fluorescence, nectar production and gas exchange contribute to secondary patterns. Warmer temperatures were negatively associated with higher PC1 loadings ($F_{1,95} = 9.71$; $P = 0.002$; Figure 3.2b), as was water-deficit ($F_{1,95} = 8.62$; $P = 0.004$; Figure 3.2b). As temperature increased and water availability decreased, there was a marked shift towards decreased total biomass and flower production.

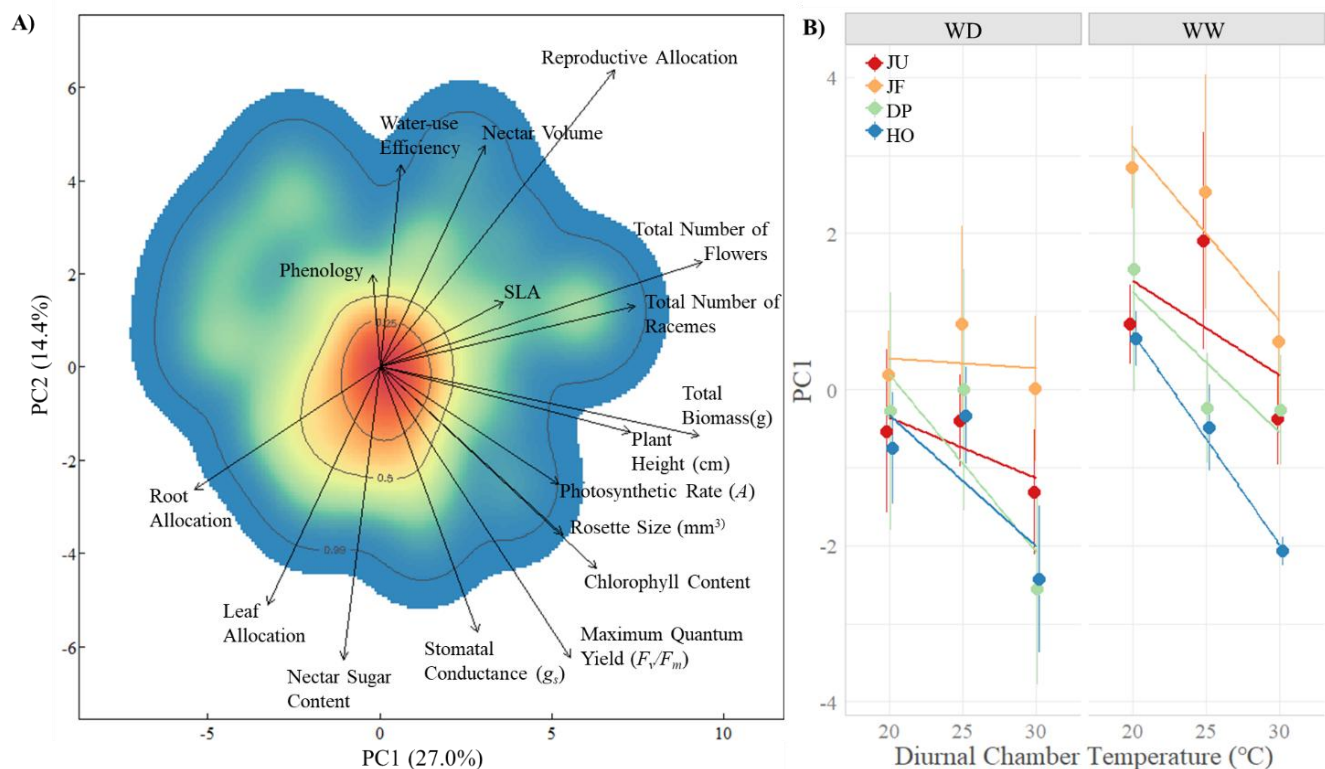


Figure 3.2 [Previous page]: a) Principal component analyses (PCA) biplot showing the multidimensional difference variation in functional traits of *Stylidium hispidum*. Vectors display the direction and weighting of each trait in the PCA. Contour lines delineate the 0.99, 0.50 and 0.25 quantiles of the multivariate probability distribution of trait values. Spectral gradient indicates the probabilistic distribution of trait combinations in the functional trait space defined by a PCA (red = high probability; blue = low probability). The proportion of variance explained by each component axis appears in parentheses; **b)** Regression of PC1 scores against temperature for each source population across both well-watered (WW) and water-deficit (WD) treatments.

2.4.2. *Functional trait diversity*

The global model for *S. hispidum* functional diversity estimated a functional richness and divergence of 124.5 and 0.44, respectively. Functional richness was significantly driven by source population ($F_{3,17} = 4.69$; $p = 0.014$) whereby the southernmost population (10–20°C: 37.6 ± 1.84), on average, had significantly reduced functional richness compared to northern counterparts (JU: 46.5 ± 2.57 ; JF: 52.7 ± 5.02 ; DP: 54.6 ± 4.14). Across populations and treatments, plants from the southernmost population and plants grown under the warmest and wettest conditions presented the lowest richness at 31.6, while the greatest richness (69.5) was observed in the southern-central population under the coolest and wettest conditions (Figure 3.3). Functional divergence ranged between 0.35–0.73. Source population (KW $\chi^2_{3,20} = 8.62$, $P = 0.034$) had a significant effect while water-deficit had a marginally significant effect (KW $\chi^2_{1,22} = 3.00$, $P = 0.083$). Divergence was generally reduced under well-watered conditions (0.47 ± 0.04) compared to water-deficit (0.56 ± 0.04) and reduced in the southern-most population (0.39 ± 0.02) compared to the other populations (range: 0.52–0.59). Temperature did not have a significant effect on functional divergence (KW $\chi^2_{2,21} = 0.785$, $P = 0.675$).

The functional niche hypervolume was significantly reduced in the southern-most population (hypervolume: 49.56) compared to all other populations (hypervolume range: 67.97–96.78; Figure 3.4). The degree of trait space overlap between populations indicated that the northern-central population presented the largest fractions of unique space with the Jaccard overlap index ranging between 0.24–0.44 and 53% represented unique space occupied by this population. The southernmost population also had the lowest fraction of unique space (0.28 ± 0.07), indicating this population trait space is largely nested within the other three.

2.4.3. *Functional trait strategies*

Characterisation of the dominant functional strategies demonstrated that the mean competitive index was significantly lower in the northern-most population (0.41 ± 0.03) compared to the other populations (range: 0.46–0.58; $F_{3,81} = 3.37$; $p = 0.023$; Figure 3.5; Figure S3.3). The mean competitive index was greater under well-watered (0.54 ± 0.03) treatments compared to the water-deficit (0.42 ± 0.03) treatments ($F_{1,81} = 10.7$; $p = 0.002$). The mean competitive index was also greater in the coolest temperature conditions (0.54 ± 0.03) by comparison to the warmest conditions (0.39 ± 0.04 ; $F_{2,81} = 6.06$; $p = 0.003$; Figure 3.5; Figure S3.3). The mean tolerance index was significantly affected by temperature treatments ($F_{2,81} = 6.01$; $p = 0.003$; Figure 3.5; Figure S3.3), with tolerance strategies being maximised under the warmest conditions (0.67 ± 0.03), and reduced under the coolest conditions (0.51 ± 0.04). The mean avoidance index was driven by watering treatments ($F_{1,81} = 8.36$; $p = 0.005$; Figure 3.5; Figure S3.3), and temperature ($F_{2,80} = 3.27$; $p = 0.043$) with avoidance strategies being more prevalent under water-deficit treatments (0.51 ± 0.02) compared to the well-watered conditions (0.41 ± 0.02) and more dominant within the warmer treatments (0.53 ± 0.03) compared to the coolest counterpart (0.42 ± 0.02).

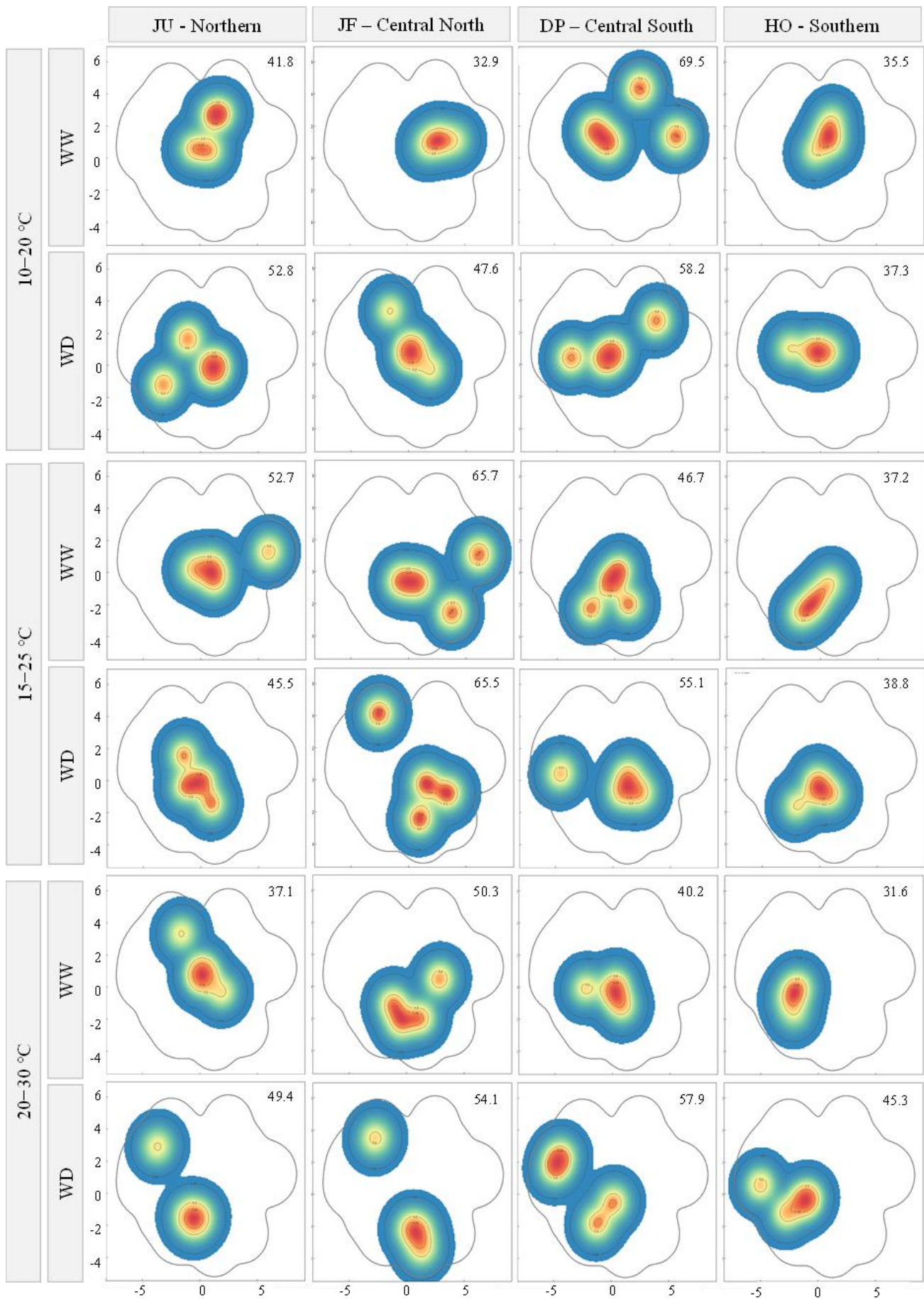


Figure 3.3 [Previous page]: Trait probability distribution represented for each *Stylidium hispidum* population [JU: Julimar Conservation Reserve; JF: John Forrest National Park; DP: State Forest (Dwellingup); HO: State Forest (Hoffman)] across three thermal conditions and two watering treatments (WW: well-watered; WD: water-deficit). Contour lines delineate the 0.99, 0.50 and 0.25 quantiles of the multivariate probability distribution of trait combinations for each population:treatment. Spectral gradient indicates the probabilistic distribution of trait combinations in the functional trait space defined by a PCA (red = high probability; blue = low probability). The thick grey polygon represents the 99.9% probability quantile of the global trait probability distribution (Figure 3a), to provide a common reference. Inset numbers reflect the functional richness of for each population: treatment combination derived from *funspace* analysis.

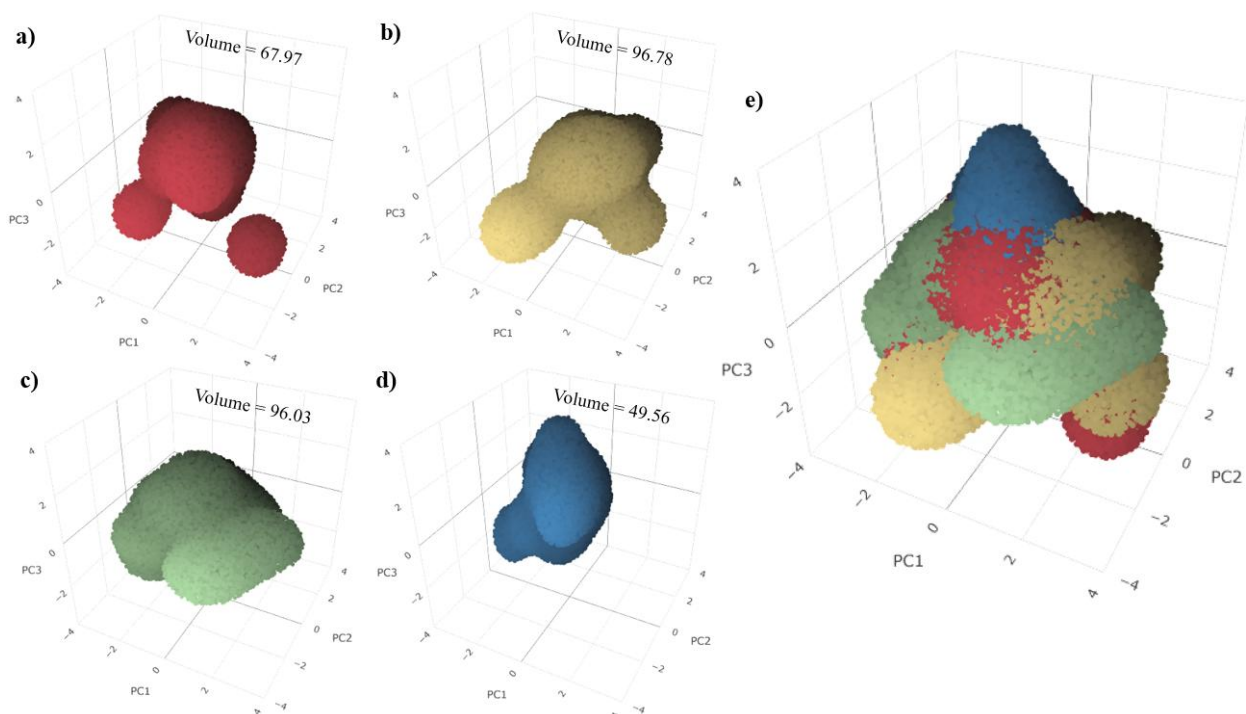


Figure 3.4: Hypervolume trait space represented for each *Stylidium hispidum* population **a)** Julimar Conservation Reserve, **b)** John Forrest National Park, **c)** State Forest (Dwellingup), **d)** State Forest (Hoffman) and **e)** total trait space for the species showcasing population overlap. Hypervolumes were constructed using the first three axes established in a principal component analysis for 27 plant traits (see Figure 3.2a). The combined hypervolume shows substantial overlap between populations with the most unique space attributed to Julimar Conservation Reserve population (a; red).

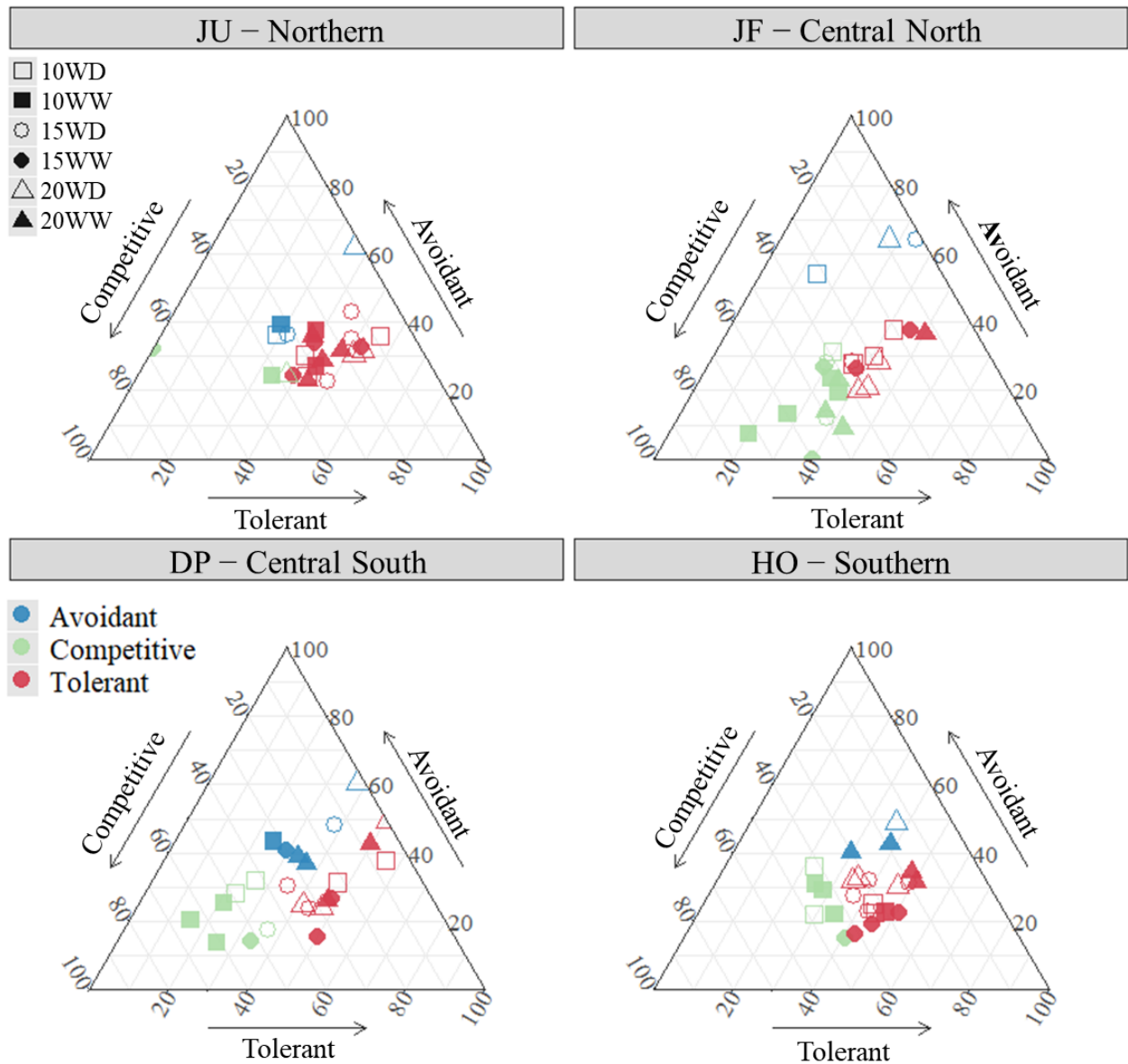


Figure 3.5: The relative proportion (%) of competitive, avoidant and tolerance trait selection for *Stylidium hispidum* plants grown from four populations [JU: Julimar Conservation Reserve; JF: John Forrest National Park; DP: State Forest (Dwellingup); HO: State Forest (Hoffman)] under three temperature treatments (10: 10-20°C, 15: 15-25°C, 20: 20-30°C) and two watering treatments (WW: well-watered; WD: water-deficit) as defined by 11 morphological, phenological and physiological traits (see methods) and calculated as the difference between the sums of positive and negative trait contributors. Each point represents an individual plant.

2.5. Discussion

The varying climate regimes present along the distribution of *Stylidium hispidum* are expected to constitute strong, differential selective pressures between populations. We observed that both elevated temperature and water deficit induced significant shifts in individual plant traits, including reductions in growth, reproductive investment, and photosynthetic rates, alongside shifts in tissue allocations. Beyond these univariate responses, multivariate analyses of trait combinations provided deeper insight into how these traits coalesce into distinct adaptive patterns. Our findings reveal that a spectrum of functional strategies exist between populations distributed along this climate gradient when grown under experimental conditions. Specifically, tolerance-avoidance strategies were found to be dominant in the northern distributions, while competitive strategies are more prominent in the central and southern distributions. Increasing temperatures and water-deficit also increased tolerance and avoidance indices within and between populations while plants grown under well-watered conditions presented higher competitive indices compared to plants grown under water-deficit. Collectively, our results provide further support to the inference of local adaptation in *S. hispidum*. However, the southern-most population exhibited significantly reduced functional richness compared to all other populations, which may reflect a constrained genotype, decreased evolutionary potential and a reduced capacity to respond to changing climate.

2.5.1. *Strategic manoeuvres and coordinated trait shifts*

Our approach has enabled powerful insights that would not be possible by observational studies alone and showcases integrated phenotypes that reflect local adaptations to climate of origin, as well as coordinated trait shifts occurring in response to experimentally induced warming and drying in *S. hispidum*. Our results demonstrated that the competitive index was significantly lower in the northern-most population, in favour of a tolerant-avoidant functional strategy defined by traits linked to maintenance of plant function under stress. This aligned with a trade-off between growth, which is advantageous in resource-rich environments, and physiological compensations which are beneficial in harsher conditions (Adler *et al.* 2014; Monson *et al.* 2022; Hu *et al.* 2023). By presenting an integrated tolerant phenotype, the plants in the most climatically stressful northern distributions can survive, albeit at the cost of reduced growing period and biomass production. Such strategies may decrease the plants' vulnerability

to short-term environmental extremes but may also lead to lower reproductive output and slow population growth, potentially limiting the ability for dispersal or to recover from disturbances (Rehm *et al.* 2015). As such, these populations are at greater risk if those disturbances or environmental extremes become more frequent or intense, as expected under climate change scenarios (IPCC 2023).

A competitive strategy was shown more prominently in the central-northern population and is advantageous in milder climates where competition for resources is a primary selective pressure (Rosado & de Mattos 2017; Astuti *et al.* 2019). This strategy is characterised by traits that enhance aboveground resource acquisition and rates of utilisation, such as larger leaf area, higher photosynthetic rates, and greater biomass accumulation and reproductive investment as a proportion of total biomass (Grime 1979; Grime 2006). The dominance of competitive strategies suggests that plants can allocate more resources towards growth without the requirement of physiological or morphological compensations to offset abiotic stress. Given that population differentiation highlighted functional shifts that confer advantages in local climates, and that these shifts emerged under standardised conditions, these results provide further evidence in support of the genetic basis to this trait variation in *S. hispidum*. This has also been demonstrated in other species in response to water availability and temperature (see Jump *et al.* 2006; Franks 2011; Ravenscroft, Fridley & Grime 2014), contributing to evidence of plant adaptation to climate.

Our results detail the coordinated trait shifts that occur in response to both temperature and water availability during the growth and flowering phase of *S. hispidum* development. While the differences between populations showcase the genetic basis for intraspecific variation and local adaptation, the differences within populations but between treatments work to showcase the capacity for phenotypic plasticity to compensate for abiotic stress. Within populations, plants exposed to water-limitation generally had reduced overall biomass, stomatal conductance, maximum photosynthetic efficiency, chlorophyll content and fewer flowers, with greater below ground allocation into root structures compared to well-watered plants in the same population. Plants grown under warmer conditions were also quicker to flower though producing fewer flowers, reducing overall reproductive allocation. Photosynthetic efficiency, chlorophyll content and maximum assimilation rates also declined with increasing temperature, though these effects were most pronounced in the southern population. Such morphological, physiological and spatiotemporal responses to water availability and temperature can destabilise biotic interactions (reviewed in Descamps, Quinet

& Jacquemart 2020). While it was beyond the scope of this study, future work should assess the degree to which these stress-induced alterations in functional traits impact other demographic processes, such as pollination, and the concomitant effects on seed traits and recruitment (see Chapter Four). Nevertheless, integrated phenotypes between *S. hispidum* populations and coordinated trait shifts in response to treatments, demonstrates a multifaceted strategy whereby genetic differentiation equips populations to maximise performance in their local environments while plasticity provides a rapid response to short-term environmental fluctuations without genetic change. However, the extent to which the species can continue to adapt in the face of escalating environmental pressures remains unanswered, therefore, necessitating further investigation to elucidate the limits of the plastic and adaptive capacities of these populations.

2.5.2. *Distribution of functional diversity*

Conforming to our hypothesis, that functional richness would vary among populations, we found that the southern-most population presented reduced functional richness and divergence, amounting to a significantly smaller total trait space compared to all other populations. This pattern contrasts the expectations of the *physiological tolerance hypothesis*, that richness and functional diversity is highest in warm and/or wet climates allowing a wider range of functional strategies to persist (Spasojevic *et al.* 2014; Jiang, Ma & Anand 2016), given that the warmest conditions are presented in the north, and wettest in the south. Instead, we found the greatest functional diversity in the two central populations sourced from John Forrest National Park and Dwellingup State Forest. This finding aligns more closely with expectations of the *central-marginal hypothesis*, stipulating that populations at the geographic core of a species' range are more genetically diverse and phenotypically plastic than those at the periphery (Eckert, Samis & Loughheed 2008). Conforming to those expectations, range-core populations were found to display greater phenotypic variability in *Arabidopsis thaliana*, which was associated with both genotype and climatic conditions (Estarague *et al.* 2022). Similarly, range-edge populations presented limited trait diversity in two *Agrimonia* species which was attributed to climatic stressors in the range-edge populations (Mägi *et al.* 2011). Though we did not initially set out to test the *central-marginal hypothesis*, the trends observed here are potentially explained by central populations in *S. hispidum* benefiting from milder conditions, stable population sizes, and potentially increased gene flow. Such conditions, in

combination, may contribute to maintaining or maximising functional richness seen in the central populations in our study.

We also found that both functional richness and trait hypervolume was greatest in the central populations and significantly reduced in the southernmost population. This demonstrated that the southern population presented the smallest breadth of expressed trait combinations under the *ex-situ* conditions (richness), and a narrower range of *possible* trait combinations overall (hypervolume). As a result, the southern population may be less equipped to respond to changing conditions through reduced plasticity and adaptive capacity hindering the maintenance of population fitness and stability under novel environmental conditions (Rehm *et al.* 2015). This population already presents comparatively reduced genetic diversity compared to the other populations (Hufford *et al.* 2016), which may contribute to explaining the smaller functional trait space in southern population. Furthermore, the populations located at the periphery of the species' range often also encounter environmental conditions that are potentially physiologically sub-optimal from those experienced by populations in the central distributions. These sub-optimal conditions may lead to reduced population sizes which can present negative genetic impacts as a result of heightened inbreeding and decreased genetic diversity caused by processes such as genetic drift, founder effects, and the accumulation of deleterious mutations in the gene pool (Lynch, Conery & Burger 1995; Young, Boyle & Brown 1996). It is also possible that poleward range shifts have led to the recent establishment of southern populations relative to their northern counterparts, and that disturbance events, such as fires, have also influenced the gene pool. If local adaptation was equally distributed across a species range, all populations would be expected to be equally vulnerable to the same magnitude of change. However, the limited functional richness may lead the southern population to be disproportionately vulnerable to climate change, as limited trait diversity, paired with reduced tolerance to abiotic stress and constrained genetic diversity, could undermine evolutionary potential and populations' ability to cope with forecasted climate change. This can increase the risk of local extinctions if the population does not have the capacity to adapt in line with the rate of change (Rehm *et al.*, 2015).

2.5.3. *Axes of variation*

When plants were grown under the same contrasting experimental temperature and drought treatments *ex-situ*, we found clear differentiation of trait expression among treatments

and populations, while identifying two dominant dimensions of variation. In our current study, the first axis highlighted that, regardless of source population, plants grown under well-watered conditions generally expressed leaf traits that were more acquisitive, with increased above-ground investment compared to below-ground root structures. Conversely, plants subjected to water deficit expressed traits that were more conservative to maximise water uptake and minimise water loss through increased investment into root structures relative to above ground structures. The second axis was defined by phenological and physiological traits relating to resource economy, instead of morphological or architectural traits as seen in the first axis. These two axes were previously found to explain trait variation across *S. hispidum* sampled *in-situ* (Chapter Two; Tudor *et al.* 2024), and also correspond to the two principal components emergent in previous global trait datasets (Wright *et al.* 2004; Díaz *et al.* 2016; Joswig *et al.* 2022). Therefore, our results further support the definition of two main axes of variation, showcasing that they are consistent in the context of both interspecific and intraspecific comparisons, while also persisting in both controlled, *ex-situ* growth conditions and in *in-situ* contexts across natural climate gradients.

2.5.4. *Implications and conclusions*

The capacity for plants to adapt to changing environmental conditions through shifts in functional strategies, either by genetic adaptation or phenotypic plasticity, has significant implications for their resilience to climate change (Andrew *et al.* 2022; Kandlikar, Kleinhesselink & Kraft 2022). By understanding and addressing the unique ecological requirements and adaptive strategies of distinct populations, management practices could be tailored to enhance the resilience of *S. hispidum* across its relatively narrow distribution, ultimately supporting population persistence and biodiversity conservation in a changing world. Generally, efforts should focus on preserving the existing functional diversity across the species range, which involves protecting any large, continuous habitats that remain to maintain and maximise both habitat connectivity and gene flow to reduce the risk of genetic bottlenecks (Hufford, Krauss & Veneklaas 2012; Millar *et al.* 2016). Specific to the northern population, *ex-situ* conservation may be warranted as it presents unique genetic and functional diversity not found in other populations as demonstrated in the hypervolume analysis (Hoban & Schlarbaum 2014; Hufford *et al.* 2016). For the southern population, introducing genetic material from more functionally diverse populations may increase the adaptive potential of the southern population to enable it to better cope with environmental changes by following a

stepwise progression of ‘climate-adjusted provenancing’ (Prober *et al.* 2015). This approach involves progressively moving seeds from populations in hotter and drier conditions to adjacent cooler, wetter locations, resulting in a ‘domino effect’ that maximises genetic compatibility and adaptation to future climate conditions, while minimising the risk of outbreeding depression (Hufford, Krauss & Veneklaas 2012).

In-situ field studies are vital for characterising trends in complex, natural environments while *ex-situ* methods offer controlled insights into the genetic and physiological mechanisms underlying local adaptation. By applying an *ex-situ* multivariate experimental design, we showcase a unique demonstration of coordinated trait shifts (Laughlin & Messier 2015), and highlight genetic and plastic mechanisms behind trait divergence in *S. hispidum*. This establishes that trait variation corresponds to local adaptations, and further supports that population phenotypes may shift from current norms in response to changing climates (Tudor *et al.* 2024). Therefore, climatic conditions play a fundamental role in shaping the species' functional diversity, but the evidence for current local adaptation in this system also suggests that ongoing adaptation under future climate regimes will be necessary for population persistence. Increased genetic and functional diversity offers an optimistic outlook for the northern population, despite originating from the hottest and driest climates. Conversely, the reduced genetic and functional diversity in the southern population may limit the adaptive potential and increase the risk of population declines with forecasted climate change, despite the southern distributions maintaining the mildest climate. From this, we confer the importance of integrating an understanding of intraspecific variation and functional diversity as populations from milder climates may not always be those inherently ‘least at risk’ to future change.

2.6. References

- Adler, P.B., Salguero-Gómez, R., Compagnoni, A., Hsu, J.S., Ray-Mukherjee, J., Mbeau-Ache, C. & Franco, M. (2014) Functional traits explain variation in plant life history strategies. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, 740-745.
- Ahrens, C.W., Rymer, P.D. & Tissue, D.T. (2021) Intra-specific trait variation remains hidden in the environment. *New Phytologist*, **229**, 1183-1185.
- Anderegg, L.D.L., Loy, X., Markham, I.P., Elmer, C.M., Hovenden, M.J., HilleRisLambers, J. & Mayfield, M.M. (2021) Aridity drives coordinated trait shifts but not decreased trait variance across the geographic range of eight Australian trees. *New Phytologist*, **229**, 1375-1387.
- Anderson, J.T., Panetta, A.M. & Mitchell-Olds, T. (2012) Evolutionary and ecological responses to anthropogenic climate change: update on anthropogenic climate change. *Plant Physiology*, **160**, 1728-1740.
- Anderson, J.T. & Song, B.H. (2020) Plant adaptation to climate change—Where are we? *Journal of Systematics and Evolution*, **58**, 533-545.
- Anderson, M.J. (2005) Permutational multivariate analysis of variance. *Department of Statistics, University of Auckland, Auckland*, **26**, 32-46.
- Anderson, M.J. (2014) Permutational multivariate analysis of variance (PERMANOVA). *Wiley StatsRef: Statistics Reference Online* (eds N. Balakrishnan, T. Colton, B. Everitt, W. Piegorsch, F. Ruggero & J. Teugels), pp. 1-15.
- Andrew, S.C., Gallagher, R.V., Wright, I.J. & Mokany, K. (2022) Assessing the vulnerability of plant functional trait strategies to climate change. *Global Ecology and Biogeography*, **31**, 1194-1206.
- Astuti, G., Ciccarelli, D., Roma-Marzio, F., Trinco, A. & Peruzzi, L. (2019) Narrow endemic species *Bellevalia webbiana* shows significant intraspecific variation in tertiary CSR strategy. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*, **153**, 12-18.
- Basu, S., Ramegowda, V., Kumar, A. & Pereira, A. (2016) Plant adaptation to drought stress. *F1000Research*, **5**
- Bates, D., Sarkar, D., Bates, M.D. & Matrix, L. (2007) The lme4 package. *R package version*, **2**, 74.

- Bjorkman, A.D., Myers-Smith, I.H., Elmendorf, S.C., Normand, S., Rüger, N., Beck, P.S., Blach-Overgaard, A., Blok, D., Cornelissen, J.H.C. & Forbes, B.C. (2018) Plant functional trait change across a warming tundra biome. *Nature*, **562**, 57-62.
- Blonder, B. (2018) Hypervolume concepts in niche-and trait-based ecology. *Ecography*, **41**, 1441-1455.
- Blonder, B., Lamanna, C., Violle, C. & Enquist, B.J. (2014) The n-dimensional hypervolume. *Global Ecology and Biogeography*, **23**, 595-609.
- Carmona, C.P., De Bello, F., Mason, N.W. & Lepš, J. (2016) Traits without borders: integrating functional diversity across scales. *Trends in Ecology & Evolution*, **31**, 382-394.
- Carmona, C.P., Pavanetto, N. & Puglielli, G. (2024) funspace: An R package to build, analyse and plot functional trait spaces. *Diversity and Distributions*, **30**, e13820.
- Chandler, J. & Bartels, D. (2008) Drought: Avoidance and adaptation. *Encyclopedia of water science*. Taylor and Francis group, London, UK.
- Chiwocha, S.D., Dixon, K.W., Flematti, G.R., Ghisalberti, E.L., Merritt, D.J., Nelson, D.C., Riseborough, J.-A.M., Smith, S.M. & Stevens, J.C. (2009) Karrikins: a new family of plant growth regulators in smoke. *Plant science*, **177**, 252-256.
- De Frenne, P., Graae, B.J., Rodríguez-Sánchez, F., Kolb, A., Chabrierie, O., Decocq, G., De Kort, H., De Schrijver, A., Diekmann, M., Eriksson, O., Gruwez, R., Hermy, M., Lenoir, J., Plue, J., Coomes, D.A. & Verheyen, K. (2013) Latitudinal gradients as natural laboratories to infer species' responses to temperature. *The Journal of Ecology*, **101**, 784-795.
- Descamps, C., Quinet, M. & Jacquemart, A.-L. (2020) The effects of drought on plant–pollinator interactions: What to expect? *Environmental and experimental botany*, **182**, 104297.
- Díaz, S., Kattge, J., Cornelissen, J.H., Wright, I.J., Lavorel, S., Dray, S., Reu, B., Kleyer, M., Wirth, C. & Colin Prentice, I. (2016) The global spectrum of plant form and function. *Nature*, **529**, 167-171.
- Eckert, C.G., Samis, K.E. & Loughheed, S.C. (2008) Genetic variation across species' geographical ranges: the central–marginal hypothesis and beyond. *Molecular Ecology*, **17**, 1170-1188.
- Estarague, A., Vasseur, F., Sartori, K., Bastias, C.C., Cornet, D., Rouan, L., Beurier, G., Exposito-Alonso, M., Herbette, S. & Bresson, J. (2022) Into the range: a latitudinal gradient or a center-margins differentiation of ecological strategies in *Arabidopsis thaliana*? *Annals of Botany*, **129**, 343-356.

- Fick, S.E. & Hijmans, R.J. (2017) WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, **37**, 4302-4315.
- Flematti, G.R., Ghisalberti, E.L., Dixon, K.W. & Trengove, R.D. (2004) A compound from smoke that promotes seed germination. *Science*, **305**, 977.
- Fox, J., Weisberg, S., Adler, D., Bates, D., Baud-Bovy, G., Ellison, S., Firth, D., Friendly, M., Gorjanc, G. & Graves, S. (2012) Package ‘car’. *Vienna: R Foundation for Statistical Computing*,
- Franks, S.J. (2011) Plasticity and evolution in drought avoidance and escape in the annual plant *Brassica rapa*. *The New Phytologist*, **190**, 249-257.
- Funk, J.L., Larson, J.E., Ames, G.M., Butterfield, B.J., Cavender-Bares, J., Firn, J., Laughlin, D.C., Sutton-Grier, A.E., Williams, L. & Wright, J. (2017) Revisiting the Holy Grail: using plant functional traits to understand ecological processes. *Biological Reviews*, **92**, 1156-1173.
- Geber, M.A. & Dawson, T.E. (1990) Genetic variation in and covariation between leaf gas exchange, morphology, and development in *Polygonum arenastrum*, an annual plant. *Oecologia*, **85**, 153-158.
- Genty, L., Kazakou, E., Metay, A., Baude, M., Gardarin, A., Michelot-Antalik, A., Leroy, A., Sotillo, A., Crouzet, J. & Barkaoui, K. (2023) Flowers of ruderal species are numerous but small, short and low-rewarding. *Oikos*, **2023**, e10219.
- Grime, J. (1979) Primary strategies in plants. *Transactions of the Botanical Society of Edinburgh*, pp. 151-160. Taylor & Francis,
- Grime, J.P. (1988) The CSR model of primary plant strategies—origins, implications and tests. *Plant evolutionary biology*, pp. 371-393. Dordrecht: Springer, Netherlands.
- Grime, J.P. (2006) *Plant strategies, vegetation processes, and ecosystem properties*. John Wiley & Sons,
- Hamilton, N.E. & Ferry, M. (2018) ggtern: Ternary diagrams using ggplot2. *Journal of Statistical Software*, **87**, 1-17.
- Hassan, T., Gulzar, R., Hamid, M., Ahmad, R., Waza, S.A. & Khuroo, A.A. (2023) Plant phenology shifts under climate warming: a systematic review of recent scientific literature. *Environmental Monitoring and Assessment*, **196**, 1-19.
- Heilmeyer, H. (2019) Functional traits explaining plant responses to past and future climate changes. *Flora*, **254**, 1-11.
- Hemantaranjan, A., Bhanu, A.N., Singh, M., Yadav, D., Patel, P., Singh, R. & Katiyar, D. (2014) Heat stress responses and thermotolerance. *Adv. Plants Agric. Res*, **1**, 1-10.

- Hervé, M. & Hervé, M.M. (2020) Package 'RVAideMemoire'. See <https://CRAN.R-project.org/package=RVAideMemoire>.
- Heschel, M.S. & Riginos, C. (2005) Mechanisms of selection for drought stress tolerance and avoidance in *Impatiens capensis* (Balsaminaceae). *American Journal of Botany*, **92**, 37-44.
- Hoban, S. & Schlarbaum, S. (2014) Optimal sampling of seeds from plant populations for ex-situ conservation of genetic biodiversity, considering realistic population structure. *Biological Conservation*, **177**, 90-99.
- Hoffmann, A.A., Rymer, P.D., Byrne, M., Ruthrof, K.X., Whinam, J., McGeoch, M., Bergstrom, D.M., Guerin, G.R., Sparrow, B., Joseph, L., Hill, S.J., Andrew, N.R., Camac, J., Bell, N., Riegler, M., Gardner, J.L. & Williams, S.E. (2019) Impacts of recent climate change on terrestrial flora and fauna: Some emerging Australian examples. *Austral Ecology*, **44**, 3-27.
- Hoffmann, A.A. & Sgrò, C.M. (2011) Climate change and evolutionary adaptation. *Nature*, **470**, 479-485.
- Hu, X., He, Y., Gao, L., Umer, M., Guo, Y., Tan, Q., Kang, L., Fang, Z., Shen, K. & Xia, T. (2023) Strategy trade-off of predominant stress tolerance relative to competition and reproduction associated with plant functional traits under karst forests. *Forests*, **14**, 1258.
- Hufford, K.M., Krauss, S.L. & Veneklaas, E.J. (2012) Inbreeding and outbreeding depression in *Stylidium hispidum*: implications for mixing seed sources for ecological restoration. *Ecology and Evolution*, **2**, 2262-2273.
- Hufford, K.M., Veneklaas, E.J., Lambers, H. & Krauss, S.L. (2016) Genetic delineation of local provenance defines seed collection zones along a climate gradient. *AoB Plants*, **8**, 1-12.
- IPCC (2023) *Summary for Policymakers. In: Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]*. IPCC c/o World Meteorological Organization (WMO), Geneva, Switzerland.
- Jiang, Z., Ma, K. & Anand, M. (2016) Can the physiological tolerance hypothesis explain herb richness patterns along an elevational gradient? A trait-based analysis. *Community Ecology*, **17**, 17-23.

- Joswig, J.S., Wirth, C., Schuman, M.C., Kattge, J., Reu, B., Wright, I.J., Sippel, S.D., Rüger, N., Richter, R. & Schaepman, M.E. (2022) Climatic and soil factors explain the two-dimensional spectrum of global plant trait variation. *Nature Ecology & Evolution*, **6**, 36-50.
- Jump, A.S., Hunt, J.M., Martínez-Izquierdo, J.A. & Peñuelas, J. (2006) Natural selection and climate change: temperature-linked spatial and temporal trends in gene frequency in *Fagus sylvatica*. *Molecular Ecology*, **15**, 3469-3480.
- Kandlikar, G.S., Kleinhesselink, A.R. & Kraft, N.J.B. (2022) Functional traits predict species responses to environmental variation in a California grassland annual plant community. *Journal of Ecology*, **110**, 833-844.
- Kooyers, N.J. (2015) The evolution of drought escape and avoidance in natural herbaceous populations. *Plant Science*, **234**, 155-162.
- Laughlin, D.C., Gremer, J.R., Adler, P.B., Mitchell, R.M. & Moore, M.M. (2020) The net effect of functional traits on fitness. *Trends in Ecology & Evolution*, **35**, 1037-1047.
- Laughlin, D.C. & Messier, J. (2015) Fitness of multidimensional phenotypes in dynamic adaptive landscapes. *Trends in Ecology & Evolution*, **30**, 487-496.
- Lê, S., Josse, J. & Husson, F. (2008) FactoMineR: an R package for multivariate analysis. *Journal of statistical software*, **25**, 1-18.
- Lortie, C.J. & Hierro, J.L. (2021) A synthesis of local adaptation to climate through reciprocal common gardens. *The Journal of Ecology*, **110**, 1015-1021.
- Lüdecke, D., Ben-Shachar, M.S., Patil, I., Waggoner, P. & Makowski, D. (2021) performance: An R package for assessment, comparison and testing of statistical models. *Journal of Open Source Software*, **6**
- Lynch, M., Conery, J. & Burger, R. (1995) Mutation accumulation and the extinction of small populations. *The American Naturalist*, **146**, 489-518.
- Mägi, M., Semchenko, M., Kalamees, R. & Zobel, K. (2011) Limited phenotypic plasticity in range-edge populations: a comparison of co-occurring populations of two *Agrimonia* species with different geographical distributions. *Plant Biology*, **13**, 177-184.
- Magnusson, A., Skaug, H., Nielsen, A., Berg, C., Kristensen, K., Maechler, M., van Benthem, K., Bolker, B., Brooks, M. & Brooks, M.M. (2017) Package ‘glmmTMB’. *R Package Version 0.2. 0*, **25**
- Matos, I.S., Eller, C.B., Oliveras, I., Mantuano, D. & Rosado, B.H. (2021) Three eco-physiological strategies of response to drought maintain the form and function of a tropical montane grassland. *Journal of Ecology*, **109**, 327-341.

- Merilä, J. & Hoffmann, A.A. (2016) Evolutionary impacts of climate change. *Oxford Research Encyclopedia of Environmental Science*.
- Millar, M.A., Byrne, M., Coates, D.J. & Roberts, J.D. (2016) Contrasting diversity and demographic signals in sympatric narrow-range endemic shrubs of the south-west Western Australian semi-arid zone. *Biological Journal of the Linnean Society*, **118**, 315-329.
- Monson, R.K., Trowbridge, A.M., Lindroth, R.L. & Lerda, M.T. (2022) Coordinated resource allocation to plant growth–defense tradeoffs. *New Phytologist*, **233**, 1051-1066.
- Negreiros, D., Le Stradic, S., Fernandes, G.W. & Rennó, H.C. (2014) CSR analysis of plant functional types in highly diverse tropical grasslands of harsh environments. *Plant Ecology*, **215**, 379-388.
- Nicolson, S.W., Nepi, M. & Pacini, E. (2007) *Nectaries and nectar*. Springer Science & Business Media,
- Nicotra, A.B., Atkin, O.K., Bonser, S.P., Davidson, A.M., Finnegan, E.J., Mathesius, U., Poot, P., Purugganan, M.D., Richards, C.L., Valladares, F. & van Kleunen, M. (2010) Plant phenotypic plasticity in a changing climate. *Trends in Plant Science*, **15**, 684-692.
- Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M.H.H., Oksanen, M.J. & Suggests, M. (2007) The vegan package. *Community ecology package*, **10**, 631-637.
- Osmond, C., Austin, M., Berry, J., Billings, W., Boyer, J., Dacey, J., Nobel, P., Smith, S. & Winner, W. (1987) Stress physiology and the distribution of plants. *Bioscience*, **37**, 38-48.
- Perez-Harguindeguy, N., Diaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M.S., Cornwell, W.K., Craine, J.M. & Gurvich, D.E. (2016) Corrigendum to: new handbook for standardised measurement of plant functional traits worldwide. *Australian journal of botany*, **64**, 715-716.
- Pierce, S., Negreiros, D., Cerabolini, B.E., Kattge, J., Díaz, S., Kleyer, M., Shipley, B., Wright, S.J., Soudzilovskaia, N.A. & Onipchenko, V.G. (2017) A global method for calculating plant CSR ecological strategies applied across biomes world-wide. *Functional ecology*, **31**, 444-457.
- Prober, S.M., Byrne, M., McLean, E.H., Steane, D.A., Potts, B.M., Vaillancourt, R.E. & Stock, W.D. (2015) Climate-adjusted provenancing: a strategy for climate-resilient ecological restoration. *Frontiers in ecology and evolution*, **3**, 1-5.
- Puglielli, G., Bricca, A., Chelli, S., Petruzzellis, F., Acosta, A.T.R., Bacaro, G., Beccari, E., Bernardo, L., Bonari, G., Bolpagni, R., Boscutti, F., Calvia, G., Campetella, G.,

- Cancellieri, L., Canullo, R., Carbognani, M., Carboni, M., Carranza, M.L., Castellani, M.B., Ciccarelli, D., Coppi, A., Cutini, M., Dalla Vecchia, A., Dalle Fratte, M., de Francesco, M.C., De Frenne, P., De Sanctis, M., de Simone, L., Di Cecco, V., Fanelli, G., Farris, E., Ferrara, A., Fenu, G., Filibeck, G., Gasperini, C., Gargano, D., Kindermann, E., La Bella, G., Lastrucci, L., Lazzaro, L., Maccherini, S., Marignani, M., Mugnai, M., Naselli-Flores, L., Passalacqua, N.G., Pavanetto, N., Petraglia, A., Rota, F., Santoianni, L.A., Schettino, A., Selvi, F., Stanisci, A., Trotta, G., Vangansbeke, P., Varricchione, M., Vuerich, M., Wellstein, C. & Tordoni, E. (2024) Intraspecific variability of leaf form and function across habitat types. *Ecology Letters*, **27**, e14396.
- R Core Team (2018) Package stats. *The R Stats Package*,
R Development Core Team, R. (2023) RStudio: Integrated Development Environment for R. Boston, MA: RStudio, Inc; 2023.
- Ravenscroft, C.H., Fridley, J.D. & Grime, J.P. (2014) Intraspecific functional differentiation suggests local adaptation to long-term climate change in a calcareous grassland. *Journal of Ecology*, **102**, 65-73.
- Rehm, E.M., Olivas, P., Stroud, J. & Feeley, K.J. (2015) Losing your edge: climate change and the conservation value of range-edge populations. *Ecology and evolution*, **5**, 4315-4326.
- Reich, P.B., Wright, I.J., Cavender-Bares, J., Craine, J., Oleksyn, J., Westoby, M. & Walters, M. (2003) The evolution of plant functional variation: traits, spectra, and strategies. *International Journal of Plant Sciences*, **164**, S143-S164.
- Rosado, B.H. & de Mattos, E.A. (2017) On the relative importance of CSR ecological strategies and integrative traits to explain species dominance at local scales. *Functional ecology*, **31**, 1969-1974.
- Sato, H., Mizoi, J., Shinozaki, K. & Yamaguchi-Shinozaki, K. (2024) Complex plant responses to drought and heat stress under climate change. *The Plant Journal*, **117**, 1873-1892.
- Schellenberger Costa, D., Gerschlauer, F., Kiese, R., Fischer, M., Kleyer, M. & Hemp, A. (2018) Plant niche breadths along environmental gradients and their relationship to plant functional traits. *Diversity and Distributions*, **24**, 1869-1882.
- Schwinning, S., Lortie, C.J., Esque, T.C. & DeFalco, L.A. (2022) What common-garden experiments tell us about climate responses in plants. pp. 986-996. Wiley Online Library,

- Slimestad, R. & Vågen, I.M. (2006) Thermal stability of glucose and other sugar aldoses in normal phase high performance liquid chromatography. *Journal of Chromatography A*, **1118**, 281-284.
- Spasojevic, M.J., Grace, J.B., Harrison, S. & Damschen, E.I. (2014) Functional diversity supports the physiological tolerance hypothesis for plant species richness along climatic gradients. *The Journal of Ecology*, **102**, 447-455.
- Tudor, E.P., Lewandrowski, W., Krauss, S. & Veneklaas, E.J. (2024) Local adaptation to climate inferred from intraspecific variation in plant functional traits along a latitudinal gradient. *Conservation Physiology*, **12**, coae018.
- Violle, C. & Jiang, L. (2009) Towards a trait-based quantification of species niche. *Journal of Plant Ecology*, **2**, 87-93.
- Westerband, A., Funk, J. & Barton, K. (2021) Intraspecific trait variation in plants: a renewed focus on its role in ecological processes. *Annals of Botany*, **127**, 397-410.
- Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J., Navas, M.-L., Niinemets, U., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J. & Villar, R. (2004) The worldwide leaf economics spectrum. *Nature*, **428**, 821-827.
- Young, A., Boyle, T. & Brown, T. (1996) The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology & Evolution*, **11**, 413-418.

2.7. Supplementary information for Chapter Three

Table S3.1: The 11 traits integrated into the characterisation of competitive (CI), avoidance (AI), and tolerance (TI) indexes to reflect functional strategies for *Stylidium hispidum* grown under controlled temperature and watering treatments. Traits were allocated as positive (+) or negative (-) components for each index. Each index was calculated as the difference between the sums of positive and negative trait contributors. The calculated indices were then normalised to range between zero and 100. The highest index value was assigned as the dominant strategy per plant.

Trait	CI	AI	TI	Rationale
Specific Leaf Area (SLA)	+	-	-	Lower SLA, which signifies thicker leaves, benefits competitive plants by improving light capture and photosynthetic efficiency. This trait supports rapid growth and resource acquisition. Additionally, lower SLA aligns with avoidance and tolerance strategies, helping plants to survive under prolonged stress conditions. Thicker leaves with lower SLA can conserve water, reduce transpiration, and maintain photosynthesis.
Total Biomass	+	-	-	High total biomass reflects greater resource capture and growth aligning with competitive dominance. In avoidance and tolerance strategies, lower total biomass is beneficial as it reduces resource demands, allowing plants to sustain growth and function under stress. By maintaining lower biomass, tolerant plants can conserve water and nutrients, enhancing their ability to withstand prolonged drought and heat stress.
Root Allocation		+	+	Competitive plants prioritise above-ground growth to maximise light capture and resource acquisition, often investing less in their root systems. However, higher root allocation is essential for avoidance strategies to access deeper water reserves and avoid deficits. In tolerance strategies, greater root allocation enhances water and nutrient uptake, supporting sustained growth and function under stress conditions.
Leaf Allocation (rosette)	+	-	-	High leaf allocation enhances competitive growth and resource acquisition. In avoidance strategies, plants prioritise rapid life cycle completion to avoid adverse conditions. Reduced vegetative growth allocation enables resource diversion to reproduction to facilitate a quicker life cycle. In tolerance strategies, reduced leaf allocation conserves energy, supporting sustained physiological functions under prolonged stress.
Reproductive Allocation	+	+	-	Competitive plants allocate significant resources to reproductive structures to maximise fitness in resource-rich environments. Plants exhibiting avoidance strategies often flower early and allocate substantial resources to seed production, ensuring they can reproduce before conditions deteriorate. Tolerant plants aim to maintain their physiological functions and survive under prolonged stress conditions. High reproductive allocation can detract from the resources needed for maintaining essential functions and surviving stress, so tolerance strategies often show comparatively lower reproductive allocation in favour of sustaining growth and function.

Phenology (week to first flower)	+	+		Under stressful conditions like drought or warming, plants with avoidance strategies will exhibit earlier flowering to complete their life cycle quickly to avoid unfavourable conditions, maximising fitness before conditions are too stressful. Under prolonged stress, tolerant plants may also adjust their flowering time to coincide with periods of lower stress, such as cooler or wetter periods to maximise reproductive success without compromising plant survival.
Nectar Volume	+	-	-	Under resource-rich conditions, nectar volume is high as plants can afford to allocate more resources to attract pollinators. Plants with avoidance strategies may produce lower nectar volumes to conserve resources for rapid seed production and early life cycle completion. Tolerance strategies focus on maintaining physiological functions and surviving under prolonged stress conditions and benefit from reduced nectar volume to conserve resources, increasing the likelihood that they can maintain overall plant health and reproductive success under stress.
Maximum quantum yield (F_v/F_m).	+	-	+	By maintaining high F_v/F_m , competitive plants can increase their productivity and outcompete other species in resource-abundant environments. In avoidance strategies, plants prioritise rapid life cycle completion to escape adverse conditions. High F_v/F_m is less critical in this context, as the focus is on completing the life cycle quickly rather than maintaining long-term photosynthetic efficiency. Under prolonged stress, tolerant plants strive to maintain high F_v/F_m values to ensure efficient photosynthesis and energy production. This ability helps them sustain growth and function despite adverse conditions, enhancing their stress tolerance.
Photosynthetic Rate (A)	+	-	+	Under favourable conditions, competitive plants exhibit high photosynthetic rates, promoting rapid growth and resource capture. Under stress conditions like drought or heat, photosynthetic rates may decrease as plants prioritise rapid reproduction and allocate fewer resources to photosynthetic maintenance. Tolerance strategies focus on maintaining physiological functions and surviving under prolonged stress conditions. High photosynthetic rates are essential for tolerance strategies, as they indicate the plant's ability to sustain efficient energy production and growth under stress.
Water Use Efficiency (WUE)	-	+	+	Competitive plants often thrive in resource-abundant environments where water is not a limiting factor. In such conditions, high WUE is less critical for their success. Under prolonged stress, tolerant and avoidant plants exhibit high WUE to optimise water use and maintain growth and function. This high WUE allows them to survive extended periods of drought by making the most efficient use of available water resources
Stomatal Conductance (g)	+	-	+	Efficient gas exchange supports higher photosynthetic rates and resource acquisition, giving competitive plants an edge in growth and biomass production. However, this comes at the cost of higher water loss through transpiration, which is less of a concern in resource-abundant environments. High stomatal conductance can lead to excessive water loss, which is detrimental in environments where water is scarce. Therefore, plants with avoidance strategies often exhibit lower stomatal conductance to minimise water loss and conserve resources. Under prolonged stress, tolerant plants exhibit moderate stomatal conductance, balancing gas exchange with water conservation. This balanced conductance allows them to maintain photosynthesis and growth, even under adverse conditions, enhancing their stress tolerance.

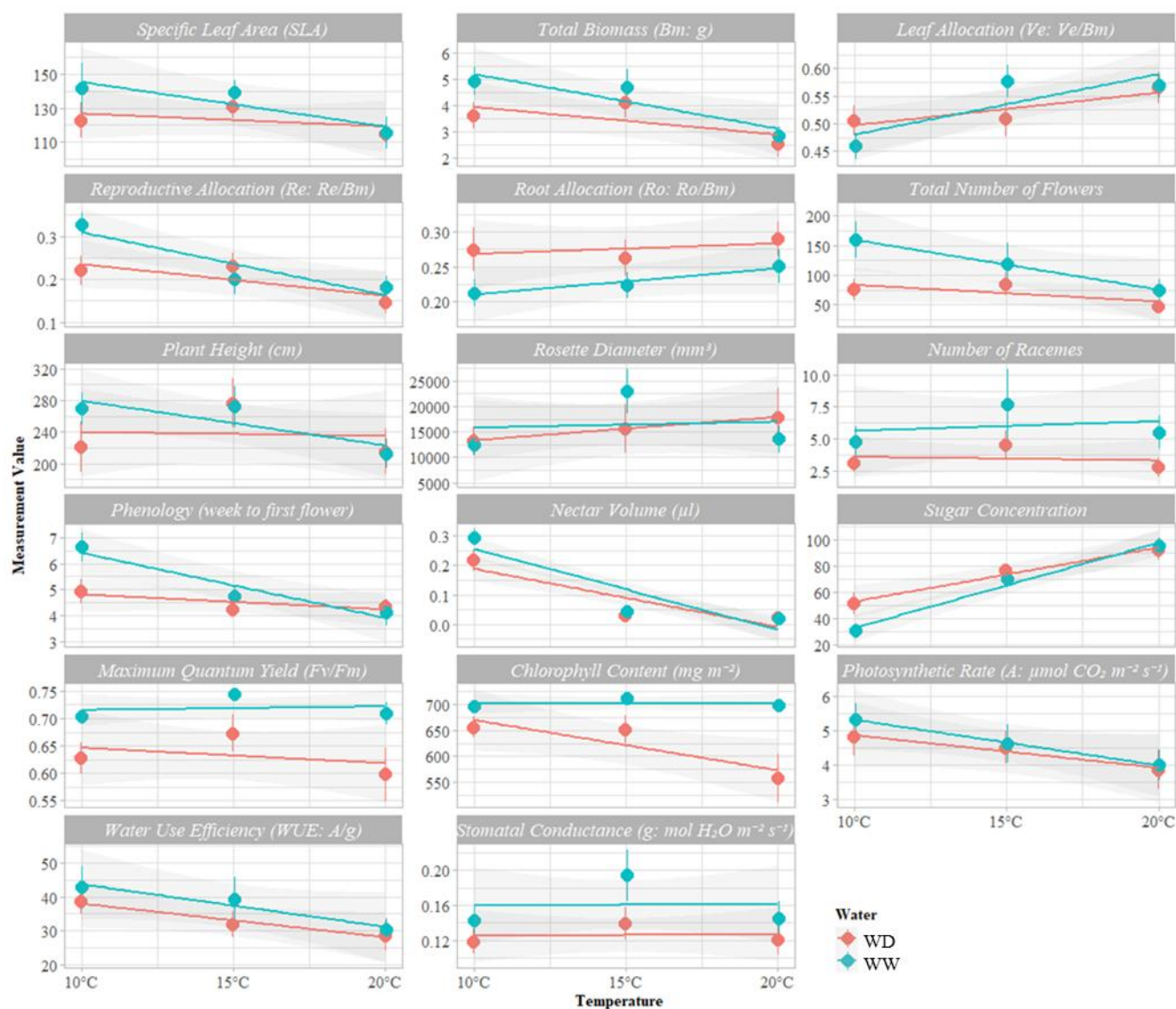


Figure S3.1: Mean plant trait responses of *Stylidium hispidum* under combined temperature treatments (10: 10-20°C, 15: 15-25°C, 20: 20-30°C) and two watering treatments (WW: well-watered; WD: water-deficit).

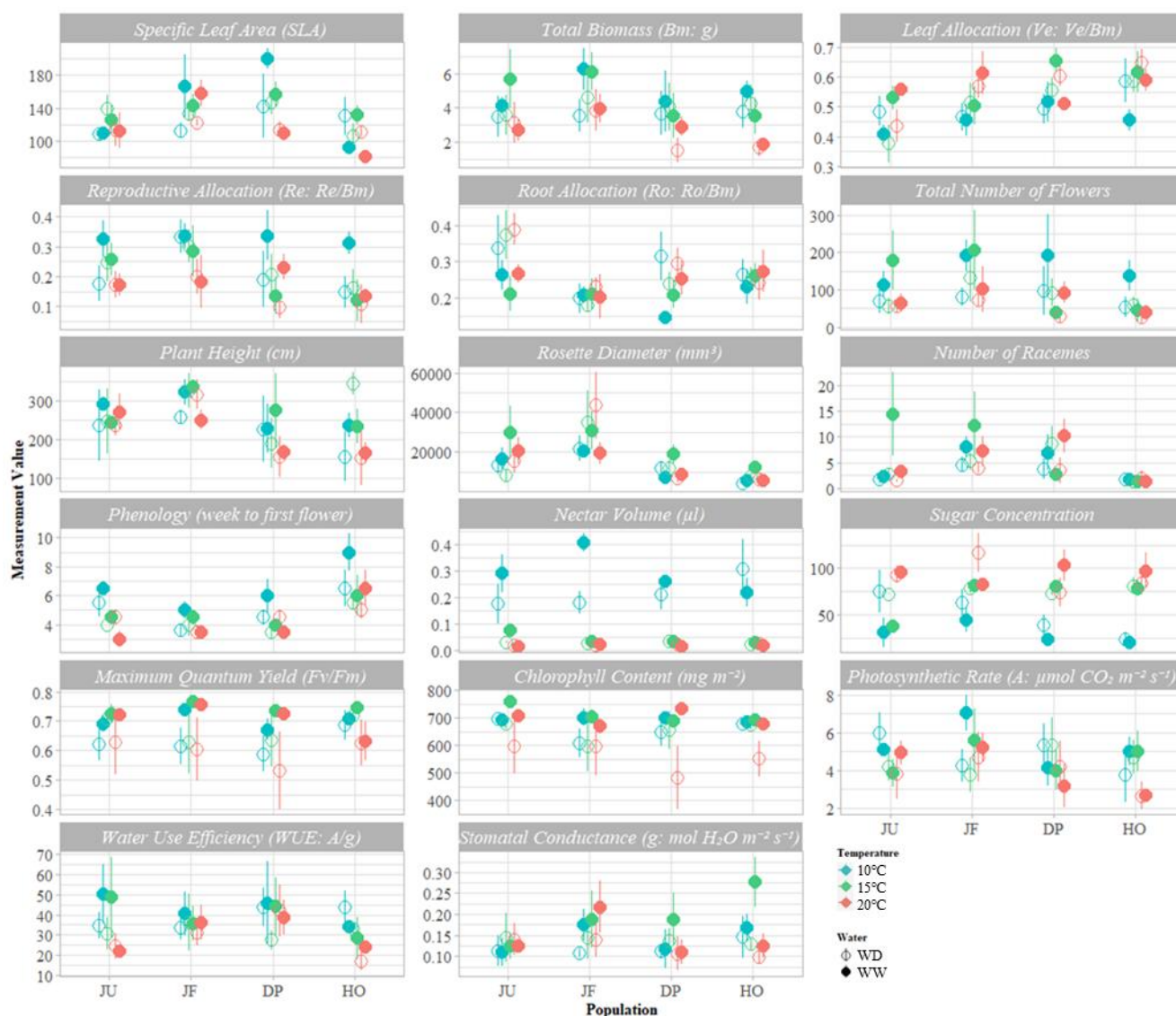


Figure S3.2: Plant trait responses across four populations [JU: Julimar Conservation Reserve; JF: John Forrest National Park; DP: State Forest (Dwellingup); HO: State Forest (Hoffman)] of *Stylidium hispidum* under three temperature treatments (10: 10-20°C, 15: 15-25°C, 20: 20-30°C) and two watering treatments (WW: well-watered; WD: water-deficit).

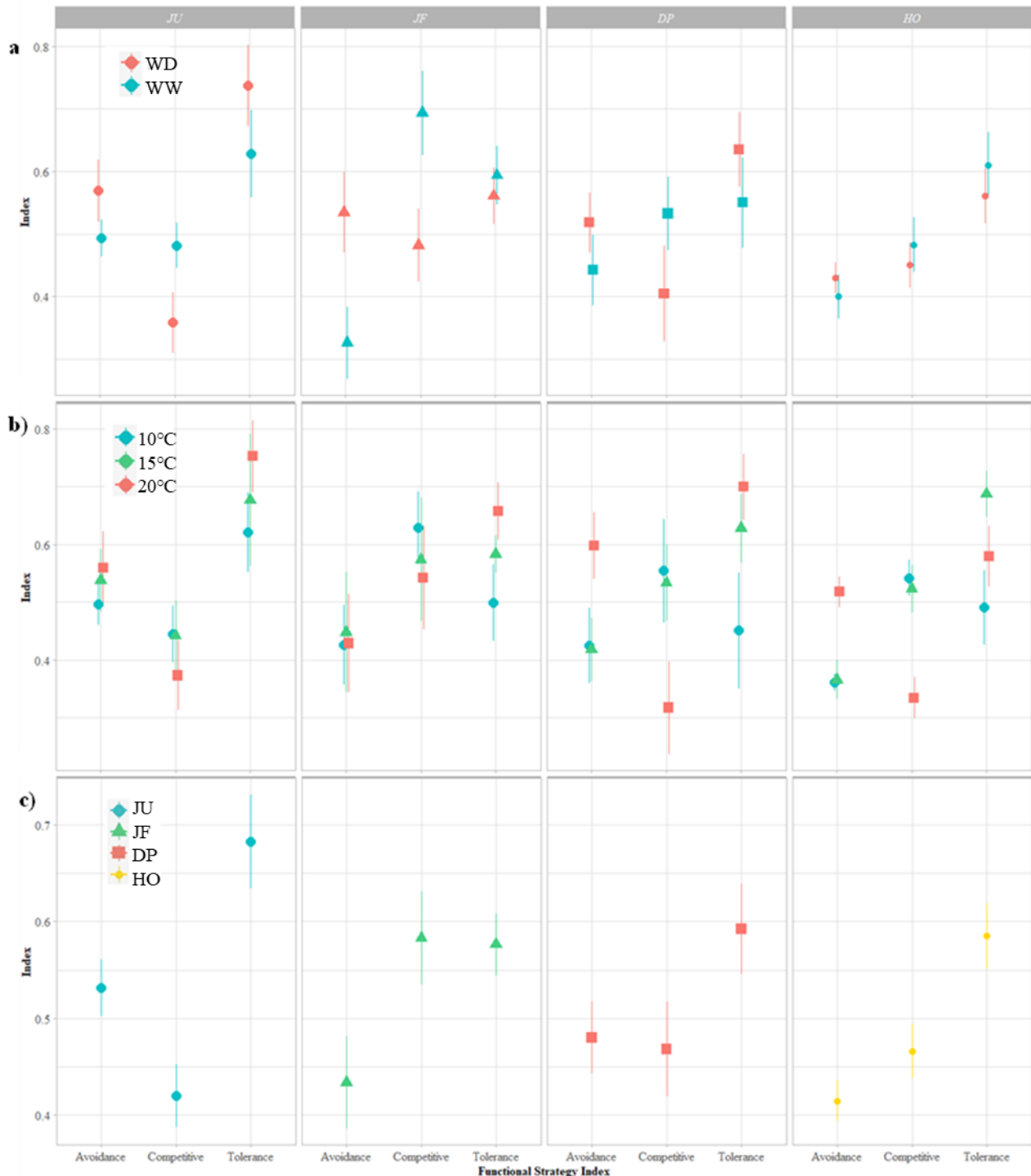


Figure S3.3: Mean and standard error for functional strategy indices (Avoidance, Competitive, and Tolerance) for *Styliidium hispidum* plants under **a)** well-watered (W; blue) and water-deficit (D; red); **b)** across temperature treatments (10: 10-20°C, 15: 15-25°C, 20: 20-30°C) and **c)** across all treatment combinations for each source population [JU: Julimar Conservation Reserve; JF: John Forrest National Park; DP: State Forest (Dwellingup); HO: State Forest (Hoffman)].