DR LEANDER D. L. ANDEREGG (Orcid ID : 0000-0002-5144-7254)

Article type : Regular Manuscript

Aridity drives coordinated trait shifts but not decreased trait variance across the geographic range of eight Australian trees

Leander DL Anderegg[1,2*], Xingwen Loy[3], Ian P Markham[4], Christina M Elmer[5], Mark J Hovenden[6], Janneke Hille Ris Lambers[7], Margaret M Mayfield[5,8]

Affiliations:

[1] Department of Integrative Biology, University of California, Berkeley, Berkeley, CA, USA 94720

[2] Department of Global Ecology, Carnegie Institution for Science, Stanford, CA, USA 94304

[3] Department of Environmental Sciences, Emory University, Atlanta, GA, USA 30322

[4] Wild Hope Collective

[5] School of Biological Sciences, The University of Queensland, St Lucia, QLD 4072, Australia

[6] Biological Sciences, School of Natural Sciences, University of Tasmania, Hobart, TAS 7005, Australia

[7] Department of Biology, University of Washington, Seattle, WA, USA 98195

[8] School of Biological Sciences, The University of Western Australia, Crawley, WA 6009, Australia

[*] Corresponding author: Leander DL Anderegg, leanderegg@gmail.com, +1 541.790.1096, 260 Panama St., Stanford, CA 94305

Received: 7 March 2020

Accepted: 28 June 2020

Social Media:

LDL Anderegg (ORCID ID: 0000-0002-5144-7254)
X Loy (ORCID ID: 0000-0003-4626-6747)
MJ Hovenden (ORCID ID: 0000-0001-7208-9700)
MM Mayfield (ORCID ID: 0000-0002-5101-6542)

Key words:

Eucalyptus, functional traits, intraspecific trait variation, water availability gradient, trait variance

Article Type: Full paper
Full Text: 5886 words
Introduction:
Methods:
Results:
Discussion
Figures: 6
Tables: 0
Supporting Information: Online SI – 1 file

Author Contributions: LDLA, MMM, MJH, JHRL designed the study, LDLA, IPM, XL, CME, collected data, LDLA performed the statistical analysis and wrote the first draft of the manuscript; all authors contributed substantially to revisions and approved of the final version.

Data accessibility statement:
All unpublished data is archived on Dryad (DOI: https://doi.org/10.6078/D1QQ5X) and relevant data and code is posted on Github (https://github.com/leanderegg/EucTraits)

Summary:

- Large intraspecific functional trait variation strongly impacts many aspects of communities and ecosystems, and is the medium upon which evolution works. Yet intraspecific trait variation is inconsistent and hard to predict across traits, species, and locations.
- We measured within-species variation in leaf mass per area (LMA), leaf dry matter content (LDMC), branch wood density (WD), and allocation to stem area vs. leaf area in branches (branch Huber value, HV) across the aridity range of seven Australian eucalypts and a co-occuring *Acacia* species to explore how traits and their variances change with aridity.
- Within-species, we found consistent increases in LMA, LDMC and WD, and HV with increasing aridity, resulting in consistent trait coordination across leaves and branches. However, this coordination only emerged across sites with large climate differences. Unlike trait means, patterns of trait variance with aridity were mixed across populations and species. Only LDMC showed constrained trait variation in more xeric species and drier populations that could indicate limits to plasticity or heritable trait variation.
- Our results highlight that climate can drive consistent within-species trait patterns, but that patterns might often be obscured by the complex nature of morphological traits, sampling incomplete species ranges, or sampling confounded stress gradients.

Introduction:

Land plants exhibit astounding variation in both form and physiological function. The identification of 'functional traits' as easily measured plant attributes that are proxies for plant physiological function and performance has spurred the rise of the field of 'plant functional ecology' and revealed some of the key causes and consequences of plant functional diversity (Mooney *et al.*, 1978; Field, 1988; Reich *et al.*, 1997; Díaz *et al.*, 2016; Ma *et al.*, 2018). In particular, across-species studies of plant traits have revealed global 'trait spectra' or 'trait syndromes'—correlations between different plant traits indicative of coordination across various aspects of plant physiology—that both illuminate trade-offs shaping plant evolution and provide powerful tools for community and ecosystem ecological studies (Wright *et al.*, 2004; Reich, 2014; Ma *et al.*, 2018).

While functional ecology has largely been built on trait patterns among species, our understanding of trait variation and trait coordination within individual species remains more limited. Ecologists increasingly recognize that within-species trait variation can be a large fraction of total trait variation (Albert *et al.*, 2010b; Siefert *et al.*, 2015), and that within-species trait variation has large consequences for ecological and evolutionary processes (Laforest-Lapointe *et al.*, 2014; Ahrens *et al.*, 2019a). Within-species variation in functional traits linked to stress tolerance has been increasingly used to predict plant responses to global change (Blackman *et al.*, 2017; Ahrens *et al.*, 2019a). Even as our appreciation of the importance of intra-specific variation grows, a mounting body of perplexing results reveals the limits to our understanding of within-species variation. For example, within-species trait responses to environmental gradients have defied generalization by proving highly trait-specific and species-specific (e.g. Schulze *et al.*, 1998; Albert *et al.*, 2010b,a; Vilà-Cabrera *et al.*, 2015; Rosas *et al.*, 2019), and sometimes even study specific (e.g. Martinez-Vilalta *et al.*, 2009; Laforest-Lapointe *et al.*, 2014). Some possible explanations for these inconsistencies are: 1) that different taxa employ different strategies of trait adjustment (Anderegg & HilleRisLambers, 2015) in which case closely related species will show more consistent trait responses, 2) that geographic stress gradients often confound multiple stressors, particularly drought and cold stress, and 3) that trait adjustments over only a portion of a species range are often subtle and hard to detect without sampling a species entire distribution (López *et al.*, 2016).

The between-species trait-trait coordination (consistent correlation among multiple traits) that underpins theory about trait spectra does not necessarily hold within individual species (Messier *et al.*, 2017; Anderegg *et al.*, 2018; Messier *et al.*, 2018). For instance, a recent analysis of intra-specific trait coordination in saplings of temperate tree species found that essentially none of the canonical trait relationships behind three classic theories of trait coordination held among individuals within species (Messier *et al.*, 2018). In another example, strong between-species trait-by-environment relationships and trait coordination didn't hold across populations within those same species in northern Spain (Rosas *et al.*, 2019). Indeed, some important trait-trait relationships can even reverse direction within- versus between-species (Anderegg *et al.*, 2018). This contrasting within- verse between-species trait coordination suggests that classical explanations of trait correlations do not necessarily hold within-species, limiting their applicability for predicting species' functional responses to climate change.

Additionally, patterns of trait variances within-species remain poorly understood. The study of trait variance (rather than trait means) has a long history in community ecology (Kraft *et al.*, 2014) and evolutionary studies, where heritable trait variance is the necessary precondition for evolution. Yet trait variances have often been overlooked in the ecophysiological literature. Ultimately, predicting plant responses to a shifting environment requires an improved understanding of the amount of heritable trait variation, the capacity for trait plasticity, and the trait-fitness links causing evolutionary selection within a species and/or performance differences among species in a community (Richter *et al.*, 2011; Chevin *et al.*, 2012; Alberto *et al.*, 2013; Franks *et al.*, 2014; Valladares *et al.*, 2014). As a first step towards this understanding, observations of the size and spatial patterns of trait variances in different species and between populations of the same species are critical (Molina-Montenegro & Naya, 2012; Lemke *et al.*, 2012; Siefert *et al.*, 2015). For instance, decreased amounts of trait variation in range-edge populations of a species could indicate fundamental limits to trait plasticity or that the strength of directional selection surpasses the rate of generation of genetic diversity (e.g. immigration, mutation). Either would indicate limited potential for trait change in those populations in a changing environment. Among species, environmental stress has been predicted to constrain phenotypic plasticity (Valladares *et al.*, 2007) but also possibly increase the expression of genetic variability in traits (Hoffmann & Merilä, 1999), and current evidence for either increasing or decreasing within-species trait variation with drought stress is weak (Siefert *et al.*, 2015). Thus, understanding trait variances may be more critical to predicting potential plant responses to climate change than trait means themselves.

While many of the plant physiological traits known to be most related to drought resistance are time consuming to measure (e.g. xylem vulnerability to embolism, leaf turgor loss point), a number of morphological “soft traits” have less direct but still clear links to drought resistance and can be tractably assessed across hundreds of individuals and large geographic areas. For instance, across species, increased leaf robustness quantified by Leaf Mass per Area (LMA) and Leaf Dry Matter Content (LDMC) and stem robustness quantified by Wood Density (WD) are often associated with xeric environments because their variation is partly driven by anatomical adjustments that allow plants to maintain hydraulic function under increasingly negative xylem pressures (Schulze *et al.*, 1998; Niinemets, 1999; Schulze *et al.*, 2006; Chave *et al.*, 2009; Poorter *et al.*, 2009; John *et al.*, 2017; Li *et al.*, 2018b). The ratio of stem sapwood area to leaf area or Huber value (HV) reflects the balance of hydraulic supply (sapwood area) relative to hydraulic demand (leaf area), with high HV typically indicating increased hydraulic efficiency and thus increased drought avoidance (Tyree & Ewers, 1991; Mencuccini & Grace, 1995; Li *et al.*, 2019; Mencuccini *et al.*, 2019). Even though these morphological traits integrate numerous drought- and non-drought-related anatomical traits, multiple tree species have been found to adjust at least one of these traits depending on water availability (Martinez-Vilalta *et al.*, 2009; Anderegg & HilleRisLambers, 2015; Rosas *et al.*, 2019). Moreover, in recent studies of *Eucalyptus* species, changes in LMA, HV and WD were associated with more physiologically direct mechanisms of drought tolerance such as changes in xylem anatomy and vulnerability to embolism (Pfautsch *et al.*, 2012; Zolfaghar *et al.*, 2015; Pfautsch *et al.*, 2016; Li *et al.*, 2018b; Pritzkow *et al.*, 2019), suggesting that they are relevant markers of drought resistance in eucalypts. These traits are also central to our understanding of plant carbon allocation and carbon stocks. For example, LMA is a key model parameter in many vegetation models for translating carbon allocated to leaves into leaf area and WD is a critical component of carbon stock estimation and prediction (Nabuurs *et al.*, 2008; Kovenock & Swann, 2018).

Within-species trait variation at landscape-scales is the result of some unknown combination of genetic or ‘ecotypic’ variation among populations (G effects), plastic adjustments to environmental gradients (E effects) and GxE interactions. Definitively disentangling these components requires experiments such as provenance trials or common gardens (McLean *et al.*, 2014; Ahrens *et al.*, 2019b). However, because genetic variation among species is traditionally larger than ecotypic variation within species, trait variation among related species in their native habitat is often ascribed primarily to G effects. Thus, by sampling within-species and across closely-related species, both genetic + plastic trait changes and primarily genetic trait changes can be explored.

Here, we examine within-species variation in leaf and stem robustness and allocation within closely related tree species across large gradients in water availability in the absence of confounding freezing stress. We present a controlled test of predictions about intraspecific trait variation across nested scales of organization, focusing on trait variation across aridity gradients in Western Australia and Tasmania. Further, we minimize differences in species life history by holding phylogenetic history relatively constant for seven core species ('eucalypts' from the closely related *Eucalyptus* and *Corymbia* genera). We compare these patterns within eucalypts to an unrelated species (*Acacia acuminata*) that co-occurs with the most xeric sampled eucalypt.

The specific questions we ask are:

1. Do leaf and stem tissues, and leaf vs stem allocation show consistent relationships with water availability across the full aridity range of multiple closely related species? Or are trait-environment relationship context dependent (e.g. present in xeric species but not in mesic species)?
2. Do species consistently show coordination between leaf and stem robustness, and leaf to stem allocation, and if so at what scale does this coordination emerge?
3. Is the total amount of within-species variation in leaf and stem traits more constrained in dry sites (both within species across sites and across sister species with different aridity niches), indicating potential limits to trait change in a drying climate?

Given their association with drought resistant phenotypes, we expected LMA, LDMC, WD and HV to increase with aridity, resulting in coordinated trait changes across tissues. We predicted consistent trait-environment relationships among closely related eucalypts, regardless of whether they were mesic or xeric, but potentially less consistency between the eucalypts and an unrelated *Acacia.* Alternatively, if trait adjustments are not consistent among eucalypts, we predicted that xeric eucalypts and the xeric *Acacia* would show stronger trait patterns than mesic eucalypts due to the increased biogeographic importance of drought stress in harsh environments (MacArthur, 1972). In addition, assuming ongoing directional selection and a limit to both plasticity and genetic variation near each species' dry range edge, we predicted that within-species variation in these traits would decrease in higher aridity populations within a species. If plasticity is constrained in harsh environments, we also predicted that xeric species would show less within-species trait variation than mesic species.

Methods:

Study site

We collected trait data along two temperate aridity gradients (Figure S1), one in southwest Western Australia (sampled November 2014) and one in Tasmania (sampled February 2016). Along each gradient, we identified three or four dominant eucalypt tree species (from the *Eucalyptus* or *Corymbia* genera of the Myrtaceae family) that are easily identified in the field and do not widely form cryptic hybrids or have notable subspecies within the sampled regions. In Western Australia, we sampled *Eucalyptus marginata* Donn ex Sm., *Eucalyptus salmonophloia* F.Muell., and *Corymbia calophylla* (Lindl.) K.D. Hill & L.A.S. Johnson. We also opportunistically sampled the non-eucalypt *Acacia acuminata* Benth., which broadly co-occurs with the most xeric sampled eucalypt, *E. salmonophloia.* In Tasmania we sampled *Eucalyptus amygdalina* Labill*., Eucalyptus obliqua* L'Hér*., Eucalyptus ovata* Labill*.,* and *Eucalyptus viminalis* subsp. *viminalis* Labill, all of which cover the majority of their global precipitation range within Tasmania*.* All focal species are evergreen, dominant or co-dominant canopy trees with the exception of *Acacia acuminata*, which is a small tree/tall shrub. All focal species are common in multiple vegetation types and are both habitat generalists (i.e. not riparian-affiliated) and soil type generalists with the exception of *E. marginata*, which is a habitat generalist but principally occurs on ironstone-derived soils. Collectively, sampled sites spanned a mean annual precipitation range of 328 to 1574mm/year (328 to 1189 mm in Western Australia, 584 to 1574 mm in Tasmania). Mean annual temperature spanned 8-20°C and elevation ranged from 24-620 m.a.s.l, with no site experiencing significant frost (mean coldest month minimum temperature >0°C for all sites). Average site climate, soil, DBH (diameter at breast height as a proxy for tree size) and stand basal area (measured for each tree with a variable radius forestry wedge prism, Tasmania only) can be found in Table S1. Sampled tree size and (where measured in Tasmania) stand Basal Area did not vary strongly with aridity for most species (Table S1). Climate data for sampled plots, including mean annual precipitation (PPT), potential evapotranspiration (PET), and moisture deficit (MD = PET – PPT), were extracted from the CHELSA 30 arc second (~1km) gridded climate database of 1979-2013 climatologies (Karger *et al.*, 2017). Aridity Index (P/PET) was also calculated but found to be almost perfectly collinear with MD(Figure S2). Soil properties including soil depth and regolith depth, as well as % sand, silt and clay, total nitrogen by mass, total phosphorus by mass, average water holding capacity, bulk density, and effective cation exchange capacity (averaged over the top 60cm soil depth) were downloaded from the 3 arcsecond resolution (~90m) Soil and Landscape Grid of Australia (Grundy *et al.*, 2015), using the *slga* R package (O'Brien 2019). Because soil properties were strongly collinear, we performed a Principal Component Analysis (PCA) on the soil variables and used the first two principal components (PCs) in analyses. The first PC explained 67% of soil variation and was interpreted as 'soil fertility' because it loaded strongly (>0.3) with everything except depth of regolith, depth of soil and water holding capacity. The second PC captured 12% of variation, loaded strongly with water holding capacity and soil depth and was interpreted as 'soil depth'.

Trait measurement

We measured branch wood density (WD, g dry mass per cm^3 fresh volume), leaf mass per area (LMA, g dry mass per cm^2 fresh leaf area) and leaf dry matter content (LDMC, g dry mass per g fresh mass) as metrics of stem and leaf robustness, and terminal branch Huber value (HV), the ratio of sapwood area to leaf area (mm^2 per cm^2), as a metric of investment in water transport versus light capture. Trait measurements were collected in a nested hierarchical design with four to five sites sampled per species to capture broad climate gradients, three plots per site to capture topographic/edaphic variation, five trees per plot to capture within-population variation, and three samples per tree to capture within-individual variation (Figure S1). For each species, four to five forestry reserves, National Parks, State Forests, Nature Reserves, or Conservation Areas were selected to cover as much of each species' precipitation range as possible. Each species' precipitation range was determined using collection locations of herbarium specimens, initially downloaded from Australia Virtual Herbarium (www.avh.chah.org.au) for identifying sampling locations and later validated with expanded occurrence records from the Atlas of Living Australia (see species climate distributions and sampling coverage in Figure S3, ala.org.au). Edaphic variation within sites was captured by locating three plots that were >500 m but <5 km apart and each containing more than five individuals of the focal species within a 30 m radius. In each of the three plots, we sampled within-population variation by collecting three sun exposed branches from the north side of each of five mature, healthy individuals using pole clippers and pull ropes. Sampled individuals in a plot were apparently healthy, canopy dominant trees at least 5m but less than 60m apart. Our sample design resulted in 180-225 trait measurements per species.

From each branch, we collected a section ~8 mm in diameter for WD measurement, and a terminal branch (first order branch collected at the point of branching) for leaf and HV measurements. We selected terminal branches (typically ~1mm in diameter, see Table S2 for details) with intact 'mature' leaves (i.e. fully expanded, not soft green new growth), though most of the study species flush sporadically throughout the year (Davison & Tay, 1989; Heatwole *et al.*, 1997) so it was not possible to perfectly control for leaf age. Sampling periods (Nov. in Western Australia and Feb. in Tasmania) avoided large leaf flush events for all species with the exception of *Corymbia calophylla* at two of its five sample sites. Samples were rehydrated in moist ziplock bags in a cooler for at least 12 hours prior to trait measurement (Pérez-Harguindeguy *et al.*, 2013). Bark was peeled from branch sections and WD quantified from segments roughly 7 cm in length by dividing dry mass (following 72+ hrs drying at 70°C). WD was weakly related to branch diameter for six species (Likelihood Ratio Test of a linear mixed effects model with a fixed effect for diameter and site and random effects for plot and tree was significant at alpha = 0.05 compared to an identical model without diameter), so diameter was included as a covariate in models of WD for these species.

All leaves subtending the selected terminal branch were collected for measurement of leaf area, LMA and LDMC. Total fresh one-sided leaf area (including petioles) of terminal branch samples was measured with a flatbed scanner and ImageJ image processing software (Schneider *et al.*, 2012). Leaves were then oven dried at 70°C to a constant weight (typically 48+ hrs) and their dry mass measured. Terminal twig basal diameter was measured just above the swelling at the branch base after gently peeling back bark (except in *A. acuminata*, where bark was difficult to distinguish from woody tissue). For each terminal branch HV, LMA, and LDMC was calculated. Multivariate trait outliers were visually diagnosed by plotting all traits against each other for each species and removed (n<10 per trait), as were LMA and LDMC values from still expanding leaves (<10% of measurements).

Statistics

Q1 – Trait-aridity relationships: We tested for significant trait-environment relationships using information-theory based model selection. For each species, we fit candidate linear mixed effects models relating each trait to plot mean annual PPT, PET, MD, soil fertility (soil PC1), or soil depth (soil PC2) with plot and tree random intercepts. We also included tree DBH (measured for all species except for often multi-stemmed *Acacia acuminata* and for *E. salmonophloia* which was missing measurements from two site) and the stand Basal Area around the focal tree (measured for Tasmanian species with a wedge prism) as potential individual-level covariates where measured. Because soil and climate variables were often collinear (Figure S2) and most soil and climatic variation occurred among sites (n=4-5 per species) we only included models with single soil/climate predictors and not multiple predictors or interactions to avoid overfitting. We then compared the candidate models and a null model (with only plot and tree random effect) using Akiake's Information Criterion corrected for small sample sizes (AICc) and selected the model with the fewest parameters that was within 2 AICc of the model with the lowest AICc. We quantified statistical significance of this model compared to the null model using Likelihood Ratio Tests (LRT). Where a soil variable proved the best trait predictor, we also tested the significance of the best climate model because soil and climate variables were often strongly collinear (Figure S2). We visually checked the model fit and the validity of model assumptions (e.g. normality of residuals, normality of random effects) using a variety of model criticism plots (see code at https://github.com/leanderegg/EucTraits).

Q2 – Trait coordination: We assessed trait-trait covariation using multiple approaches. First, for each species we tested for significant Pearson correlations between tree-level averaged traits for all trait pairs and visualized the relationships with Standardized Major Axis (SMA) regressions. Next, we assessed the distribution of trait-trait correlations for hierarchically nested data subsets to assess at what level trait coordination emerges. For each trait pair for each species, this involved calculating the Pearson correlations across the replicate branches within each tree, across tree averages in each plot, across plot averages in each site, and across site averages, for all eight sampled species. Lastly, we assessed the dominant mode of trait covariation across all traits and species. We performed a Principal Component Analysis (PCA) on all branch-level trait measurements with complete trait data (1400 branches), and assessed the trait loadings along the first and second PC axes. We then calculated the PC score for all site-averaged trait values, and assessed whether any PC related to site MD across species using a linear mixed effect model including a fixed effect of MD and species random slopes and intercepts.

Q3 – Constrained variance at high aridity: We first examined whether more xeric species showed less intraspecific trait variation than mesic species. For each species and trait, we quantified the amount of trait variation at each nested scale using variance decomposition by fitting a linear mixed effect model with a fixed intercept and random effects for site, plot, and tree. In this formulation, the random effect variance parameters represent the between-site, between-plot in site, and between-tree in plot variance (respectively), with the residual variance representing samples within tree. We then characterized species' aridity niche based on occurrence records in the Atlas of Living Australia, extracting the MD values for each occurrence from the CHELSA gridded climatologies and calculating the range center (median MD) and dry range edge (90th percentile MD) of each species' climatic distribution. We then used the species' range center or dry range edge as an index of how mesic or xeric each species' range is. To test for among-species patterns, we extracted the variance parameters for each eucalypt species (excluding *Acacia acuminate*) and used linear models to relate species total trait variance (sum of all variance components for a trait) to the species' dry range edge. We also tested whether individual variance components decreased with increasing aridity by fitting linear models relating species variance components to each species' driest range edge plus a fixed effect for variance component (between-site, between-plot, between-tree, or within-tree) and a component-by-MD interaction.

To test for decreasing trait variation with aridity within species (i.e. across populations), we used AICc to determine whether the best trait-aridity mixed effect model (from Q1) for each species and trait was improved by allowing the variance to change as either a power or exponential function of the dominant climate variable, or to assume a different value for each site. If AICc and LRTs suggested that a non-constant variance function improved the trait-climate model, we classified whether the variance increased with aridity, decreased with aridity, or showed variation between sites that was not aridity-related (i.e. the model with different variances per site was the best model).

All analyses were performed in the R statistical environment (R Core Team, 2019), version 3.6.0). Mixed effects models were fit using the *lme4* and *lmerTest* packages (Bates *et al.*, 2015; Kuznetsova *et al.*, 2017) for fixed variance models, or the *nlme* package for more complicated variance structures (Pinheiro *et al.*, 2019). SMA regressions were fit using the *lmodel2* package (Legendre, 2014). All data and analysis code is available in the Github repository associated with this paper (https://github.com/leanderegg/EucTraits). Data are also available in the Dryad data repository (DOI: https://doi.org/10.6078/D1QQ5X).

Results

Do traits respond to aridity?

For the majority of our examined species, most traits shifted in a way consistent with greater drought resistance (increased WD, LMA, LDMC and HV) in higher aridity plots (Figure 1). All species showed significant trait-by-environment relationships for LMA and LDMC and seven of the eight species showed significant trait-by-environment relationships for WD and HV (Table S3). A measure of aridity (PPT, PET or MD) was the best predictor in 19 of 32 trait-by-environment relationships, soil fertility in 9 of 32 and soil depth in 2 of 32. However, in all but one of the trait-by-environment relationships where soil quality or depth was the best predictor, precipitation was collinear to that soil variable and also a significant, if worse, predictor (Table S3). Precipitation, potential evapotranspiration, moisture deficit and soil fertility were correlated across plots for many, but not all species (Figure S2). Tree DBH was never a significant trait predictor in final models, and stand Basal Area was only included as a covariate for one trait (LMA) for two of the Tasmanian species (Table S3). Across the seven eucalypts, species mean trait values also showed significant or marginally significant positive relationships with species median MD, though for WD and LMA this was driven primarily by the driest species (Figure S4).

Are trait responses coordinated across tissues?

Ubiquitous trait-by-environment relationships resulted in coherent trait coordination across leaf and stem tissue, and coordination between leaf robustness and increased HV within species (Figure 2). However, while consistent and often significant, these within-species trait correlations were typically weak, with the mean within-species trait correlation being <0.5 for all trait pairs except LMA and LDMC. Across tree-level trait averages, the majority of species showed significant correlations between both WD and LMA (mean correlation of 0.33) and WD and LDMC (mean correlation of 0.38; Figure 2a, 2b), though these were typically less strong than the correlations between LMA and LDMC (mean correlation of 0.74; Figure 2c). Both leaf traits were also positively correlated with HV, with mean correlations of 0.44 and 0.32 for LMA and LDMC respectively. However, WD was only significantly correlated with HV in three species. In the seven eucalypts, most species fell in roughly the same trait space, with more trait variation within each species than across species (Figure 2). *Acacia acuminata* showed larger HV, but similar trait correlations to the seven eucalypts (Figure 2d, 2e, 2f).

Within-species trait coordination only emerged when comparing traits across the most disparate environments. The distribution of correlation coefficients at smaller spatial scales (e.g. trait-trait correlations across individuals or branches within a plot, correlations across plots or individuals within a site) typically had an interquartile range spanning zero for all trait pairs except LMA-LDMC and HV-LMA (Figure 2g, Table S4). Only when comparing across site mean trait values did the mean within-species correlation differ substantially from zero for most trait pairs (Table S4). This decrease in correlation strength at smaller spatial scales was not purely a result of smaller sampled trait variation, as there was often as much or more trait variation within plots as across sites, and funnel plots did not show strong relationships between correlation strength and sampled trait variance except for the relationship between HV and LMA (Figure S5).

Even though trait coordination only emerged across large aridity gradients, the dominant mode of trait variation in the entire dataset was a coordinated increase in tissue robustness and HV, both within and among species, likely driven by decreasing water availability. In a PCA of the entire branch-level dataset, the first principal component (PC1) explained 53% of the total variance and was loaded reasonably equally with all four traits (Figure 3a). Additionally, for each species the site-level average PC1 score was strongly related to site PPT and MD,(linear mixed-effects models, $p<0.0007$ and 0.004, marginal $R^2 = 0.51$ and 0.53 respectively) though not site PET. While the slopes differed between species (particularly for the driest eucalypt and the *Acacia*) these patterns indicate that the coordinated increase in WD, LMA and LDMC, and HV represented by PC1 was driven by water availability (Figure 3b). The second trait PC (PC2) explained 26% of the variation, was largely unrelated to climate, and primarily differentiated the seven eucalypts from *Acacia acuminata* (Figure S6).

Is trait variation constrained at higher aridity?

Evidence for increasingly constrained trait variation at higher levels of aridity was mixed, both within and among species. Variance decomposition revealed huge variability in the total amount and dominant scales of within-species trait variation (Figure 4). Variation between plots in a site was almost always the smallest variance component. The relative contribution of within-tree, within-plot and between-site variation differed drastically, however, depending on the trait and species (Figure 4). The only exception was the consistently high amount of within-tree variation in $\log_{10}$-transformed HV, which made up >40% of total trait variation in all species. *Acacia acuminata* also tended to have much larger intra-specific trait variation than any of the sampled eucalypts. Combining all trait data from all seven eucalypt species, within-species trait variation represented between 31% (WD) and 72 % ($\log_{10}$(HV)) of total trait variation, and between-site/climate-related within-species trait variation was over half the magnitude of inter-specific variation in LDMC and $\log_{10}$(HV) (Figure S7).

Across species, there was limited evidence for decreased intraspecific trait variation in more xeric species. In the seven eucalypts, total within-species trait variation was unrelated to the aridity of a species' geographic distribution (the median MD of herbarium specimen locations) for WD, LMA, and LDMC, but was marginally negatively related for LDMC ($p=0.11$; Figure 5a-d). Most individual variance components were also unrelated to species aridity niche. However, the amount of between-site variation was negatively related to species aridity niche for HV ($p=0.001$) and marginally for

LDMC (p=0.07; Figure 5e-h). Results were similar but slightly more significant using the species' dry range edge (90th percentile MD) rather than niche center (median MD). Results were also similar using trait coefficients of variation (CV=trait standard deviation divided by trait mean) rather than trait variances, though the CV of HV was no longer related to species mean MD but the CV of LMA decreased marginally with MD (p=0.067, Figure S8).

Within-species, variance patterns moving from wet sites to dry sites also showed mixed support for decreasing variance with increasing aridity. A few species did show constrained within-tree and within-plot trait variation at drier sites in a few traits, consistent with an increasingly strong environmental filter. However, most species for most traits showed no change in trait variance across sites (Figure 6). LDMC showed the most consistent variance constraint with aridity, with three of eight species showing lower trait variances at drier sites. HV showed no aridity-related variance patterns in any species (Figure 6).

Discussion

Our extensive dataset of 1620 paired trait measurements demonstrated that increasing aridity resulted in coordinated trait shifts. However, these shifts were only evident across large aridity gradients. Moreover, despite ubiquitous aridity-related increases in trait means consistent with high trait values being adaptive, we did not find decreasing trait variances with decreasing water availability except in LDMC, providing little initial evidence for constrained plasticity or limited genetic variation. Below, we discuss these results in greater detail.

Mean trait shifts

Shifts in leaf, stem, and allocation traits towards more drought resistant values at drier sites were ubiquitous across the sampled species (Figure 1). Indeed, within-species trait shifts due to some combination of local adaptation and plasticity were in the same direction and sometimes even of the same magnitude as trait relationships across species (Figure S4), particularly for the driest euclaypt (*E salmonophloia*) and for the trait HV. These shifts are consistent with increases in tissue drought tolerance, and indeed all traits but LDMC have previously been reported to show within-species patterns related to water availability either geographically or experimentally in eucalypts (Li & Wang, 2003; Schulze *et al.*, 2006; Zolfaghar *et al.*, 2014; McLean *et al.*, 2014). In *Eucalyptus obliqua,* geographic variation in LMA and HV are associated with concurrent changes in physiological traits such as leaf turgor loss point, xylem vessel wall thickness, and xylem vulnerability to embolism, primarily through plasticity rather than genetic ecotypic variation (Pritzkow *et al.* 2019). However, these same traits often do not show aridity-related variation within-species in other systems (Martinez-Vilalta *et al.*, 2009; Fajardo & Piper, 2010; Richardson *et al.*, 2013; Laforest-Lapointe *et al.*, 2014; Vilà-Cabrera *et al.*, 2015; Anderegg & HilleRisLambers, 2015; Rosas *et al.*, 2019). This may be in part due to the nature of these morphological traits themselves. HV is directly relevant to the water balance and hydraulic status of a plant (Whitehead & Jarvis, 1981; Trugman *et al.*, 2019), but traits like wood density are only partially mechanistically linked to more drought-relevant physiological traits such as xylem vulnerability to embolism (Lens *et al.*, 2010), and linkages have typically only been shown among species rather than within species. However, complicated and inconsistent trait-environment relationships are often found even for more labor intensive plant hydraulic traits (Rosas *et al.*, 2019).

In the literature, it is more common to find changes in only a subset of traits than to find the consistent trait coordination documented here. The lack of consistent trait-environment relationships in the literature might be due to fundamentally different capacities of various clades to adjust different tissue characteristics. For example, it is possible that eucalypts are a taxon with anomalously large morphological plasticity or adaptability. Indeed, eucalypts have demonstrated remarkably clean trait-environment patterns within and among species in numerous studies (Schulze *et al.*, 1998; 2006; Pfautsch *et al.*, 2016; Li *et al.*, 2018a). However, other methodological causes of the discrepancies in the literature warrant mentioning.

This study was unique in that it explicitly sampled as much of each focal species' geographic aridity niche as possible, and because the aridity gradients in Australia are largely unconfounded by freezing stress. Given that between-site, or climate-related trait variation is often less than half of total within-species trait variation (Figure 4), sampling as broad of climate space as possible may be critical to ensure that one can detect the climate signal from the considerable noise. With the exception of *E. obliqua*, our collections covered the vast majority of the precipitation space inhabited by all study species and the bulk of potential evapotranspiration space (Figure S3).

Additionally, the confounding effect of cold stress may weaken trait-climate relationships and obscure trait coordination. Morphological traits such as LMA are known to vary with multiple environmental signals, including water availability, nutrient availability, and cold stress (Poorter *et al.*, 2009). In our study, none of our sites experienced significant cold stress, though soil quality and water availability co-varied (Table S1, Figure S2). While some patterns documented here may be due to changes in nutrient rather than water availability (soil quality or depth was the best trait predictor in ~1/3 of trait-environment relationships), these stresses tend to have similar effects on morphology that may reinforce each other in our study. For example, low nutrient availability and low water availability both tend to increase LMA in isolation (Poorter *et al.*, 2009). However, in cold temperate study systems, cold stress and low water availability tend to have the similar effect of increasing tissue robustness but are *negatively* correlated on the landscape. We posit that studies focused on elevational gradients (Fajardo & Piper, 2010; Anderegg & HilleRisLambers, 2015) and latitudinal gradients (Martinez-Vilalta *et al.*, 2009) in the cold temperate zones are likely to see confounding effects of cold stress and drought stress, particularly on leaf traits (González-Zurdo *et al.*, 2016; Niinemets, 2016). If stem versus leaf allocation (i.e. HV) is less sensitive to cold stress than other morphological traits, this could explain why HV shows more ubiquitous within-species patterns than other morphological adjustments, as HV is responding to only a single stress gradient while leaf traits are responding to the complex interactions between multiple stressors.

Trait coordination

We found that coordination across leaf, stem, and allocation traits related to aridity was consistent across species and the dominant mode of trait variation in our study (Figure 2 & 3). One implication of this coordination is that the effects of water stress are scaled to species physiology, such that both mesic and xeric species must respond similarly to increasing water stress at their dry range edge regardless of large differences in total water availability. Our seven eucalypt species differed in the wetness of their range center by over 1100 mm of moisture deficit (Figure 5). Yet all of them showed significant trait-by-aridity relationships and trait-trait coordination.

The consistent trait coordination across leaf, stem, and allocation traits found here is also reasonably unique in the literature. It is far more common for within-species trait coordination to show variable and often unexpected patterns (Richardson *et al.*, 2013; Laforest-Lapointe *et al.*, 2014; Anderegg *et al.*, 2018; Messier *et al.*, 2018; Rosas *et al.*, 2019). However, while present in our entire dataset (Figure 3), trait coordination only emerged at the largest of spatial and ecological scales (Figure 2g). Indeed, even though variation between branches in a canopy and between individuals within a plot constituted the majority of trait variation in the majority of traits and species (25 of 32 species by trait combinations, Figure 4). Despite this, consistent trait correlations only emerged across site-level trait averages in five of six trait pairs (Table S4).

These large-scale trait correlations suggests that leaf, stem, and allocation traits are at best weakly mechanistically linked within species. Even when many axes of variation are held constant by looking only within a species, the potential for compensating trait variation (e.g. between roots and leaves) and the important but ultimately weak relationships of many ‘functional traits’ with either physiological rates or demographic outcomes should make weak trait-trait relationships the norm and strong coordination the exception in land plants. Moreover, given that functional traits may respond independently to different environmental stresses (Anderegg *et al.*, 2018), it should be no surprise that consistent within-species trait coordination has been so elusive in the literature.

Patterns in trait variance

In contrast to the ubiquitous patterns in trait means, we found less evidence for consistent patterns in trait variances with aridity. Looking across the seven eucalypt species (i.e. focusing primarily on the between species or G component), we found that HV and to a lesser extent LDMC tended to be more constrained in xeric than mesic species but the same was not true of LMA and WD. This pattern was more statistically significant for between-site variance than total variance, suggesting that the component of trait variation controlled by climate was indeed increasingly constrained at low water availability (Figure 5), perhaps indicating constrained plasticity in harsh environments (Valladares *et al.*, 2007). However, this pattern only sporadically scaled down to populations within species (where G, E and GxE effects are possible), with almost half of species showing marked variance patterns across sites for LDMC, rare variance constraints in HV, LMA and WD (Figure 6).. LDMC was somewhat unique among the four traits, showing the most prevalent within-species variance patterns (Figure 6) and decreasing climate-linked trait variances (Figure 5) and CVs (Figure

S8) with aridity across species. LDMC may therefore be a worthwhile trait to investigate alongside more detailed physiological measurements in common garden work with *Eucalyptus* (Bourne *et al.*, 2017) and other trees. For other traits, GxE effects (an adaptive increase in plasticity) may maintain plasticity in dry-adapted populations, as found for LMA in a common garden study of *Eucalyptus tricarpa* (L.A.S. Johnson) L.A.S. Johnson & K.D. Hill (McLean *et al.*, 2014).

If LMA, WD and HV are under selection in a warming world (which is likely given the trait-by-aridity relationships within and between species), their variance patterns may be good news for the adaptive and/or acclimatory potential of these species. The acclimatory potential for HV may be particularly high, given the consistently high within-tree variation in this trait (Figure 4). Meanwhile, depending on the heritability of WD and LMA, which has often proven to be high in angiosperms (Zobel & Jett, 1995; Poorter *et al.*, 2009) and specifically high for WD but not LMA in *C. calophylla* (Ahrens *et al.*, 2019a), the reliably high within-plot variation (Figure 4) and lack of variance-by-aridity relationships (Figure 5 & 6) may indicate considerable adaptive potential.

It should be noted, however, that a likely explanation for both the weak trait coordination and the mixed variance patterns documented here is that selection is not happening on any of these four traits directly, but rather on underlying anatomical traits that collectively determine gross morphology. All four of the studied 'functional traits' integrate signals from many different anatomical attributes that have a multitude of influences on actual physiological function (Niinemets, 1999; Chave *et al.*, 2009; Poorter *et al.*, 2009; 2011). Thus, it is common for trait variation in different environments to be driven by disparate anatomical changes that have drastically different physiological consequences but result in identical trait values (e.g. Baird *et al.*, 2017).

Within eucalypts, our results might indicate a constraint on the underlying anatomical properties that drive variation in LDMC and HV, the two traits that did show decreased variance in xeric species (Figure 5). However, a considerable amount of the total variation in both traits is non-climatic (Figure 4, Figure S7), making it difficult to detect changes in trait variation at the population level (Figure 6). This further highlights the importance of understanding the underlying anatomical drivers of variation of these traits (Niinemets, 1999; Onoda *et al.*, 2017). The trait-trait and trait-climate relationships documented here are unlikely to prove mechanistic in the manner necessary for the parameterization of dynamic ‘trait-based’ vegetation models without gaining a greater understanding of the root causes of this trait variation.

Conclusion

We found consistent and coordinated trait shifts towards drought resistance across the aridity range of eight tree species. These findings are unique in the literature, in part because we were able to explicitly sample complete aridity gradients that were not confounded by cold stress. However, the compound nature of the gross morphological traits we measured resulted in 1) within-species trait coordination that only emerged across the most climatically disparate individuals in a species and 2) fewer consistent patterns in the size of trait variances with aridity than between trait means and aridity. Our findings imply considerable capacity for these species to adapt and/or acclimate to increasing aridity with future climate change thanks to the substantial within-species variation in multiple traits that is significantly related to climate. Our work highlights outstanding questions about the anatomical mechanisms driving functional trait variation within species, as well as the need to disentangle conflicting effects of different environmental constraints (e.g. temperature versus nutrient versus water) on trait variation to develop a multi-scale understanding of plant functional ecology.

Acknowledgements:

We acknowledge the Traditional Custodians and Owners of Australia—the Aboriginal and Torres Strait Islander people—and recognize their continuing connection to land and waters on which this research was conducted. Specifically we recognize the Paredarerme, Palawa, Tyerrernotepanner, Wajuk, Wiilman, Kaniyang, Amangu, Bibbulman, Minang, Goren, and Nyaki-nyaki lands on which this work was done. We thank H Wauchope, HR Lai, and J Park for field assistance, T Britton for lab assistance, and G Badgley and A Trugman for comments on the analysis and manuscript. This work was supported by a National Geographic Society Young Explorer Grant (to LDLA). This material is also based upon work supported by the National Science Foundation Graduate Research Fellowship Program under Grant No.s DGE-1256082; DDIG-1500837, an NSF international travel allowance through the Graduate Research Opportunities Worldwide and an NSF Postdoctoral Research

Fellowship Grant No. DBI-1711243 and a National Oceanic and Atmospheric Administration Climate and Global Change Fellowship (to LDLA). Any opinions, findings and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

Works Cited:

Ahrens CW, Andrew ME, Mazanec RA, Ruthrof KX, Challis A, Hardy G, Byrne M, Tissue DT, Rymer PD. **2019a**. Plant functional traits differ in adaptability and are predicted to be differentially affected by climate change. *Ecology and Evolution* **10**: 232–248.

Ahrens CW, Mazanec RA, Paap T, Ruthrof KX, Challis A, Hardy G, Byrne M, Tissue DT, Rymer PD. **2019b**. Adaptive variation for growth and resistance to a novel pathogen along climatic gradients in a foundation tree. *Evolutionary Applications* **12**: 1178–1190.

Albert CH, Thuiller W, Yoccoz NG, Douzet R, Aubert S, Lavorel S. **2010a**. A multi-trait approach reveals the structure and the relative importance of intra- vs. interspecific variability in plant traits. *Functional Ecology* **24**: 1192–1201.

Albert CH, Thuiller W, Yoccoz NG, Soudant A, Boucher F, Saccone P, Lavorel S. **2010b**. Intraspecific functional variability: extent, structure and sources of variation. *Journal of Ecology* **98**: 604–613.

Alberto FJ, Aitken SN, Alía R, González-Martínez SC, Hänninen H, Kremer A, Lefèvre F, Lenormand T, Yeaman S, Whetten R, *et al.* **2013**. Potential for evolutionary responses to climate change - evidence from tree populations. *Global Change Biology* **19**: 1645-1661.

Anderegg LD, HilleRisLambers J. **2015**. Drought stress limits the geographic ranges of two tree species via different physiological mechanisms. *Global Change Biology* **22**: 1029–1045.

Anderegg LDL, Berner LT, Badgley G, Sethi ML, Law BE, Hillerislambers J. **2018**. Within-species patterns challenge our understanding of the leaf economics spectrum (J Peñuelas, Ed.). *Ecology letters* **21**: 734–744.

Baird AS, Anderegg LDL, Lacey ME, Hillerislambers J, Van Volkenburgh E. **2017**. Comparative leaf growth strategies in response to low-water and low-light availability: variation in leaf physiology underlies variation in leaf mass per area in Populus tremuloides. *Tree Physiology* **37**: 1140–1150.

Bates D, Mächler M, Bolker B, Walker S. **2015**. Fitting linear mixed-effects models using lme4.

Journal of Statistical Software **67**: 1–48.

Blackman CJ, Aspinwall MJ, Tissue DT, Rymer PD. **2017**. Genetic adaptation and phenotypic plasticity contribute to greater leaf hydraulic tolerance in response to drought in warmer climates. *Tree Physiology* **37**: 583–592.

Bourne AE, Creek D, Peters JMR, Ellsworth DS, Choat B. **2017**. Species climate range influences hydraulic and stomatal traits in *Eucalyptus* species. *Annals of botany* **120**: 123–133.

Chave J, Coomes D, Jansen S, Lewis SL, Swenson NG, Zanne AE. **2009**. Towards a worldwide wood economics spectrum. *Ecology letters* **12**: 351–366.

Chevin L-M, Collins S, Lefèvre F. **2012**. Phenotypic plasticity and evolutionary demographic responses to climate change: taking theory out to the field (A Hoffmann, Ed.). *Functional Ecology* **27**: 967–979.

Davison ME, Tay F. **1989**. Phenology of *Eucalyptus marginata* on sites infested with *Phytophthory cinnamomi*. *Aust. J. Bot.* **37**: 193–206.

Díaz S, Kattge J, Cornelissen JHC, Wright IJ, Lavorel S, Dray S, Reu B, Kleyer M, Wirth C, Prentice IC, *et al.* **2016**. The global spectrum of plant form and function. *Nature* **529**: 167–171.

Fajardo A, Piper FI. **2010**. Intraspecific trait variation and covariation in a widespread tree species (*Nothofagus pumilio*) in southern Chile. *New Phytologist* **189**: 259–271.

Field CB. **1988**. On the role of photosynthetic responses in constraining the habitat distribution of rainforest plants. *Functional Plant Biology* **15**: 343-358.

Franks SJ, Weber JJ, Aitken SN. **2014**. Evolutionary and plastic responses to climate change in terrestrial plant populations. *Evolutionary Applications* **7**: 123–139.

González-Zurdo P, Escudero A, Babiano J, García-Ciudad A, Mediavilla S. **2016**. Costs of leaf reinforcement in response to winter cold in evergreen species (U Niinemets, Ed.). *Tree Physiology* **36**: 273–286.

Grundy MJ, Rossel RAV, Searle RD, Wilson PL, Chen C, Gregory LJ. **2015**. Soil and Landscape Grid of Australia. *Soil Research* **53**: 835.

Heatwole H, Lowman MD, Donovan C, McCoy M. **1997**. Phenology of leaf-flushing and macroarthropod abundances in canopies of *Eucalyptus* saplings. *Selbyana* **18**: 200–214.

Hoffmann A, Merilä J. **1999**. Heritable variation and evolution under favourable and unfavourable conditions. *Trends in Ecology & Evolution* **14**: 96–101.

John GP, Scoffoni C, Buckley TN, Villar R, Poorter H, Sack L. **2017**. The anatomical and compositional basis of leaf mass per area (H Maherali, Ed.). *Ecology letters* **20**: 412–425.

Karger DN, Conrad O, Böhner J, Kawohl T, Kreft H, Soria-Auza RW, Zimmermann NE, Linder HP, Kessler M. **2017**. Data Descriptor: Climatologies at high resolution for the Earth's landsurface areas. *Nature Publishing Group* **4**: 170122.

Kovenock M, Swann ALS. **2018**. Leaf trait acclimation amplifies simulated climate warming in response to elevated carbon dioxide. *Global Biogeochemical Cycles* **32**: 1437–1448.

Kraft NJB, Adler PB, Godoy O, James EC, Fuller S, Levine JM. **2014**. Community assembly, coexistence and the environmental filtering metaphor (J Fox, Ed.). *Functional Ecology* **29**: 592–599.

Kuznetsova A, Brockhoff PB, Christensen R. **2017**. lmerTest Package: tests in linear mixed effects models*. Journal of Statisitcal Software* **82**: 1-12. R package version 2.0-30.

Laforest-Lapointe I, Martinez-Vilalta J, Retana J. **2014**. Intraspecific variability in functional traits matters: case study of Scots pine. *Oecologia* **175**: 1337-1348.

Legendre P. **2014**. lmodel2: Model II Regression. R package version 1.7-3. https://CRAN.R-project.org/package=lmodel2

Lemke IH, Kolb A, Diekmann MR. **2012**. Region and site conditions affect phenotypic trait variation in five forest herbs. *Acta Oecologica* **39**: 18–24.

Lens F, Sperry JS, Christman MA, Choat B, Rabaey D, Jansen S. **2010**. Testing hypotheses that link wood anatomy to cavitation resistance and hydraulic conductivity in the genus *Acer*. *New Phytologist* **190**: 709–723.

Li C, Wang K. **2003**. Differences in drought responses of three contrasting Eucalyptus microtheca F. Muell. populations. *Forest Ecology and Management* **179**: 377–385.

Li X, Blackman CJ, Choat B, Duursma RA, Rymer PD, Medlyn BE, Tissue DT. **2018a**. Tree hydraulic traits are co-ordinated and strongly linked to climate-of-origin across a rainfall gradient. *Plant, Cell & Environment* **41**: 646-660.

Li X, Blackman CJ, Choat B, Duursma RA, Rymer PD, Medlyn BE, Tissue DT. **2018b**. Tree hydraulic traits are coordinated and strongly linked to climate-of-origin across a rainfall gradient. *Plant, Cell & Environment* **41**: 646–660.

Li X, Blackman CJ, Peters JMR, Choat B, Rymer PD, Medlyn BE, Tissue DT. **2019**. More than iso/anisohydry: Hydroscapes integrate plant water use and drought tolerance traits in 10 eucalypt species from contrasting climates (R Oliveira, Ed.). *Functional Ecology* **33**: 1035–1049.

López R, Cano FJ, Choat B, Cochard H, Gil L. **2016**. Plasticity in vulnerability to cavitation of *Pinus canariensis* occurs only at the driest end of an aridity gradient. *Frontiers in Plant Science* **7**: 535.

Ma Z, Guo D, Xu X, Lu M, Bardgett RD, Eissenstat DM, McCormack ML, Hedin LO. **2018**. Evolutionary history resolves global organization of root functional traits. *Nature* **555**: 94–97.

MacArthur D. **1972**. *Geographical Ecology: Patterns in the Distribution of Species*. New York: Harper & Row.

Martinez-Vilalta J, Cochard H, Mencuccini M, Sterck F, Herrero A, Korhonen JFJ, Llorens P, Nikinmaa E, Nol A, Poyatos R, *et al.* **2009**. Hydraulic adjustment of Scots pine across Europe. *New Phytologist* **184**: 353–364.

McLean EH, Prober SM, Stock WD, Steane DA, Potts BM, Vaillancourt RE, Byrne M. **2014**. Plasticity of functional traits varies clinally along a rainfall gradient in *Eucalyptus tricarpa*. *Plant, Cell & Environment* **37**: 1440–1451.

Mencuccini M, Grace J. **1995**. Climate influences the leaf area/sapwood area ratio in Scots pine. *Tree Physiology* **15**: 1–10.

Mencuccini M, Rosas T, Rowland L, Choat B, Cornelissen H, Jansen S, Kramer K, Lapenis A, Manzoni S, Niinemets U, *et al.* 2019. Leaf economics and plant hydraulics drive leaf : wood area ratios. *New Phytologist* **224**: 1544–1556.

Messier J, Lechowicz MJ, McGill BJ, Violle C, Enquist BJ. **2017**. Interspecific integration of trait dimensions at local scales: the plant phenotype as an integrated network (H Cornelissen, Ed.). *Journal of Ecology* **152**: 1775–1790.

Messier J, Violle C, Enquist BJ, Lechowicz MJ, McGill BJ. **2018**. Similarities and differences in intrapopulation trait correlations of co-occurring tree species: consistent water-use relationships amid widely different correlation patterns. *American Journal of Botany* **105**: 1477–1490.

Molina-Montenegro MA, Naya DE. **2012**. Latitudinal patterns in phenotypic plasticity and fitness-related traits: assessing the climatic variability hypothesis (CVH) with an invasive plant species. *PloS one* **7**: e47620.

Mooney HA, Ferrar PJ, Slatyer RO. **1978**. Photosynthetic capacity and carbon allocation patterns in diverse growth forms of *Eucalyptus*. *Oecologia* **36**: 103–111.

Nabuurs GJ, van Putten B, Knippers TS, Mohren GMJ. **2008**. Comparison of uncertainties in carbon sequestration estimates for a tropical and a temperate forest. *Forest Ecology and Management* **256**: 237–245.

Niinemets U. **1999**. Research review. Components of leaf dry mass per area–thickness and density–alter leaf photosynthetic capacity in reverse directions in woody plants. *New Phytologist* 144: 35-47.

Niinemets U. **2016**. Does the touch of cold make evergreen leaves tougher? (D Way, Ed.). *Tree Physiology* **36**: 267–272.

O'Brien L. **2019.** slga: Data Access Tools for the Soil and Landscape Grid of Australia. R package version 1.1.0. https://CRAN.R-project.org/package=slga

Onoda Y, Wright IJ, Evans JR, Hikosaka K, Kitajima K, Niinemets U, Poorter H, Tosens T, Westoby M. **2017**. Physiological and structural tradeoffs underlying the leaf economics spectrum. *New Phytologist* **113**: 1–17.

Pérez-Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte MS, Cornwell WK, Craine JM, Gurvich DE, *et al.* **2013**. New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany* **61**: 167.

Pfautsch S, Harbusch M, Wesolowski A, Smith R, Macfarlane C, Tjoelker MG, Reich PB, Adams MA. **2016**. Climate determines vascular traits in the ecologically diverse genus *Eucalyptus* (F Lloret, Ed.). *Ecology letters* **19**: 240–248.

Pfautsch S, Macfarlane C, Ebdon N, Meder R. **2012**. Assessing sapwood depth and wood properties in *Eucalyptus* and *Corymbia* spp. using visual methods and near infrared spectroscopy (NIR). *Trees* **26**: 963–974.

Pinheiro J, Bates D, DebRoy S, Sarkar D. **2019**. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-140. https://CRAN.R-project.org/package=nlme

Poorter H, Niinemets U, Poorter L, Wright IJ, Villar R. **2009**. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist* **182**: 565–588.

Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L. **2011**. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytologist* **193**: 30–50.

Pritzkow C, Williamson V, Szota C, Trouvé R, Arndt SK. **2019**. Phenotypic plasticity and genetic adaptation of functional traits infuences infra-specific variation in hydraulic efficiency and safety (J Martinez-Vilalta, Ed.). *Tree Physiology* **40**: 215–229.

Reich PB. **2014**. The world-wide ‘fast–slow’ plant economics spectrum: a traits manifesto. *Journal of Ecology* **102**: 275-301.

Reich PB, Walters MB, ellsworth DS. **1997**. From tropics to tundra: global convergence in plant functioning. *Proceedings of the National Academy of Sciences* **94**: 13730–13734.

Richardson SJ, Allen RB, Buxton RP, Easdale TA, Hurst JM, Morse CW, Smissen RD, Peltzer DA. **2013**. Intraspecific Relationships among Wood Density, Leaf Structural Traits and Environment in Four Co-Occurring Species of *Nothofagus* in New Zealand (NG Swenson, Ed.). *PloS one* **8**: e58878.

Richter S, Kipfer T, Wohlgemuth T, Calderón Guerrero C, Ghazoul J, Moser B. **2011**. Phenotypic plasticity facilitates resistance to climate change in a highly variable environment. *Oecologia* **169**: 269–279.

Rosas T, Mencuccini M, Barba J, Cochard H, Saura-Mas S, Martinez-Vilalta J. **2019**. Adjustments and coordination of hydraulic, leaf and stem traits along a water availability gradient. *New Phytologist* **223**: 632-646.

Schneider CA, Rasband WS, Eliceiri KW. **2012**. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **9**: 671–675.

Schulze E-D, Turner NC, Nicolle D, Schumacher J. **2006**. Leaf and wood carbon isotope ratios, specific leaf areas and wood growth of Eucalyptus species across a rainfall gradient in Australia. *Tree Physiology* **26**: 479–492.

Schulze ED, Williams RJ, Farquhar GD, Schulze W, Langridge J, Miller JM, Walker BH. **1998**. Carbon and nitrogen isotope discrimination and nitrogen nutrition of trees along a rainfall gradient in northern Australia. *Australian Journal of Plant Physiology* **25**: 413.

Siefert A, Violle C, Chalmandrier L, Albert CH, Taudiere A, Fajardo A, Aarssen LW, Baraloto

C, Carlucci MB, Cianciaruso MV, *et al.* 2015. A global meta-analysis of the relative extent of intraspecific trait variation in plant communities (J Chase, Ed.). *Ecology letters* **18**: 1406–1419.

R Core Team. **2019**. R: A language and environment for statistical computing. version 3.6.0. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Trugman AT, Anderegg LDL, Sperry JS, Wang Y, Venturas M, Anderegg WRL. **2019**. Leveraging plant hydraulics to yield predictive and dynamic plant leaf allocation in vegetation models with climate change. *Global Change Biology* **25**: 4008-4021.

Tyree MT, Ewers FW. **1991**. The hydraulic architecture of trees and other woody plants. *New Phytologist* **119**: 345–360.

Valladares F, Gianoli E, Gómez JM. **2007**. Ecological limits to plant phenotypic plasticity. *New Phytologist* **176**: 749–763.

Valladares F, Matesanz S, Guilhaumon F, Araújo MB, Balaguer L, Benito-Garzón M, Cornwell W, Gianoli E, van Kleunen M, Naya DE, *et al.* 2014. The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change (W Thuiller, Ed.). *Ecology letters* **17**: 1351–1364.

Vilà-Cabrera A, Martinez-Vilalta J, Retana J. **2015**. Functional trait variation along environmental gradients in temperate and Mediterranean trees. *Global ecology and biogeography* **24**: 1377–1389.

Whitehead D, Jarvis PG. **1981**. Coniferous forests and plantations. Kozlowski TT ed. Woody Plant Communities. New York, NY USA: Academic Press.

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, *et al.* 2004. The worldwide leaf economics spectrum. *Nature* **428**: 821–827.

Zobel BJ, Jett JB. **1995**. *Genetics of Wood Production*. Berlin: Springer-Verlag.

Zolfaghar S, Villalobos-Vega R, Cleverly J, Eamus D. **2015**. Co-ordination among leaf water relations and xylem vulnerability to embolism of Eucalyptus trees growing along a depth-to-groundwater gradient. *Tree Physiology* **35**: 732-743.

Zolfaghar S, Villalobos-Vega R, Cleverly J, Zeppel M, Rumman R, Eamus D. **2014**. The influence of depth-to-groundwater on structure and productivity of Eucalyptus woodlands. *Australian Journal of Botany* **62**: 428.

Supporting information:

Table S1: Summary of site characteristics
Table S2: Huber Value branch characteristics
Table S3: Summaries of Trait-by-environment relationships
Table S4: Summary of trait-trait correlations at different nested scales
Figure S1: Diagram of sampling strategy
Figure S2: Correlation between between environmental variables across sites
Figure S3: Coverage of study species' climate distributions
Figure S4: Inter-specific trait-climate relationships
Figure S5: Funnel Plots of trait covariation
Figure S6: Trait PC2 versus site MD
Figure S7: Variance decomposition across 7 eucalypts
Figure S8: Trait CVs versus climate niche

Figure Legends

Figure 1: Relationships between four leaf and stem traits and plot mean annual precipitation (PPT) for eight tree species. Points show tree averages. Red crosses show *Acacia acuminata,* the one non-eucalypt species. Trend lines show simple linear regressions of tree averages against MD for each species. Species abreviations: *A. acu – Acacia acuminata, E. sal – Eucalyptus salmonophloia, E. mar – E. marginata, C. cal – Corymbia calophylla, E. ova – E. ovata, E. vim – E. viminalis, E. amy – E. amygdalina, E. obl – E. obliqua*

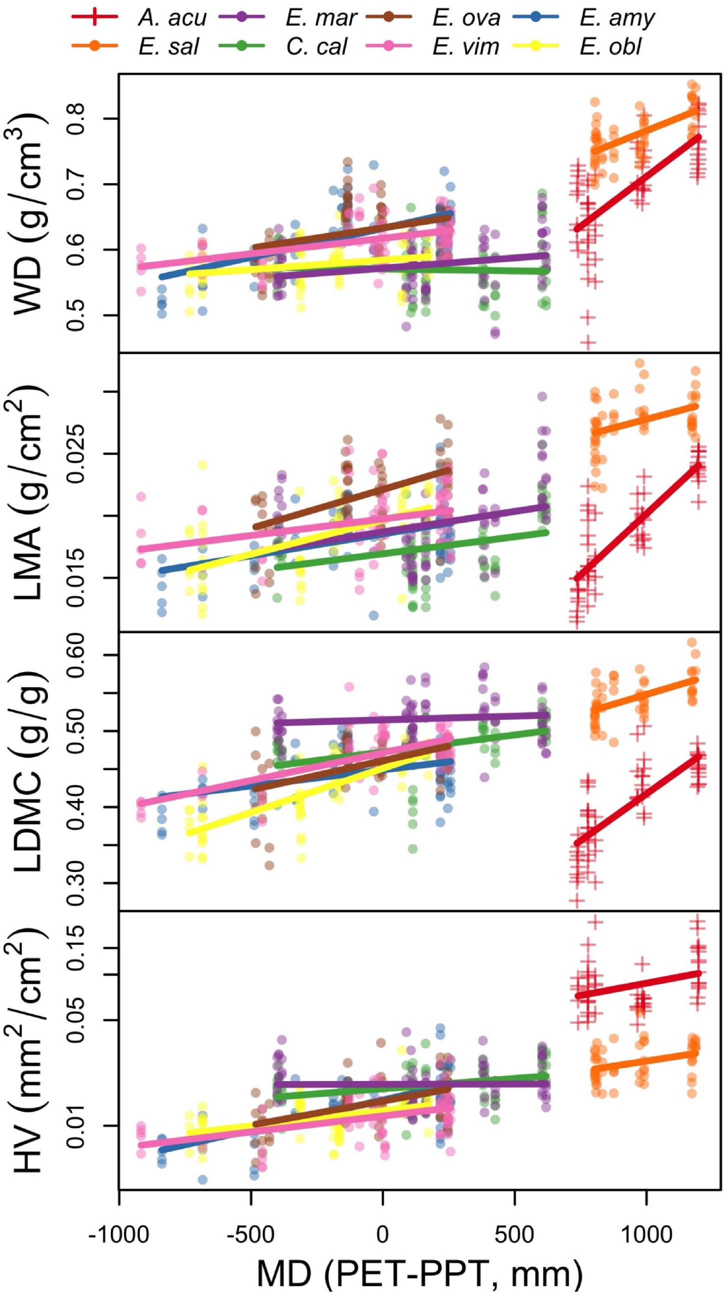
Figure 2: Correlations between leaf and stem traits across the aridity range of eight tree species (a-f). Points show tree average trait values, and lines show Major Axis Regressions (solid lines show significant correlations). Crosses show *A. acuminata*, the one non-eucalypt species. Trend lines show SMA regressions per species (n.s. correlations are dashed lines). Numbers in the upper left corners report the fraction of species showing significant trait-trait correlations with the range of Pearson correlation coefficients in parentheses. Panel (g) shows the distribution of correlation coefficients across all species for two example trait pairs, LMA vs WD (black) and LMA vs LDMC (blue). Trait correlations typically had a mean near zero across branches or across individuals within a site for all trait pairs except LMA vs LDMC. Species abreviations: *A. acu – Acacia acuminata, E. sal – Eucalyptus salmonophloia, E. mar – E. marginata, C. cal – Corymbia calophylla, E. ova – E. ovata, E. vim – E. viminalis, E. amy – E. amygdalina, E. obl – E. obliqua*

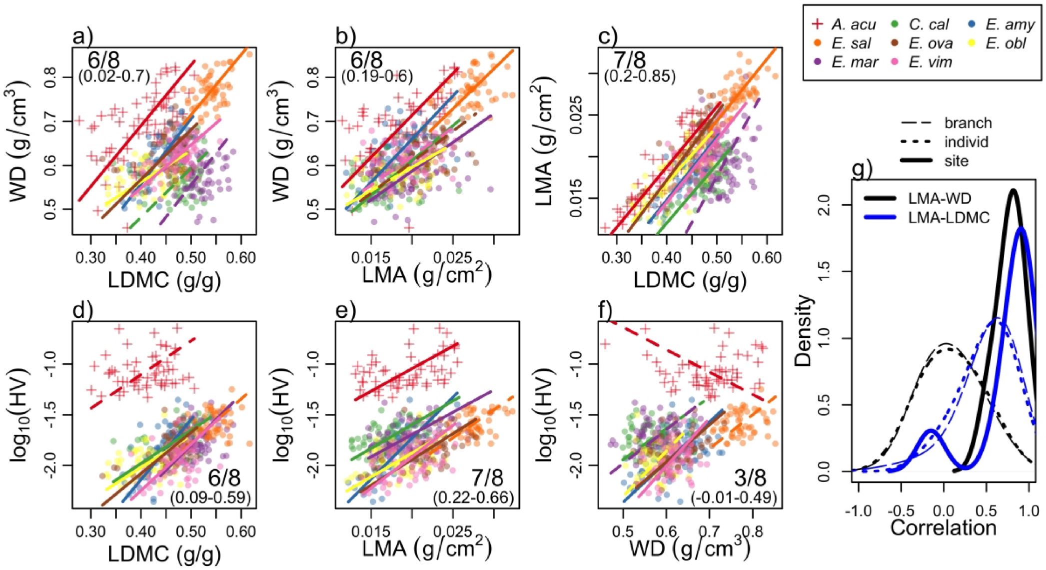
Figure 3: PC loadings of a PCA including all branch measurements (a). Site average trait PC1 scores are strongly related to site moisture availability across eight tree species (b). PET = potential evapotranspiration, PPT = precipitation. Trend lines show SMA regressions per species. Species abreviations: *A. acu – Acacia acuminata, E. sal – Eucalyptus salmonophloia, E. mar – E. marginata, C. cal – Corymbia calophylla, E. ova – E. ovata, E. vim – E. viminalis, E. amy – E. amygdalina, E. obl – E. obliqua*

Figure 4: Variance decomposition of WD (a), LMA (b), LDMC (c), and $\log_{10}$-transformed HV (d) measured across the aridity range of eight tree species. Colored bars show proportion of total trait variance ("% trait Var") while gray bar shows absolute amount of variance per species ("Tot trait Var"). The amount and dominant scale of trait variance differs considerably between species for the same trait and between traits. However, variation between plots at a site was almost universally the smallest variance component for all traits and species. Within-tree variation was also always larger for $\log_{10}$-transformed HV than for all other traits. Species are ordered from driest on the left to wettest on the right. Species abreviations: *A. acu – Acacia acuminata, E. sal – Eucalyptus salmonophloia, E. mar – E. marginata, C. cal – Corymbia calophylla, E. ova – E. ovata, E. vim – E. viminalis, E. amy – E. amygdalina, E. obl – E. obliqua*

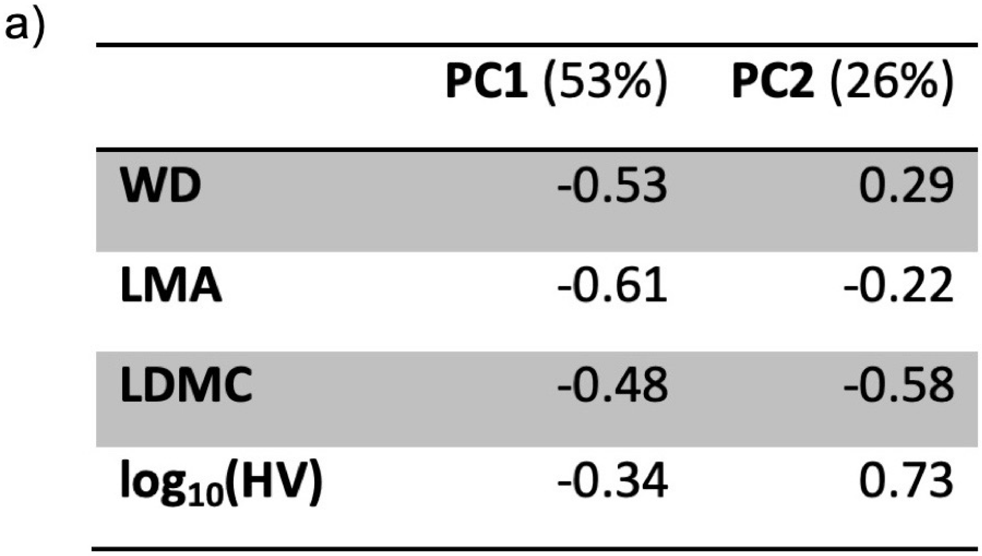
Figure 5: The total amount of within-species trait variation (top row) and individual variance components (bottom row, variance components multiplied by 100 for axis labels) of WD (a,e), LMA (b,f), LDMC (c,g) and $\log_{10}$(HV) (d,h) of seven eucalypt species (excluding *A. acuminata*) were rarely related to species aridity niche (here shown as the median moisture deficit of each species' geographic distribution based on occurrence records in the Atlas of Living Australia). Total trait variation in HV decreased marginally significantly in drier species, and climate-related (between site) trait variation in LDMC and HV decreased significantly in drier species, consistent with environmental filtering limiting constraining trait variation. Solid lines indicate significant trends (alpha<0.05), dashed lines indicate near significant trends (0.15<alpha<0.05).

Figure 6: Summary of within-species variance patterns. Few of the seven eucalypt and one *Acacia* species showed evidence of decreasing trait variation (red) at dryer sites, with even LDMC (the trait in which this pattern is most prevalent) showing decreases in only 37.5% of species and increases in 12.5% of species. "Non-aridity" signifies species that showed significant site-to-site differences in trait variance that could not be explained by site aridity.


A. acu
E. sal
E. mar
C. cal
E. ova
E. vim
E. amy
E. obl
WD (g/cm^3)
0.5
0.6
0.7
0.8
LMA (g/cm^2)
0.015
0.025
LDMC (g/g)
0.30
0.40
0.50
0.60
HV (mm^2/cm^2)
0.01
0.05
0.15
-1000
-500
0
500
1000
MD (PET-PPT, mm)

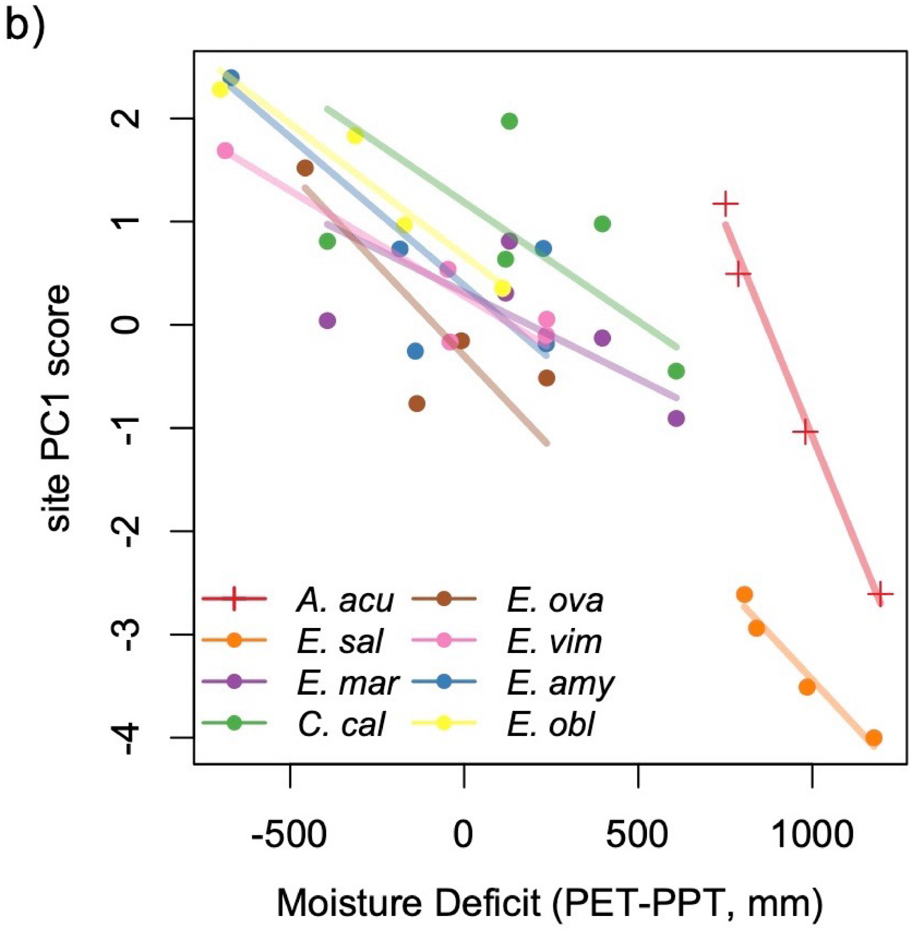


nph_16795_f2.jpg

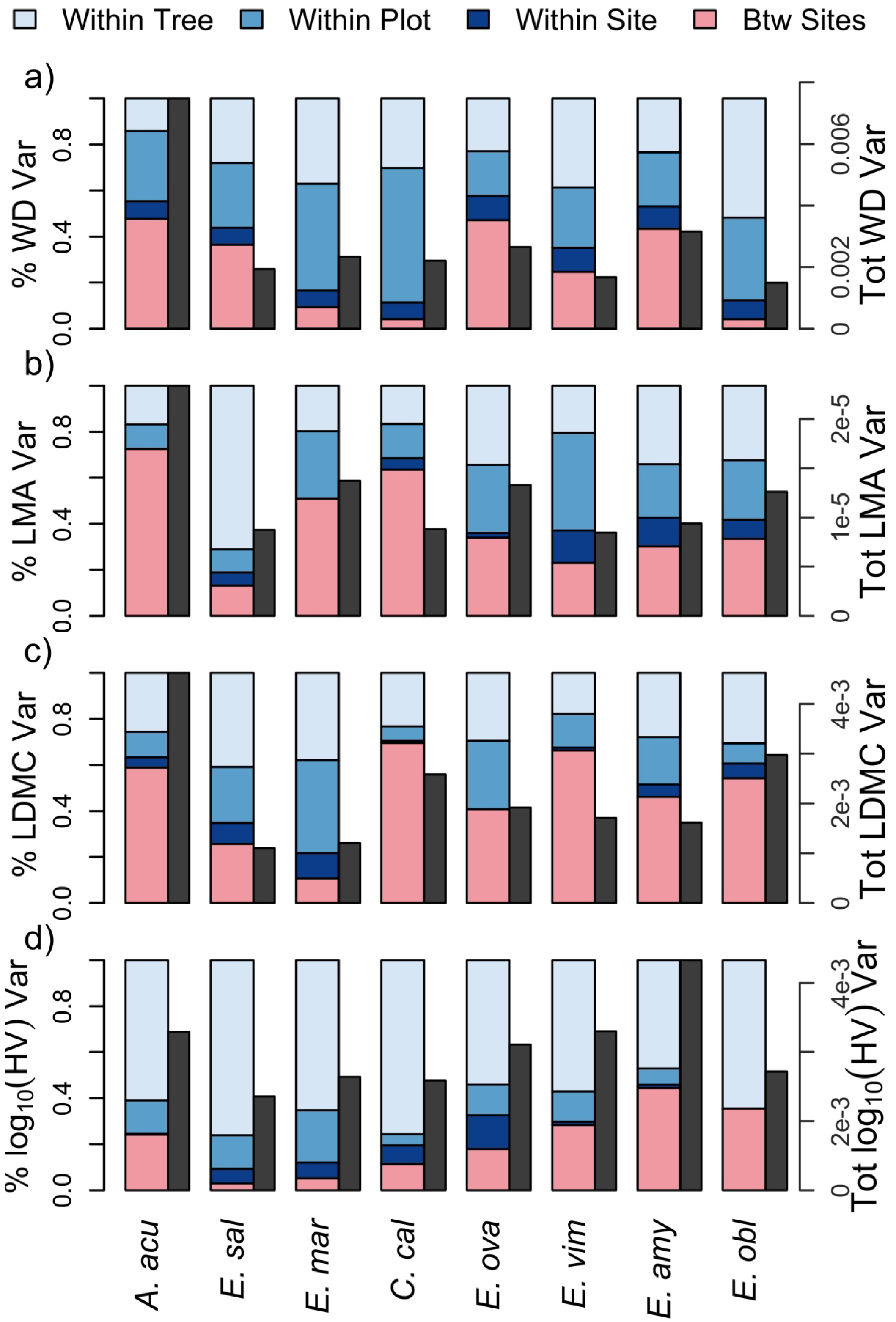


a)

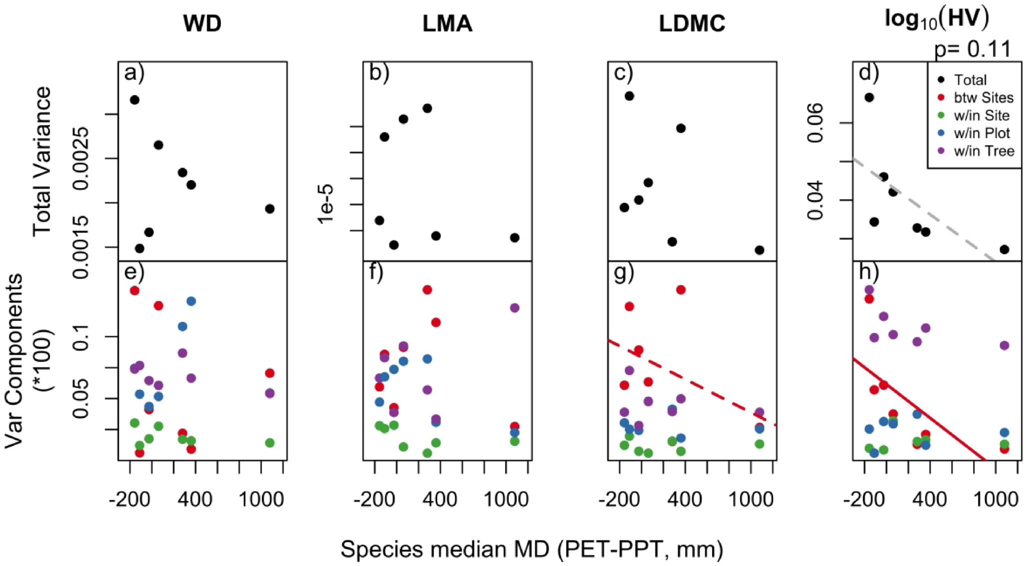
	PC1 (53%)	**PC2** (26%)
WD	-0.53	0.29
LMA	-0.61	-0.22
LDMC	-0.48	-0.58
log$_{10}$(HV)	-0.34	0.73



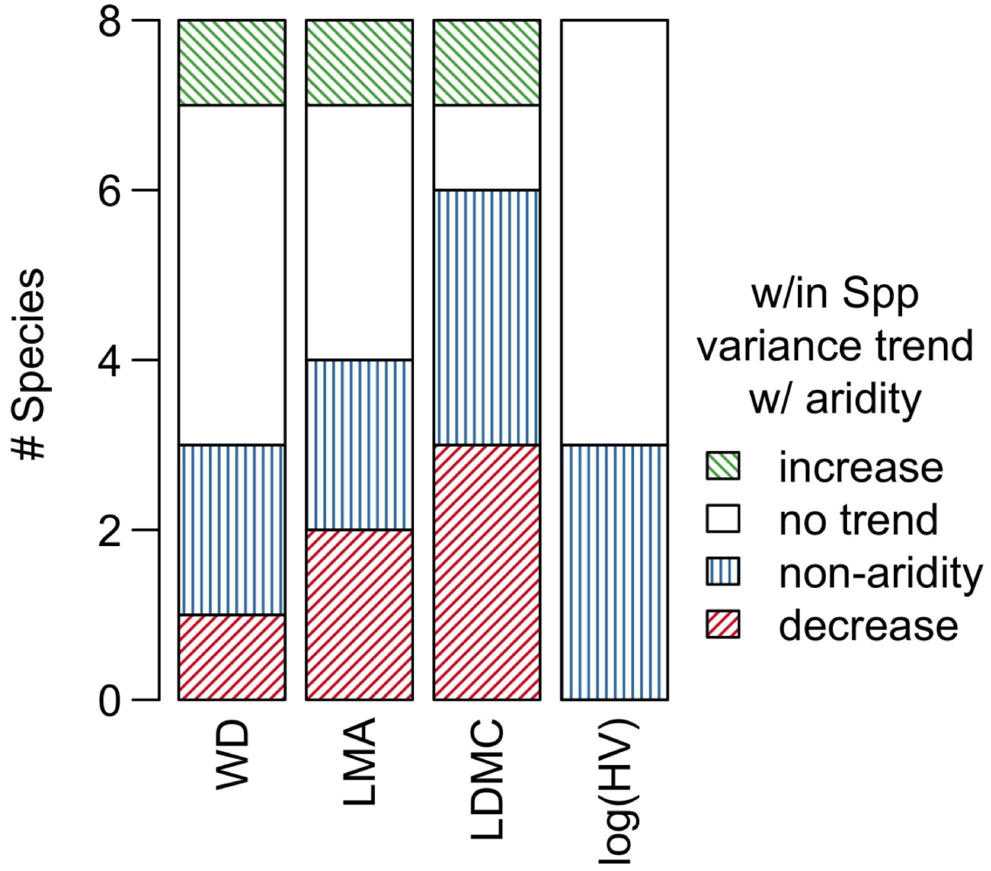
nph_16795_f3.jpeg



nph_16795_f4.jpg



nph_16795_f5.jpg



nph_16795_f6.jpg