**Title:**

Hydroscapes, hydroscape plasticity, and relationships to plant functional traits and mesophyll photosynthetic sensitivity in *Eucalyptus* species

**Running Title:** Hydroscapes, plasticity, and functional traits

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**Abstract:**

The isohydric-anisohydric continuum describes the relative stringency of stomatal control of leaf water potential (*leaf*) during drought. Hydroscape area (*HA*) – the water potential landscape over which stomata regulate *leaf* – has emerged as a useful metric of the iso/anisohydric continuum because it is strongly linked to several hydraulic, photosynthetic, and structural traits. Previous research on *HA* focused on broad ecological patterns involving several plant clades. Here we investigate relationships of *HA* to climatic conditions and functional traits across ecologically diverse but closely related species while accounting for phylogeny. Across a macroclimatic moisture gradient, defined by the ratio of mean annual precipitation to mean annual pan evaporation (*P/Ep*), *HA* decreased with *P/Ep* for ten *Eucalyptus* species. Greater anisohydry reflects lower turgor loss points and greater hydraulic safety, mirroring global patterns. More isohydric species have mesophyll photosynthetic capacity that is more sensitive to *leaf*, consistent with an earlier model for optimal stomatal behavior. Hydroscapes exhibit little plasticity in response to variation in water supply, and the extent of plasticity does not vary with *P/Ep* of native habitats. These findings strengthen the case that *HA* is a useful metric for characterizing drought tolerance and water-status regulation.

**Keywords:** anisohydry, drought, hydraulic conductance, isohydry, non-stomatal limitation, photosynthesis, plasticity, stomatal regulation, water relations.

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**Author contributions:** The project was conceived and designed by KAM, TJG, and AMS. AMS, DDS, MAA, and SGG carried out measurements. AMS analyzed data with assistance from KAM, TJG, and DDS. AMS, KAM, and TJG wrote the manuscript with help from DDS and MAA.

**Introduction**

Characterizing drought tolerance of land plants has become increasingly important over recent decades, in light of continued increases in drought and heat under global climate change (Allen *et al.* 2010; Perkins-Kirkpatrick & Lewis 2020). Understanding effects of drought on plants is needed to predict how both individual species and ecosystems will respond to changing conditions. Several traits have been used to categorize species as more or less drought tolerant. For example, physiological traits such as the water potentials that induce loss of leaf turgor and substantial declines in stomatal, branch, and whole plant hydraulic conductance, and anatomical traits like SLA, leaf vein architecture, and stomatal size and density, are all commonly used traits in evaluating plant drought tolerance (e.g., Bartlett , Klein, Jansen, Choat & Sack 2016; de Boer *et al.* 2016). The stringency of stomatal behavior in regulating leaf water potential (*leaf*) has emerged as a valuable measure of species-specific responses to drought (Tardieu & Simonneau 1998) as it integrates other physiological characteristics and is strongly linked to traits - photosynthetic, hydraulic, structural - that affect whole-plant responses to drought (Fu *et al.* 2019; McCulloh, Domec, Johnson, Smith & Meinzer 2019).

Stomatal regulation of *leaf* can be described as falling along an isohydric-anisohydric continuum. Species with greater isohydry exhibit tight control of stomatal conductance and leaf water potential as soil water potential (*soil*) declines. In contrast, more anisohydric species tend to maintain stomatal conductance in response to drought, resulting in greater reductions in *leaf* with declining *soil* (Meinzer *et al.* 2016). Many metrics have now been used to quantify the strictness of stomatal regulation of *leaf* (Martínez-Vilalta, Poyatos, Aguadé, Retana & Mencuccini 2014; Skelton, West & Dawson 2015; Meinzer *et al.* 2016; Hochberg, Rockwell, Holbrook & Cochard 2018). Hydroscape area in particular has been strongly linked to various plant hydraulic traits and has proven useful in bridging hydraulic behavior and species drought tolerance (Fu & Meinzer 2018; Fu *et al.* 2019; Li *et al.* 2019; McCulloh *et al.* 2019). Hydroscape area (*HA*) is illustrated in Fig. 1 and defined as the area of the triangle formed when mid-day ψ*leaf*(ψ*MD*) as a function of pre-dawn leaf water potential (ψ*PD*) intersects with ψ*PD*= 0 (soil saturation) and the maximum possible ψ*MD* as a function of ψ*PD* (i.e. ψ*MD* = ψ*PD* due to stomatal closure and cessation of midday transpiration). We define the point of intersection between actual and maximum possible *ψMD* (i.e. the point of actual total stomatal closure) as ψ*\*.* *HA* thus describes a water potential land­scape over which stomata regulate leaf water potential prior to complete, drought-induced stomatal closure (Meinzer *et al.* 2016). More anisohydric species have more negative **\*’s and larger *HA* (Fig. 1). The range from zero to **\* is established during days when stomata remain open. Similarly, the range **PD to **MD is established over days when stomata fully close. Theoretically, species with contrasting degrees of isohydry could have the same *HA*. However, maintaining the same area across the water potential landscape would require shifts in water potential parameters that may be biologically implausible. For example, for a more anisohydric species (i.e., one with more negative **\*, achieved across days) to have the same *HA* as a more isohydric species, it would have to operate at higher *MD* values under well-watered conditions. This might be achieved via reduced stomatal conductance or evaporative surface (relative to root absorptive surface). However, both would run counter to anisohydric species being more plastic with respect to water potential. Given that hydroscape area, **\*, and slope of **MD vs **PD are correlated (Fig. S1; Meinzer *et al.* 2013), we interchangeably use the terms hydroscape area, hydroscape, and the degree of anisohydry.

Previous studies on hydroscapes have analyzed species selected to span the iso- to anisohydric continuum (Meinzer *et al.* 2016; Fu *et al.* 2019), global biomes (Fu & Meinzer 2018), and woody vegetation communities along an aridity gradient (Li *et al.* 2019). In general, species from drier areas function across larger hydroscape areas. These more anisohydric species tend to have xylem vasculature with reduced hydraulic conductance that are less vulnerable to drought (Fu & Meinzer 2018; Fu *et al.* 2019). We do not yet know how closely related species from different habitats are distributed along the iso- to anisohydric continuum, and how their hydroscape areas are related to other key functional traits when analyzed in a phylogenetic context. Do hydroscapes track phylogeny or ecology more closely? Universal application of functional trait relationships in predicting plant behavior or stress tolerance can be problematic because relationships between traits and resource use strategies are often context- or scale-dependent. For example, trait correlations across the leaf economic spectrum often show opposite signs within species or genera versus those across genera and families (Anderegg *et al.* 2018). Vein architecture can vary with env­ironment in different ways in different lineages due to phylogenetic constraints (de Boer *et al.* 2016). Hydroscape area may also exhibit novel relationships to functional traits and climatic conditions when investigated across closely related species that inhabit a wide range of environments.

We also do not yet know the extent to which hydroscape areas vary within species. Trait variation within a species – whether encoded as phenotypic plasticity or non-plastic genetic polymorphisms – can have profound effects on the resiliency of a species and populations to rapid climatic changes (Lande 2009), and can explain why some species can tolerate certain environmental conditions but others cannot (Bartlett *et al.* 2014; Scoffoni *et al.* 2015). It is thus useful to characterize hydroscape plasticity across a set of closely related species that are differently distributed along a natural gradient in macroclimatic water availability and exhibit differences in a range of functional traits.

Most research on plant responses to drought has focused on hydraulics and/or the decline in photosynthesis caused by stomatal closure. However, non-stomatal limitations to photosynthesis reflect another cost of drought stress (Dewar *et al.* 2017; Salmon *et al.* 2020; Salvi, Smith, Adams, McCulloh & Givnish 2021). Non-stomatal limitations to photosynthesis are those that reduce the maximum photosynthetic rate at a given light level and internal CO2 concentration (Tezara, Mitchell, Driscoll & Lawlor 1999; Flexas, Bota, Loreto, Cornic & Sharkey 2004; Zhou, Medlyn, Sabaté, Sperlich & Prentice 2014). Examples include declines in mesophyll conductance of CO2 and reduced rates of ribulose-1,5-biphosphate regeneration, and these limitations tend to increase as *leaf* declines during drought (Zhou *et al.* 2014; Dewar *et al.* 2017; Salmon *et al.* 2020). Species with greater sensitivity to leaf water potential of non-stomatal controls on photosynthesis (what Salvi *et al.* 2021 term mesophyll photosynthetic sensitivity, or MPS) should benefit from traits that limit declines in *leaf*.

Here we investigate relationships of *HA* to climatic conditions and functional traits across ecologically diverse but closely related species, by measuring hydroscape area, leaf functional traits, whole-plant hydraulic conductance and vulnerability, and MPS across the 10 upland *Eucalyptus* species studied by Salvi *et al.* (2021). Species were stratified by dominance of different portions of a macroclimatic gradient in relative moisture supply (*P/Ep*, the ratio of precipitation to pan evaporation over a species’ range) and by phylogenetic position in subgenus *Eucalyptus* vs. subgenus *Symphyomyrtus*. We stratified by subgenus partly to eliminate effects due to close relationships, and partly to detect such effects if they exist. For example, while both subgenera are distributed widely along climatic gradients, members of subgenus *Symphyomyrtus* are frequently restricted to arid mallee habitats (Nicolle 2006). We might therefore expect members of *Symphyomyrtus* to show larger *HA* and other drought-adapted traits at a given species' mean *P/Ep* if phylogeny is important, and for members of both subgenera to show the same *HA* and other drought-adapted traits at a given species' mean *P/Ep* if subgeneric membership is unimportant. We assess relationships among hydroscapes, functional traits, MPS, hydraulic vulnerability, and *P/Ep* in a phylogenetic context to test hypothesized trait-trait and trait environment correlations.

We hypothesized four main trends in hydroscape area. First, plants should be more isohydric – that is, *HA* should be smaller – when whole-plant hydraulic conductance, maintenance of leaf turgor, and mesophyll photo­synthetic capacity are more sensitive to drops in leaf water potential. We expect this association between MPS and HA because plants with greater MPS should have lower stomatal conductance and therefore be more isohydric (i.e., have smaller *HA*; Givnish 1986; Salvi *et al.* 2021). Second, *HA* should be correlated with anatomical traits associated with aridity and/or that affect leaf hydraulic conductance, such as SLA, maximum theoretical stomatal conductance, vein density, and the ratio of vein investment to leaf thickness. Specifically, we predict that SLA and the ratio of vein investment to leaf thickness should decrease with increasing *HA*, while maximum theoretical stomatal conductance and vein density should increase with *HA*.

Third, hydroscape area should increase with species range aridity, given that highly isohydric plants are likely to die under prolonged or frequent droughts (McDowell *et al.* 2008; Meinzer *et al.* 2017), while these same plants may have an advantage in moister habitats because they can maintain greater leaf water potentials and, therefore, high mesophyll photosynthetic capacity (Givnish 1986; Salvi *et al.* 2021). Fourth, we expect that *HA* plasticity might be greater in species adapted to more arid environments, given that the shift to a smaller *HA* when growing in a moister environment may be simpler physiologically than expanding *HA* in a more arid one. We also expect that more anisohydric species should occupy environments with greater variability in precipitation and moisture availability (seen in McDowell *et al.* 2008), meaning *HA* plasticity might increase with the standard deviation and/or coefficient of variation in *P/Ep* within the species’ range, to the extent that such plasticity can allow species to expand their distribution along a moisture gradient. These trends, however, might fail to materialize if the competitive advantages of isohydry under favorable conditions outweigh the survival advantages of anisohydry under drier conditions, or if fixed genetic differences rather than plasticity govern variation in anisohydrocity across a species range.

**Materials and methods**

***Study System and Growing Conditions***

Ten *Eucalyptus* species were selected for this study: *Eucalyptus arenacea, E. dives, E. dumosa, E. macrorhyncha, E. microcarpa, E. nitens, E. obliqua, E. regnans, E. sideroxylon,* and *E. viminalis*. These species often dominate upland, non-riparian sites in different vegetation types ranging from cool temperate rainforest to wet sclerophyll forest, open forest, woodland, and arid mallee in Victoria, Australia (Table S1; Salvi *et al.* 2021). Species are stratified by their dominance of different portions of a macroclimatic gradient in moisture supply vs. demand (as measured by *P/Ep,* the ratio of annual precipitation to pan evaporation) as well as by subgenus (*Eucalyptus* vs. *Symphyomyrtus;* Thornhill *et al.* 2019). Species means for *P* range from 350 to 1043 mm, while those for *P/Ep* vary from 0.19 to 0.98 (Salvi *et al.* 2021; Table S1). Seeds were obtained from Forest Seeds Australia (Bacchus Marsh, VIC), Goulburn Broken Indigenous Seedbank (Dookie, VIC), and CSIRO Australian Tree Seed Centre (Black Mountain, ACT). Precise provenances are not known for most species. At a glasshouse in UW-Madison in early 2018, plants were germinated simultaneously in flat beds of field soil and transplanted to 4” pots filled with Pro-mix® BX potting medium (Premier Tech Horticulture, Québec, Canada). Once a substantial rooting system had developed and seedlings were about 5 cm tall, seedlings were moved to 14” plastic pots filled with equal parts sand:field soil:Promix® BX, and fertilized one to two times per week. Plants were hand-watered to maintain a non-limiting water supply during this initial growth. Espoma Organic Soil Acidifier was added to maintain neutral pH as irrigation water was somewhat calcified. Experiments were conducted from May to October 2018. Air temperature was regulated ranging from 25-30℃ during the day to 14-20℃ at night. Artificial overhead lighting was added if environmental PAR fell below 600 μmol m-2 s-1. Plants were randomly rotated biweekly to minimize effects of differences in micro-climate conditions across the glasshouse.

***Species Placement along the Isohydric to Anisohydric Continuum***

We placed study species on the iso/anisohydry continuum by quantifying their hydroscape areas (Meinzer *et al.* 2016). Four individuals per species were measured for predawn and midday leaf water potential (*PD*and *MD*, respectively) during a slow dry-down over the course of several weeks during summer 2018 until *MD* = *PD*, or as close as possible to this point. *PD* was measured 1-2 hours before sunrise and *MD* was measured at 1200 – 1300 hrs when *leaf* was at a daily minimum on the youngest fully developed leaves or apical shoots using a Scholander-type pressure chamber (PMS Instruments, Albany, OR, USA). The degree of anisohydry was defined according to the area between the 1:1 line (*PD*= *MD*) and the slope of the regression of *MD*vs. *PD* when stomata (rather than e.g., irradiance) have most control over *MD*; perfect isohydry would result from constant *MD*. First, we found the data point closest to where *MD*= *PD*, beyond where drought has induced complete stomatal closure (Fu & Meinzer 2018), and removed any more negative data points. Then, we created a linear regression using that point and the two points with the next greatest *MD*. We added single water potential measurements of greater *MD* to the hydroscape linear regression until the r2 of the linear regression reached a maximum (Meinzer *et al.* 2016; Fu & Meinzer 2018). This linear regression of *MD*vs. *PD* was then used to calculate the hydroscape area (*HA*, MPa2) for each species as:

*HA* = *b2* */ 2(1 – m)* (1)

where *b* is the y-intercept of the *MD* vs. *PD* regression, which represents *MD* when *PD* = 0, and *m* is the slope of the regression. Smaller hydroscape areas are associated with more stringent control of *leaf*, and thus, with more isohydric behavior and greater isohydricity.

***Turgor Loss Point***

Turgor loss point (TLP; MPa), the *leaf* at turgor loss, was calculated for 5 leaves or small leafy shoots of each species from pressure-volume curves generated using the bench drying method (Tyree & Hammel 1972; Tyree, Dainty & Benis 1973). Briefly, leaves or apical shoots were cut from the branch early in the morning when transpiration was low, and placed in distilled water and out of light to hydrate for 20-30 minutes. We repeatedly measured *leaf* and leaf mass until a plot of -1/*leaf* vs. leaf water mass lost formed a linear region, where it is assumed that turgor pressure equals zero. The equation of this linear region can be used to calculate pressure (*P*)and osmotic potentials (****) at any given *leaf*. TLP is the highest *leaf* for which *P* = 0.

***Whole-plant Hydraulic Conductance***

We quantified whole-plant hydraulic conductance (*Kplant*) on the same plants on which we measured hydroscapes. Pots were bagged to ensure any loss of water was due to transpiration from the plant, not from the soil. When leaves were collected to measure predawnand midday *leaf*, potted plants were weighed with a 0.1 g resolution balance. Changes in plant mass and *leaf* were caused by transpiration. Whole-plant hydraulic conductance (g H2O MPa-1 m-2 min-1) was calculated as

(2)

where *Δ mass* is the change in plant mass (g) between dawn and midday, *Δ time* is the time (minutes) between dawn and midday measurements of mass and *leaf*, and *LA* is the total leaf area (m2) of the plant. *LA* was estimated from the total number of leaves per plant multiplied by average leaf area per species (n = 4 plants, 5 leaves per plant). Measurements started with saturated soils and were repeated every few days for several weeks as plants slowly dried down; measurements were made along with those for quantifying hydroscape areas. We then fit an exponential response curve of *Kplant* to*leaf* for each species across time, using function “lm” from package *stats* within R version 3.6.2 (R Core Team 2019)with a log transformation of *Kplant*. The resulting equation is:

*Kplant = B\*exp(Slope Kplant \* MD*)(3)

where *Slope Kplant* is the exponential decay coefficient, and *B* is the *Kplant* extrapolated to *MD*=0*.* Instead of using *B*, maximum *Kplant* (*Max Kplant*) was calculated at *leaf* = maximum *MD* per species to avoid overestimation of *Max Kplant* given the exponential nature of the function. *50 Kplant* was calculated as the *MD* at which the *Kplant* was equal to half *Max Kplant.*

***Mesophyll Photosynthetic Sensitivity***

*50 Amax*, the leaf water potential at which there was a 50% loss of light-and-CO2-saturated photosynthetic capacity was taken from Salvi *et al.* (2021). Briefly, we withheld water from a separate set of 8-11 plants per species in the same glasshouse conditions over a period of several weeks in May through October 2018, repeatedly conducting CO2 response curves and measuring *leaf* as plants were slowly dried down. *Amax* of each CO2 response curve was calculated as the predicted *A* at *ci*= 6000 µmol mol-1, including day respiration. *Amax* is thus effectively the maximum net photo­synthetic rate of each curve. We fit a sigmoidal response curve of *Amax* to *leaf* for each species as:

*Amax*= (*Max Amax)/ (1+ exp(β* \*(*50 Amax*- *leaf* )))(4)

where *Max Amax* is the value of *Amax* under wet conditions [*leaf* = 0; effectively the maximum photosynthetic capacity], *β* is a decay coefficient (termed * Amax*), and *50 Amax* is the *leaf* at which *Amax* equals half *Max Amax*. Equation fitting was done with non-linear least squares using the R function “SSlogis” in the *stats* package of R version 3.6.2 (R Core Team 2019). Photosynthetic safety margin was calculated as the difference (MPa) between TLP and *50 Amax.*

***Leaf Anatomy***

Before drought treatments began, the youngest fully expanded leaf from six individuals per species was collected to measure leaf thickness, stomatal density and size, vein density, and maximum theoretical stomatal conductance. Leaf thickness (μm) was measured using a thickness gauge (B.C. Ames, Framingham, MA, USA) at the center of the leaf lamina, avoiding major veins. The *Eucalyptus* species under study are amphistomatous, so we collected leaf surface impressions of adaxial and abaxial surfaces by pressing fresh leaves onto Magic Scotch tape (3M, St. Paul, MN, USA) softened with acetone. Stomatal densities (*D,* mm-2) of leaf imprints were calculated in ImageJ (Rasband, 1997–2014) by counting the number of stomata per 0.47 mm2, averaged across three images per leaf taken at 10x magnification with a Nikon DS-Fi2 microscope (Nikon, Inc., Melville, NY, USA). Leaves were then dried in an oven at 75℃ for 24 hours and analyzed for other vein and stomatal traits. Sections from the middle of the lamina were cut from dried whole leaves and cleared: leaves were digested with 5% NaOH, bleached (10% household bleach), put through an ethanol dehydration series, dyed with 0.5% Safranin O ethanol solution, and mounted in glycerol. Total vein length per unit leaf area (VLA, units mm mm-2)was calculated from a 2 mm2 image per leaf at 4x magnification with the same microscope as above, and measured using ImageJ. Compared to fresh leaves, *Eucalyptus* leaves that undergo this protocol increase in area by 3.6% ± 5.4%. The intervein distance (*dx­*, in μm) was obtained from the same vein network following de Boer *et al.* (2016), by randomly drawing 10,000 random 1 mm cross-sectional lines across each traced image in ImageJ. For each cross-sectional line, we counted the number of locations where the line intersected with a traced vein, and then calculated the average of these distances. The metric *dx­* is the grand mean distance between veins of these 10,000 lines, calculated per leaf sample. The vein-epidermis distance (*dy*, in μm) was calculated as half leaf thickness, which is justified by the fact that all species were amphistomatous, meaning veins are equidistant from adaxial and abaxial epidermises (de Boer *et al.* 2016). We used these measurements to calculate *dx/dy*, a measure of the investment in veins vs. the investment in leaf thickness, where a ratio of 1 is theorized to optimize vein placement for water transport through leaves (Zwieniecki & Boyce 2014). Mean *dx/dy* per species was quantified as species mean *dx* divided by species mean *dy*. *dx/dy* could not be calculated for each individual, as the corresponding plant identifier was not recorded for leaf thickness measurements. We cannot report the standard deviation of *dx/dy*.

Following Franks & Beerling (2009), for 4 randomly selected stomata per leaf, we measured guard cell length (*L*, μm), total width of guard cell pair *(W,* μm), and stomatal pore length (*p*, μm). We calculated stomatal size (*S,* μm2*)* as *L* x *W*, and stomatal pore depth (*l*, μm) for fully open stomata as *W*/2. Maximum theoretical stomatal conductance to CO2 as defined by stomatal anatomy (*gsmax.theo*, mol m-2 s-1) was calculated using the equation (from Franks & Beerling 2009, adapted from Franks & Farquhar 2001):

(5)

where *d* is the diffusivity of water vapor in air (2.82 x 10-5 m2 s-1 at 25°C), *v* is the molar volume of air (2.4465 x102 m3 mol-1), *D* is stomatal density (mm-2), *a*max is the maximum stomatal pore area (μm2, calculated as π(*p*/2)2), *l* is stomatal pore depth (μm), π is the mathematical constant, and 1.6 converts conductance of water to conductance of CO2. For amphistomatous *Eucalyptus* spp. leaves*,* *gsmax.theo* is calculated as the sum of abaxial and adaxial *gsmax.theo*.

***Hydroscape Plasticity***

Using the methods described above, we also quantified hydroscape areas for the same 10 *Eucalyptus* species at two common gardens in Victoria, Australia: Hattah (most arid; *P/Ep* = 0.155, MAT = 16.9℃; -34.74◦N, 142.25 ◦E) and Bealiba (semi-arid; *P/Ep* = 0.374, MAT =13.8℃; -36.73 ◦N 143.61 ◦E). At these sites, ca. 5 month-old seedlings (mean height: 13.1 cm) were planted in June 2018 at 30 cm spacing through holes in weed matting to reduce competition. In October and November 2018, after 4-5 months of establishment, we erected rain-out shelters over the plants and began collecting water potentials (as above) as they declined due to rain exclusion and seasonal warming. Measurements concluded in February 2019. The UW-Madison glasshouse can be considered the least arid of the three sites, as plants were kept well-watered until the hydroscape experiment began (see Fig. S2 for temperature, relative humidity, and vapor pressure deficit recorded during glasshouse and common garden measurements). For each species, the plasticity of hydroscape areas across the three sites of variable aridity was calculated as the relative distance plasticity index (RDPI; Valladares *et al.*, 2006), where

(6)

and *HA* is hydroscape area, *i* and *i’* denote different sites (and *i ≠* *i’*) within a single species, *n* is the number of sites and *m* is the number of distances (i.e. *n*(*n*-1)/2). RDPI ranges from 0 (no plasticity) to 1 (maximal plasticity).

We used parametric bootstrapping using package *mvtnrom* and function “rmvnorm” in R version 3.6.2 (R Core Team 2019)to simulate 500,000 hydroscapes per species per site using the estimated slopes and intercepts and their covariation from the original hydroscape linear regressions described above (Genz *et al.* 2021). These simulations were used to estimate the standard error of hydroscape area for each species at each site.

***Data Analyses***

To calculate native range aridity, species occurrences were obtained from Atlas of Living Australia on 19 May 2018. We rounded latitude and longitude to the nearest 0.25 degree, and removed duplicate species-location combinations to avoid effects of oversampling. For each location, we extracted climate variables (mean annual precipitation [P] and temperature) from WorldClim (Fick & Hijmans 2017) using QGIS 3.0 (QGIS Development Team 2018). Pan evaporation (*Ep*) was extracted for each location from the Bureau of Meteorology’s (BOM) 0.25 degree resolution dataset (http://www.bom.gov.au/jsp/ncc/climate\_averages/evaporation/index.jsp?period=anu#maps) using R version 3.6.2 (R Core Team 2019). We calculated this ratio of mean annual precipitation to mean annual evaporation (*P/Ep*) for each occurrence location and then took the mean for each species. See Salvi *et al.* (2021) for more information and raw data. We calculated both the standard deviation and the coefficient of variation (standard deviation / mean) of *P/Ep* for each species to represent the variability in macroclimatic moisture across each species’ native range.

We used the R package *phytools* (Revell 2012) to obtain relationships and times of divergence among our study species from the time-calibrated molecular phylogeny of [Thornhill *et al.* (2019); see Salvi et al. (2021)]. We used both ordinary and phylogenetically structured linear regressions to test for significant relationships between traits. Ordinary linear regressions were done using function “lm” from package *stats* within R version 3.6.2 (R Core Team 2019); r2 values presented are adjusted for number of predictors in the model.Phylogenetically structured linear regressions used Pagel’s lambda, a Brownian motion-derived model, and the R function “phylolm” within package *phylolm* (Ho & Ané 2014).

To investigate correlations among  *Amax*, *Max Amax, 50 Kplant, Max Kplant,* TLP, VLA, SLA, and *gsmax.theo* (but excluding *dx:dy*, photosynthetic safety margin, and *Slope Kplant* because they correlated strongly with other traits), we used phylogenetically structured principal component analyses (pPCA) of mean trait values for each species, using Pagel’s lambda and R function “phyl.pca” within package *phytools* (Revell 2012). The traits of interest *50 Amax, HA,* and *P/Ep* were overlaid on this pPCA using function “envfit” in order to compare how other traits correlate with these traits. We found there were no phylogenetic effects (< 0.0001), so a non-phylogenetic PCA function was used (base R functions) to visualize the PCA for simplicity. Using both forwards and backwards selection and the R function “step" in the *stats* package*,* and *50 Kplant,* TLP, *50 Amax*, and/or *P/Ep* as possible predictor variables, we found the best model for predicting *HA*.

To test for differences in hydroscape areas across sites and species, we used an F test on df = 2, ∞, using the mean hydroscape area estimated from the linear regression and the standard error estimated from parametric bootstrapping, and allowing for species differences (i.e., *HA ~ site\*species)*, followed by a Fisher’s pairwise post-hoc test.To test for differences in hydroscape slopes and intercepts across sites and species, we used multiple linear regression and performed ANOVA model comparisons between the reduced model assuming no species or site differences or interactions (*MD* ~ *PD)* to models that add additional terms, where the full model allows for all possible differences and interactions between species and sites (*MD* ~ *PD\*site\*species*). If added terms were considered statistically significant on a level of  < 0.05, they were accepted in the best model. The best model was used to compare slope and intercepts across sites. To further test hydroscape plasticity, we tested if RDPI differed among species, and with species’ native *P/Ep*. All analyses were performed using R version 3.6.2 (R Core Team 2019).

**Results**

***Hydroscape area, functional traits, and their correlations***

Hydroscape area ranged from 1.8 - 9.1 MPa­­2 across our ten species (Table 1; Fig. S3), a five-fold variation that compares with a 13-fold variation seen across plants worldwide (Fu & Meinzer 2019). *Kplant­* declined exponentially with **MD in each species (Fig. S4). Across species, *Max* *Kplant* ranged from 0.3 to 1.5 g H2O MPa-1 m-2 min-1, *50 Kplant*, from -3.7 to -2.0 MPa, and *Slope Kplant* from 0.3 to 1.2 (Table 1). TLP varied from -1.0 to -2.3 MPa and photosynthetic safety margin from 0.5 to 1.8 MPa. Among anatomical traits, VLA ranged from 8.8 to 14 mm mm­-2; SLA, from 89.6 to 190.2 cm2 g-1; *gsmax.theo*, from 0.6 to 1.5 mol m-2 s-1; and *dx/dy*, from 0.7 to 1.6 μm μm -1 (Table 1).

Species with contrasting hydroscapes exhibited different responses of turgor loss and hydraulic disfunction to water stress. More anisohydric species tended to have lower TLP, greater VLA, less sensitivity of *Amax* and *Slope* *Kplant* to *leaf* (Fig. 1; Table 1). As hydroscape area increased, species showed significant declines in *50 Kplant* (Pord/phy = 0.006; r2 = 0.59), *50 Amax* (Pord/phy = 0.01; r2 = 0.52), *Amax*decay coefficient ( *Amax*) (log-log transform­ation, Pord/phy = 0.04; r2 = 0.35), and TLP (Pord/phy = 0.02/0.04; r2 = 0.45) (Fig. 2). An increase in photosynthetic safety margin with hydroscape area was marginally significant (Pord/phy = 0.06; r2 = 0.30), and a decrease in Slope *Kplant* with hydroscape area was weakly significant (Pord/phy = 0.08, r2 = 0.25) (Fig. 2). Hydroscape area showed no correlation with *Max* *Kplant* (Pord/phy = 0.51; r2 = 0.06) (Fig. 2). *Max Amax* tended to decrease with hydroscape areas, but this relation­ship was not statistically significant (Pord/phy = 0.21; r2 = 0.09) (Fig. 2). *Max* *Amax* and *Max* *Kplant­*, had no relationship (Pord/phy = 0.25, r2 = 0.06; Fig. S5a). When *Max Amax* was analyzed on a per area basis, we found a significant positive correlation with *Max Kplant* (Pord/phy = 0.01, r2 = 0.54; Fig. S5b). TLP decreased with *50 Kplant*, although the relationship was weakly significant and there were phylogenetic effects (Pord/phy = 0.06/0.14; r2 = 0.29; Fig. 3). This relationship was more pronounced in the subgenus *Symphyomyrtus.*

Among the aspects of leaf anatomy investigated, only VLA showed a significant increase with hydroscape area (Pord/phy = 0.03/0.03; r2 = 0.425; Fig. 4). SLA tended to increase with hydroscape area (Pord/phy = 0.09, r2 = 0.24), and *gsmax.theo* tended to decrease (Pord/phy = 0.06/0.21, r2 = 0.30), but the correlations were only marginally significant for SLA and non-significant for phylogenetically structured analyses of *gsmax.theo* (Fig. 4). Using an ANCOVA to test for differences between the two subgenera in the relationship between *gsmax.theo* and hydroscape area, we found that intercepts were different between subgenera (Pord/phy = 0.03) but slopes were not (Pord/phy = 0.12), and this positive relationship between *gsmax.theo* and hydroscape was significant when including subgenus in the model (Pord/phy = 0.08). The expected decline in *dx/dy* with hydroscape area was not significant when all species were included (Pord/phy = 0.15, r2 = 0.15), but was significant when the outlier *E. viminalis* was excluded (Pord/phy = 0.02, r2 = 0.49).

A PCA identified two major axes which cumulatively explained 68.1% of the variation of eight parameters included across our ten species (Fig. 5). The first component (PC1) accounted for 47.0% of total variation, and was negatively associated with *gsmax.*theo and VLA, while positively associated with *Amax,* TLP, and SLA (Table S2). The second component (PC2) accounted for 21.1% of total variation, and was positively associated with *Max Kplant* and *50 Kplant* (Table S2). In PCA space, species were arranged in three loose groups: *regnans, nitens,* and *viminalis*, all of which are abundant in tall mesic forests; *dives, macrorhyncha,* and *obliqua*, all characteristic of eucalyptus forests and woodlands; and *sideroxylon, microcarpa, arenacea,* and *dumosa,* abundant in woodlands and mallee. *50 Amax* was tightly and positively correlated with TLP,  *Amax* and negatively to *gsmax.theo*, but orthogonal and therefore not related to *Max Kplant*. *P/Ep* and *HA* fell on opposite sides of PC1, suggesting similar correlations with traits related to PC1, but of opposite signs. The best model for predicting *HA*, using *50 Kplant,* TLP, *50 Amax*, and/or *P/Ep* as possible predictors, was *HA = -2.6(50 Kplant) – 4.4(P/Ep) + 0.7* (Table S3; P = 0.002, r2 = 0.77).

***Relating hydroscapes to climatic moisture gradient***

Hydroscape area decreased with increasing *P/Ep* (Pord/phy = 0.01; r2 = 0.50; Fig. 6a). However, hydroscape area did not correlate with the variability of *P/Ep* across each species’ native range, whether quantified with standard deviation (Pord/phy = 0.11; Fig. 6b) or coefficient of variation (Pord/phy = 0.75/0.66; Fig. 6c). Using an ANCOVA to test for differences in the relationships between hydroscape area and *P/Ep* between the subgenera *Eucalyptus* and *Symphyomyrtus*, we found that neither slope (Pord/phy = 0.24) nor intercept (Pord/phy = 0.14) differed between the two subgenera.

***Plasticity of Hydroscapes***

We found evidence that hydroscape areas differed among the glasshouse and two field sites (F2, ∞ = 4.65, P < 0.001), but only for 3 (of 10) species (Fig. 7a, Table S4). These species are endemic to mesic (*viminalis*) and drier (*microcarpa* and *sideroxylon*) portions of the climatic gradient. In these three species, hydroscape area was larger in the glasshouse than common garden populations. Slope and intercept of the hydroscape linear regression (*MD* vs. *PD*) differed among sites when allowing for differences between species (F40 = 14.29, P < 0.001). Slope differences between sites were equal across all species (F18 = 1.53, P = 0.08). Specifically, we found that slopes of hydroscape linear regressions are steepest at the intermediately dry site, Bealiba, (F41, 296 = 171.5, P < 0.001; Table S5-6) within all ten species. Intercept differences between sites were variable across species (F18 = 2.80, P < 0.001), where the best model was *MD* ~ *PD\*site + PD\*species + site\*species* (see Table S6), and differences between sites were found within all ten species (Table S5; Fig. 7b,c). Post-hoc comparison of intercepts revealed that the glasshouse was the site with the most negative intercept in 5 of 10 species, while Hattah and the glasshouse congruently had the most negative intercept in the other species. In 7 of 10 species, Bealiba was the site with the least negative intercept (Fig. 7b,c).

RDPI, a measure of the plasticity in hydroscape area, ranged from 0.07 – 0.38 across the species, but did not differ statistically among species (ANOVA F9,20 = 1.5, P = 0.20). RDPI did not vary with native *P/Ep* (Pord/phy = 0.84/0.88; r2 = -0.12; Fig. 6d), the standard deviation of *P/Ep* across the species’ geographic range (Pord/phy = 0.85/0.66 ; r2 = -0.12; Fig. 6e), or the coefficient of variation of *P/Ep* across the species’ geographic range (Pord/phy = 0.31/0.23; r2 = 0.02; Fig. 6f).

**Discussion**

Previous studies at continental to global scales established that species with greater anisohydry from a range of lineages tend to inhabit drier climates, have xylem networks with lower branch hydraulic conductivity that are less vulnerable to drought, and have more negative leaf wilting points compared with more isohydric species (Fu & Meinzer 2018; Fu *et al.* 2019; Li *et al.* 2019). Despite these emergent properties, we still know remarkably little about variation along the isohydric-anisohydric continuum for groups of closely related species and the habitats they inhabit. Including accounting for phylogenetic relationship, the integrating trait of hydroscape area helped clarify and simplify broad-scale relationships between the iso/anisohydric continuum and key functional traits. Species showing greater anisohydry and with greater hydroscape areas were native to drier habitats. These species were less vulnerable to water stress-induced hydraulic and photosynthetic dysfunction, tended to have slower rates of photosynthesis, and had greater whole-plant hydraulic conductance under well-watered conditions. Hydroscape areas are a robust tool in studies of drought response within plant groups at both very broad (*e.g.* global) and local (*e.g.* ranging from metres to hundreds of km) scales. We found that hydroscape areas were relatively fixed, with the degree of plasticity not related to macroclimatic moisture variability.

***Plant physiology and hydroscapes at broad vs. fine scales***

Theory suggests a tradeoff between hydraulic safety and efficiency, because wider conduits with greater conductance tend to have a greater chance of embolism (Tyree & Sperry 1988). Supporting this tradeoff, a recent global analysis of the iso/anisohydric continuum found that more isohydric species tended to have greater branch conductivity and greater vulnerability to embolism formation (Fu & Meinzer 2018). Within our closely related species we found that more anisohydric species, with safer hydraulic transport, were as efficient as more vulnerable isohydric species. A possible explanation for the lack of smaller whole-plant hydraulic conductance for more anisohydric species is that they likely had greater root-to-shoot ratios (than the more isohydric species). Greater root-to-shoot ratios generally increase whole-plant hydraulic conductance by minimizing the greater hydraulic resistance of leaves (as integrated across the entire hydraulic network; Sperry, Adler, Campbell & Comstock 1998). In addition, individual components of hydraulic networks do not always vary in accordance with all other components. For example, under well-watered conditions stem hydraulic conductivity can be greater in more isohydric plants. When study species were grown in the field, more anisohydric species showed lower branch conductivity (D.D. Smith, unpublished data­). Thus, we emphasize the importance of measuring multiple traits across the plant body when considering variations in hydraulic traits among species (Sperry *et al.* 1998; Meinzer, McCulloh, Lachenbruch, Woodruff & Johnson 2010; McCulloh *et al.* 2019). Another possible explanation is that anisohydric eucalypts may be highly opportunistic in their water use, very rapidly transpiring until leaf water potential limits photosynthetic capacity, and then shutting down the stomata. Indeed, in our experiment, the more anisohydric species tended to dry out faster than their more isohydric relatives (observational data). These analyses of hydraulic conductance come with the caveat that our estimates of whole plant hydraulic conductance are likely conservative, given that our methods underestimate the rate of hydraulic transport and overestimate the difference in ** between the soil and leaves at midday.

Theory and empirical observations also point to a second tradeoff between maximum photosynthetic rates and resistance to drought-induced declines in photosynthesis (Givnish & Vermeij 1976; Orians & Solbrig 1977; Givnish 1986; Temme, Kerr & Donovan 2019). We found support for this tradeoff among our 10 species, where more isohydric species that were more vulnerable to water stress tended to have greater photosynthetic rates per unit mass during non-stressful conditions (Fig. 2). Salvi *et al.* (2021) found that mesophyll photosynthetic sensitivity to drops in *leaf* (MSP) declined significantly with mean *P/Ep* measured over species’ ranges, but at the cost of reduced photosynthetic rates per unit leaf mass at or near full hydration; *Amax* increased with species *P/Ep* in four common gardens in Victoria.

Meinzer et al. (2017) found that more anisohydric species have higher carbon assimilation rates under well-watered conditions than more isohydric species (Meinzer *et al.* 2017), but they measured *Amax* on a per area basis. Area- and mass-based rates are often inversely related, with leaves with high SLA having higher photosynthetic rates per unit leaf mass (Reich et al. 1997), presumably as a result of less competition for light and CO2 within the leaf cross-section and of shorter diffusive distances, while leaves with low SLA (usually much thicker leaves) have higher aggregate rates per unit area (e.g., see Chabot & Hicks 2008). Had maximum photosynthetic rates been presented on a per mass basis, it is likely Meinzer *et al.* 2017 would have found a trend parallel to ours, that maximum photosynthetic rates tend to be smaller as hydroscape areas increase. Across species, photosynthetic rates per unit leaf mass are far more strongly coupled to whole-plant growth than area-based rates, because mass-based rates reflect energetic returns per unit investment (Kruger & Volin 2006). Additionally, across our species and in agreement with broad-scale patterns, maximum photosynthetic rates are positively correlated with maximum whole plant hydraulic conductance (both per leaf area) across our species (Salvi *et al.* 2021), as expected (Reich 2014; Scoffoni *et al.* 2016).

Hydroscape area was best predicted by vulnerability of plant hydraulic conductance and macroclimatic moisture availability of species’ native range, strengthening the idea that the isohydric to anisohydric continuum is strongly coordinated with hydraulic traits and climatic conditions (Fu & Meinzer 2018; Li *et al.* 2019) both globally and at finer scales. Furthermore, our data indicate coordination of traits across the entire plant, which is evident in the trend between TLP and *50 Kplant* (Fig. 3). Whole plant hydraulic conductance is rarely measured directly due to the difficulty of estimating it in field-grown plants, but declines in this parameter due to drought stress have been shown to be tightly linked with loss of hydraulic function in roots (Domec *et al.* 2006; Domec, Schäfer, Oren, Kim & McCarthy 2010).

Franks, Drake & Froend (2007) proposed a unique behavior in which plants with strong stomatal control of leaf water potential also maintain relatively constant root to shoot water potential gradients regardless of soil water potential – that is, they are isohydrodynamic. In this context, we observe that the two most anisohydric species (*E. microcarpa* and *E. sideroxylon*) had hydroscape slopes that were extremely close to 1 (and thus close to parallel with a 1:1 relationship of *MD*vs. *PD*; Fig. S3). Therefore, in these two species, the difference between *MD*and *PD* remained relatively constant as the plants dried out, especially compared to the other species, which suggests *E. microcarpa* and *E. sideroxylon* approximate this isohydrodynamic behavior. While hydroscape areas have not yet been directly compared in isohydrodynamic plants, we expect that species with hydroscape slopes closer to 1 are more isohydrodynamic. Thus, isohydrodynamic behavior may actually fit well within the anisohydric end of the iso/anisodry continuum framework.

***Hydroscape area and MPS***

Salvi *et al.* (2021) found that *Eucalyptus* species from less arid habitats have higher mesophyll photosynthetic sensitivity, but also higher photosynthetic rates per unit leaf mass during non-water-stressed conditions. Here, we showed that species with greater MPS – as quantified by more negative *50 Amax* and steeper ** *Amax* – have smaller hydroscapes (Fig. 2). This supports our hypothesis that species with greater MPS should be more isohydric. Such stomatal behavior is not predicted by the classic ∂A/∂E model of Cowan & Farquhar (1977), which excludes any impact of leaf water potential on mesophyll photosynthetic capacity. However, it is predicted by the model of Givnish (1986), which does include the effects of stomatal conductance and leaf: root allocation on *leaf*, and of *leaf*on mesophyll photosynthetic capacity, and recovered all of the principal conclusions of Cowan & Farquhar (1977) (e.g., linear relationships of photosynthetic vs. stomatal conductance across leaves whose photosynthetic capacity varies) based on measurable parameters.

While Salvi *et al.* (2021) found that species in subgenus *Eucalyptus* had greater MPS for a given *P/Ep* than those in subgenus *Symphyomyrtus,* here we found no significant subgeneric differences in the relationship of hydroscape area to *P/Ep*. Overall, more anisohydric *Eucalyptus* species tend to have mesophyll photosynthetic capacity that is less sensitive to water stress, providing evidence of a new dimension of the generality that anisohydric species tend to be more drought tolerant (McDowell *et al.* 2008; Li *et al.* 2019). Further studies of the relationships of MPS to key functional traits, especially those related to stomatal and leaf hydraulic regulation, are needed to better understand the full range of tradeoffs with this newly framed drought tolerance trait. Based on our current study, however, members of subgenus *Symphomyrtus* had a much noisier relationship of hydroscape area declining with native *P/Ep* (Fig. 2). Beyond the species we sampled, subgenus *Symphyomyrtus* shows a strong tendency to occur in arid mallee habitats. The ecological implications of differences in hydroscape area at a given native *P/Ep* we have found should now be investigated in other mallee species.

***Hydroscape Plasticity***

Our study is the first to examine hydroscape area plasticity within species in response to relative moisture supply, and to test whether the extent of such plasticity varies with the native distribution of species along moisture gradients. When the same 10 species were grown in three experimental locations that differed in moisture availability, hydroscape areas within species seldom (*i.e.,* 3/10 species) differed across sites (Fig. 7), suggesting the degree of isohydry of a species when quantified with this metric is relatively constant no matter the environmental conditions in which the species grows. While in most cases hydroscape area did not vary across experimental sites, slopes and intercepts of the *PDMD* linear relationship did, that we interpret as evidence of coordination of traits such that the degree of isohydry is maintained across environments. While we can only speculate as to the causes of coordinated maintenance in *HA,* there is evidence that other hydraulic traits also show coordinated variation. TLP, osmotic potential and *50 Kleaf* have been shown to change through and between seasons (Maréchaux *et al.* 2015; Binks *et al.* 2016; Johnson *et al.* 2018a). Given that stomatal regulation of *leaf* also reflects numerous other physiological characteristics (Fu *et al.* 2019; McCulloh *et al.* 2019), the consistency of hydroscape area and isohydry is not surprising.

We note that the three experimental sites used to test for variability in hydroscape across environments did not provide consistent growing conditions. The two drier sites, Bealiba and Hattah, are outdoor common gardens in Victoria, Australia, subject to local variations in rainfall, temperature and humidity, while the third site was a climate-controlled glasshouse in Wisconsin, USA. An important observation is that there were few differences in hydroscape area at the species level. This provides strong support for genetic control of hydroscape areas and contrasts with studies suggesting the placement of a species within the isohydric to anisohydric continuum varies depending on environmental conditions (Domec & Johnson 2012; Hochberg *et al.* 2018). There though we recognize that isohydry was characterized using metrics other than hydroscape area (i.e., response of stomatal conductance and transpiration to changes in vapor pressure deficit, or slope of regression of *MD* vs. *PD*).

Climate is a major determinant of hydraulic strategy across species, and anisohydric species tend to occupy more drought-prone habitats (Fu & Meinzer 2018; Li *et al.* 2019). As with previous studies, we found larger hydroscape areas in species native to more arid conditions. Hence, while hydroscape plasticity does not correlate with aridity, hydroscape area does. A similar trend was found in a global analysis of TLP (a trait which correlates strongly with hydroscape area [Meinzer et al. 2016]), where species from drier climates had more negative TLP, but the change in TLP before and after a dry season did not relate to species climate of origin (Bartlett *et al.* 2014).

Neither hydroscape area nor plasticity of *HA* correlated with the variability of aridity across a species’ entire native geographic range (Fig. 6), suggesting that neither the degree of isohydry nor the plasticity of this behavior is favored in species that span a larger range in macroclimatic moisture availability. There is little known about how the iso-anisohydric continuum relates to climatic variability within environments. One study that compared precipitation variability to seasonal response of *PD* found that a more anisohydric species had a greater response in *PD* to increases in precipitation variation than a co-occurring isohydric species (Gu, Pallardy, Hosman & Sun 2016), but how this difference in hydraulic response affected growth and survival is unknown. While it is common for species with contrasting positions on the iso-anisohydric continuum to coexist (Linton, Sperry & Williams 1998; Vogt 2001; Quero, Sterck, Martínez-Vilalta & Villar 2011; Johnson *et al.* 2018b), because species-specific functional traits such as rooting depth or leaf phenology may enable species to partition hydraulic and photosynthetic niche spaces within the same habitat (Jacobsen, Pratt, Davis & Ewers 2008; McCulloh *et al.* 2019), we also expect that more anisohydric species tend to occupy environments with greater variability in precipitation and moisture availability (McDowell *et al.* 2008).

**Conclusion**

Across 10 upland species of *Eucalyptus* that dominate different portions of a macroclimatic gradient in relative moisture supply in southeast Australia, we found that the degree of anisohydry increases toward drier habitats and is associated with several drought-tolerant traits. Furthermore, we provide the first rigorous demonstration that increased mesophyll photosynthetic sensitivity to leaf water potential – associated with native distribution in moister habitats – is itself tied to increased isohydry, a trend previously predicted (Givnish 1986). Finally, we show for the first time that hydroscapes show little plasticity in response to substantial variation in moisture supply, and that the extent of plasticity does not vary significantly with the moisture availability across native distributions of different species. This last finding provides further evidence that hydroscape area is a useful metric for characterizing plant drought tolerance and water-status regulation – an increasingly important subject in understanding and predicting the consequences of climate change on plants and ecosystems.

**Figure Legends**

**Figure 1.** Comparisons of the most isohydric species (left column, *E. nitens*) to a more anisohydric species (right column, *E. dumosa*), with degree of anisohydry being classified by hydroscape area. **Top row** displays hydroscape (light blue triangle) across declines in midday leaf water potential (*MD*) and soil water potential (*PD*), with greater hydroscape area in the more anisohydric species by defini­tion. Different colored data points represent different individual plants. **Middle row** shows the response of whole plant hydraulic conductance (*Kplant,* g MPa-1 m-2 min-1) and mesophyll photosynthetic capacity (*Amax*, mol g-1 s-1) to declines in *leaf* (solid blue and purple lines, respectively). Vertical dashed lines display the *leaf* of leaf turgor loss (TLP), of 50% decline in *Amax* (*50Amax*), and of 50% decline in *Kplant* (*50 Kplant*). *Kplant* and *Amax* are less sensitive to **leaf and TLP is lower in the more anisohydric species. **Bottom row** displays vein and stomatal architecture. Vein length per area (VLA, mm mm-2) is greater in the more anisohydric species.

**Figure 2.** Relationships between hydroscape area and key hydraulic and photosynthetic traits. Traits include water potential inducting 50% loss of whole plant hydraulic conductance (*50 Kplant*), hydraulic conductance when *leaf* = 0 (*Max Kplant*), exponential slope of the decline of *Kplant* with decreasing*leaf*, *leaf* at 50% loss of mesophyll photosynthetic capacity (* Amax*), mesophyll photosynthetic capacity when *leaf* = 0 (*Max Amax*), sigmoidal slope of the decline of *Amax* with decreasing*leaf* (***Amax*), *leaf* at leaf turgor loss (TLP), and photosynthetic safety margin between *50 Amax* and TLP. Error lines indicate one standard deviation. Solid black lines are phylogenetically structured linear regressions; dashed gray lines are ordinary linear regressions; black and gray dashed lines indicated both types of linear regressions yield the same results.

**Figure 3.** Correlation between vulnerability of whole plant hydraulic conductance (*50 Kplant*) and turgor loss point (TLP).Black and gray dashed lines indicated that both ordinary and phylogenetic linear regressions yield the same statistically significant results.

**Figure 4.** Relationships between hydroscape area and leaf anatomy. Traits include specific leaf area (SLA), vein length per area (VLA), max theoretical stomatal conductance (*gsmax.theo*), and the ratio of intervein distance to vein-epidermis distance (*dx:dy*). Error lines indicate one standard deviation. Solid black lines are phylogenetically structured linear regressions; dashed gray lines are ordinary linear regressions; black and gray dashed lines indicated both types of linear regressions yield the same results. Legend is the same as Fig. 2.

**Figure 5.** Results of phylogenetic principal component analysis (PCA) of functional traits. HA is hydroscape area, VLA is vein length per area, SLA is specific leaf area, *gsmax\_theo* is maximum theoretical stomatal conductance calculated using stomatal dimensions. Blue arrows represent traits of interest overlaid on the PCA for the purpose of analyzing correlation of these traits with the PCA axes and traits therein (red arrows).

**Figure 6.** Hydroscape areas and plasticity of 10 *Eucalyptus* species. **(a-c)** Hydroscape areas grown in glasshouse conditions and their relationship to **a)** moisture availability of native habitat (mean precipitation vs. mean pan evaporation, *P/Ep*), **b)** the standard deviation of *P/Ep*across the species’ native distribution, and **c)** the coefficient of variation (mean *P/Ep* / standard deviation *P/Ep*). **(d-f)** RDPI of hydroscape area, calculated from hydroscape areas of 10 *Eucalyptus* species across 3 sites of different moisture levels, and their relationship to **d)** *P/Ep*, **e)** the standard deviation of *P/Ep*, and **f)** the coefficient of variation *P/Ep*. Black and gray dashed lines indicated that both ordinary and phylogenetic linear regressions yield the same statistically significant results. Legend is the same as Fig. 2.

**Figure 7.** Hydroscape areas (a), intercepts (b), and slopes (c) across 3 sites in ten species of *Eucalyptus*. Glasshouse (GH) represents wettest conditions, Hattah is driest. Error bars represent one standard deviation, estimated using parametric bootstrapping. Species ordered left to right by most isohydric to most anisohydric according to GH hydroscape area. **a)** Asterisks denote the three species that displayed plasticity in hydroscape area (*HA*). In all three cases, *HA* was largest in the GH (P < 0.005). **b)** All ten species displayed differences in intercepts among sites (P < 0.05), but which site had the most negative intercept depended on the species (see Table S5). **c)** All ten species displayed differences in slopes among sites, and were steepest at intermediately dry site Bealiba for all ten species (P < 0.001). Thus, while slope and intercept were found to be plastic, *HA* itself in most cases was not.

**Supporting Information**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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**Table 1.** Functional traits of 10 *Eucalyptus* species. Traits presented as means ± standard deviations. Species ordered by hydroscape area. Accurate standard deviations could not be easily estimated for *50 Kplant*, photosynthetic safety margin, and *dx/dy* given they are calculated from other estimated values. *50 Amax,  Amax*, and *Max Amax* taken from Salvi *et al.* (2021).

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Hydroscape area (MPa2)** | ***50 Amax* (MPa)** | *** Amax*** | ***Max Amax* (mol g-1 s-1)** | ***50*** ***Kplant* (MPa)** | ***Slope Kplant*** | ***Max Kplant* (g MPa-1 m-2 min-1)** | **TLP (MPa)** | **Photosynthetic Safety Margin (MPa)** | **SLA (cm2 g-1)** | **VLA (mm mm-2)** | ***gsmax.theo* (mol m-2 s-1)** | ***dx/dy* (μm μm-1)** |
| *nitens* | 1.8 ± 0.2 | -2.1 ± 0.2 | 3.2 ± 1.8 | 0.59 ± 0.11 | -2.0 | 1.2 ± 0.2 | 0.7 ± 1.5 | -1.5 ± 0.2 | 0.5 | 163.3 ± 14.9 | 9.3 ± 0.7 | 1.4 ± 0.6 | 1.1 |
| *dives* | 2.9 ± 0.2 | -2.4 ± 0.2 | 3.2 ± 2.0 | 0.43 ± 0.07 | -2.4 | 0.6 ± 0.2 | 0.9 ± 1.5 | -1.3 ± 0.4 | 1.1 | 141.9 ± 18.2 | 10.1 ± 0.6 | 0.6 ± 0.4 | 1.1 |
| *obliqua* | 3.2 ± 0.6 | -2.1 ± 0.1 | 6.7 ± 5.3 | 0.39 ± 0.04 | -2.3 | 0.8 ± 0.2 | 0.9 ± 1.6 | -1.0 ± 0.2 | 1.1 | 138.4 ± 17.0 | 9.4 ± 0.9 | 0.9 ± 0.2 | 1.1 |
| *regnans* | 3.3 ± 0.2 | -2.2 ± 0.2 | 5.9 ± 5.3 | 0.49 ± 0.06 | -2.7 | 0.6 ± 0.1 | 0.5 ± 1.5 | -1.5 ± 0.3 | 0.7 | 175.0 ± 11.6 | 9.2 ± 0.9 | 0.9 ± 0.4 | 1.4 |
| *arenacea* | 4.3 ± 0.5 | -2.6 ± 0.3 | 1.8 ± 0.9 | 0.55 ± 0.09 | -2.4 | 0.8 ± 0.1 | 0.9 ± 1.4 | -1.4 ± 0.2 | 1.3 | 129.0 ± 18.2 | 9.8 ± 1.4 | 1.0 ± 0.3 | 0.8 |
| *macrorhyncha* | 5.0 ± 0.5 | -2.7 ± 0.3 | 2.3 ± 1.4 | 0.41 ± 0.07 | -2.0 | 0.9 ± 0.2 | 1.4 ± 1.6 | -1.6 ± 0.3 | 1.1 | 109.1 ± 2.2 | 12.4 ± 3.7 | 1.1 ± 0.6 | 0.8 |
| *viminalis* | 5.3 ± 0.5 | -3.6 ± 0.6 | 1.6 ± 1.4 | 0.46 ± 0.10 | -3.0 | 0.5 ± 0.1 | 0.3 ±1.6 | -1.7 ± 0.2 | 1.8 | 190.2 ± 36.1 | 8.8 ± 0.7 | 1.1 ± 0.4 | 1.6 |
| *dumosa* | 6.5 ± 0.5 | -4.1 ± 0.4 | 1.2 ± 0.5 | 0.41 ± 0.05 | -2.9 | 0.7 ± 0.1 | 1.5 ± 1.5 | -2.3 ± 0.5 | 1.7 | 89.6 ± 7.3 | 10.6 ± 2.1 | 1.5 ± 0.3 | 0.9 |
| *sideroxylon* | 7.6 ± 2.1 | -3.0 0.3 | 2.1 ± 1.1 | 0.51 ± 0.07 | -2.8 | 0.7 ± 0.1 | 1.1 ± 1.5 | -1.9 ± 0.3 | 1.1 | 122.8 ± 9.7 | 14.0 ± 2.2 | 1.2 ± 0.3 | 0.7 |
| *microcarpa* | 9.1 ± 1.8 | -3.4 ± 0.3 | 1.8 ± 0.9 | 0.37 ± 0.04 | -3.7 | 0.3 ± 0.1 | 0.7 ± 1.5 | -2.0 ± 0.4 | 1.5 | 106.5 ± 9.6 | 11.9 ± 1.9 | 1.5 ± 0.4 | 0.8 |