Title: Intraspecific trait variation arises similarly among genotypes of *Eucalyptus camaldulensis* in response to seasonal change in environment rather than water availability or climate of genotype provenance

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Summary

- We used a widely-distributed tree *Eucalyptus camaldulensis* subsp. *camaldulensis* to partition intraspecific variation in leaf functional traits to genotypic variation and phenotypic plasticity. We examined if genotypic variation is related to the climate of genotype provenance and whether phenotypic plasticity maintains performance in a changing environment.

- Ten genotypes from different climates were grown in a common garden under watering treatments reproducing the wettest and driest edges of the subspecies’ distribution. We measured functional traits reflecting leaf metabolism and associated with growth (respiration rate, nitrogen and phosphorus concentrations, and leaf mass per area) and performance proxies (aboveground biomass and growth rate) each season over a year.

- Genotypic variation contributed substantially to the variation in aboveground biomass but much less in growth rate and leaf traits. Phenotypic plasticity was a large source of the variation in leaf traits and performance proxies, and was greater among sampling dates than between watering treatments. The variation in leaf traits was weakly correlated to performance proxies, and both were unrelated to the climate of genotype provenance.

- Intraspecific variation in leaf traits arises similarly among genotypes in response to seasonal environmental variation, instead of long-term water availability or climate of genotype provenance.

Key words: Climate, genotype, growth, intraspecific variation, leaf functional traits, phenotypic plasticity, season, water availability
Introduction

Genotypes within plant species show variation in leaf functional traits that underpin growth and survival. This intraspecific variation is often related to climate (Jonas & Geber, 1999; Albert et al., 2010; Aspinwall et al., 2013; Reich et al., 2014; Bloomfield et al., 2018), and genotypes with native trait values tend to perform better in their environment of provenance when reciprocally transplanted (Geber & Griffen, 2003). These observations suggest that genotypes are well adapted to their specific environment (Bolnick et al., 2003), expressing certain values of key functional traits that have fitness consequences. Leaf functional traits are often related to growth rate – a measure of vegetative performance and a component of fitness (Reich et al., 1997; Poorter & De Jong, 1999; Poorter & Bongers, 2006; Reich, 2014), and while rarely shown, they can also influence fitness across species (Adler et al., 2014). Some key functional traits are related to leaf metabolism, reflecting ecological strategies for resource acquisition and use (Wright et al., 2004; Diaz et al., 2016), and include photosynthesis and respiration rates, chemical composition, and the mass to area ratio.

Leaf functional traits also vary within a single genotype in response to changes in ambient conditions. The ability of a genotype to adjust trait value is known as phenotypic plasticity (Pigliucci, 2001) and has a genetic basis in altered gene expression and translation (Laitinen & Nikoloski, 2018). Therefore, genotypic variation and phenotypic plasticity together generate intraspecific trait variation observed in natural populations. Their relative importance is often examined within a genotype vs environment framework, often using analysis of variance to quantify the contribution of genotypic variation (G) independent of environment, environment (E) independent of genotype, and interaction of the two factors (G x E). Phenotypic plasticity corresponds to variance arising from E, and we define genotypic variation to constitute two components of G and G x E, with G x E corresponding to the genetic basis of phenotypic plasticity (Pigliucci, 2001) to emphasize that phenotypic plasticity has a genetic component when examined across genotypes. Understanding the source of intraspecific trait variation may improve predictions of species responses to changing environments (Schlichting & Pigliucci, 1998; Geber & Griffen, 2003; Albert et al., 2011; Violle et al., 2012). For example, intraspecific trait variation could lead to adaptive divergence and ultimately to reproductive isolation and speciation if the intraspecific variation has a large genotypic component and if the trait value and plasticity have strong fitness consequences.

The adaptive significance of phenotypic plasticity depends on whether the traits showing plasticity affect performance and ultimately fitness, the plastic response is in the
direction that maintains fitness in the environment, and the plastic response differs among
genotypes (Schlichting, 1986; Nicotra et al., 2010). Phenotypic plasticity is not necessarily
adaptive and can be neutral or maladaptive since some plastic responses are passive and
inevitable consequences of environmental effects on physiology (Bradshaw, 1965;
Schlichting, 1986; Sultan, 1987; Van Kleunen & Fischer, 2005). Examples of passive
responses include increased respiration rates during short term rises in temperature or
reduced photosynthesis rate under lower irradiance. Other responses are potentially adaptive,
reflecting physiological and developmental adjustments to maintain performance under
heterogeneous conditions, increasing the ecological niche, and maintaining fitness in a wider
range of environments (Bradshaw, 1965; Sultan, 1995; Pigliucci, 2001; Turcotte & Levine,
2016). However, adaptive plasticity is difficult to assess (Sultan, 2000). Only a few
responses, such as stem elongation under low light, are correlated with measures of fitness
(Dudley & Schmitt, 1996; Donohue et al., 2000). Instead, the adaptive significance of
phenotypic plasticity is often inferred from the variation in plastic response within natural
populations (Sultan, 2000; Valladares et al., 2000; Grassein et al., 2010). This assumes that
passive responses are unlikely to vary markedly among genotypes. For example, species
across the globe show similar short-term temperature responses in leaf respiration (Heskel et
al. 2016). It also assumes that plasticity in critical phenotypes, such as leaf functional traits,
improves plant performance if the response is in the direction appropriate under new
conditions.

Leaf functional traits are thought to reflect fundamental tradeoffs in plant strategies
for resource acquisition and use, and thus affect performance and may ultimately influence
fitness. Some of the most widely measured leaf functional traits are the ratio of leaf dry mass
per unit leaf area (LMA), leaf nitrogen (N) and phosphorus (P) concentrations, and metabolic
rates (respiration and photosynthesis). These traits are correlated with environmental
conditions when compared across sites around the globe. For example, LMA increases with
mean annual temperature but decreases with mean annual precipitation (Wright et al., 2004).
Leaf N concentration increases while P concentration decreases with mean annual
temperature (Ordonez et al., 2009), and mass-based and temperature-normalized respiration
rate decreases with aridity and increasing mean annual temperature (Atkin et al., 2015). Leaf
functional traits are also predictably correlated when compared across species. For example,
LMA is positively correlated with leaf lifespan, but negatively correlated with leaf N and P
concentrations and with mass-based rates of photosynthesis and respiration (Reich et al.,
1997; Wright et al., 2004). These correlations constitute the leaf economic spectrum (Reich
et al., 1992; Wright et al., 2004), ranging from rapid leaf metabolic rates in species with short-lived leaves to slow leaf metabolism in species with long-lived leaves. This spectrum is part of a life history spectrum, spanning from resource acquisitive but short-lived species to resource conservative but long-lived species (Lambers & Poorter, 1992; Poorter & Van der Werf, 1998; Westoby et al., 2002; Grime, 2006; Reich, 2014; Diaz et al., 2016). Indeed, leaf functional traits are related to growth rate, survival, fecundity, and ultimately fitness when examined across species, at least in some contexts (Reich et al., 1997; Enquist et al., 2007; Poorter et al., 2008; Easdale & Healey, 2009; Adler et al., 2014; Reich, 2014; Falster et al., 2018).

However, it remains unclear what selective forces are actually shaping the correlations among the traits and between the traits and environmental conditions. The correlation between traits and environmental conditions is often weak, in large part because within-site variation is large (Wright et al., 2004; Reich, 2014; Atkin et al., 2015; Shipley et al., 2016). Clearly, many trait combinations can successfully exist under similar environmental conditions. Also, correlations among traits can be contradictory when compared among closely-related species or within species (Albert et al., 2010): correlations can be identical to across species patterns (Hayes et al., 2019), weak (Muir et al., 2017), unrelated (Li et al., 2015), or even in an opposite direction (Anderegg et al., 2018).

Observations like these may be results of the linkage between leaf traits and growth rate being circumstantial. For example, across species, growth rate is related to leaf photosynthetic capacity in both seedlings and adults, whereas growth rate is related to leaf mass to area ratio in seedlings and not necessarily in adult plants in the field (Poorter et al., 2008; Anaïs et al., 2016; Wright et al., 2019).

In this study, we determine the relative contributions of genotypic variation and phenotypic plasticity to intraspecific variation in leaf functional traits, correlations among traits, linkages between traits and climate of genotype provenance and growing conditions, and relationships between traits and growth in *Eucalyptus camaldulensis* Dehn (River Red Gum). *Eucalyptus camaldulensis* is an Australian native tree commonly found in riparian habitats and has the widest natural distribution among *Eucalyptus* species. The species’ geographical distribution stretches across 12-38 °S in latitude (Brooker & Kleinig, 1983), 12 to 28 °C in mean annual temperature, and 150 to 1800 mm in mean annual rainfall, encompassing much of Australia. We start with the premise that genotypes of *E. camaldulensis* are genetically diverse, are well-adapted to the climate of their provenance, and possess the value and plasticity of leaf functional traits that increases performance. If
supported, it follows that 1) genotypic variation is a substantial source of leaf trait variation, and trait values of individual genotypes are related to climate of their provenance; 2) genotypes differ in the magnitude of plastic responses in leaf traits to experimental changes in environment (due to season and watering treatment); 3) leaf traits have trait-trait relationships consistent with leaf economic spectrum and which are maintained with plastic responses, reflecting fundamental tradeoffs among traits at the leaf level; and, 4) growth rate is higher for individuals showing high degrees of plasticity of leaf trait combinations that increase resource acquisition. The hypotheses are shown in Fig. 1. We test these hypotheses using a common garden experiment with 10 genotypes from geographically and climatically-dispersed populations of *E. camaldulensis* subsp. *camaldulensis* grown under rainout shelters with two watering regimes that resulted in differences in soil water content and across all four seasons that generated differences in environmental conditions. We used a robotic, high-throughput system to measure respiratory oxygen (O₂) uptake and its temperature dependence (Scafaro *et al.*, 2017). This system enabled 1900 individual measurements of respiration in the 10 genotypes for detailed assessment of the influence of genotypic variation, seasonal climate and watering treatments on respiration, a trait central to leaf function.

**Materials and Methods**

**Study design**

Genotypes were clones of mother trees naturally growing at 10 different sites along an aridity gradient within the geographic distribution of *Eucalyptus camaldulensis* Dehnh. subsp. *camaldulensis*, and were planted into a common garden at the Hawkesbury Institute for the Environment at Western Sydney University in Richmond, NSW (33° 36’ 38.7” S, 150° 44’ 22.2” E). An important plantation species worldwide (Eldridge *et al.*, 1994), *E. camaldulensis* is a sclerophyllous evergreen tree commonly found in permanent or ephemeral riverine sites across most of mainland Australia (Brooker & Kleinig, 1983), and the subspecies *camaldulensis* is distributed mainly throughout the Murray-Darling River basin of New South Wales and Victoria but may also be found in parts of Queensland and South Australia. *E. camaldulensis* is. Ten sites were chosen in New South Wales and Victoria to represent an aridity gradient, and ranged in mean annual temperature (MAT) from 13.8 to 19.5 °C and mean annual precipitation (MAP) from 262 to 813 mm (Table 1). At each site, seeds were collected from a single mother tree, taken to a glasshouse at the Hawkesbury Institute for the Environment, germinated and grown into seedlings. From the seedlings, clonal cuttings of similar sizes were taken, rooted, and grown in containers with potting mix
for ~2 months in a naturally lit nursery until average height and basal diameter reached 25 cm and 2 mm. The individual trees thus consist of clonal replicates of 10 genotypes from 10 unique provenances.

In a split block design, the individuals were planted into a common garden with six replicate blocks with two watering treatment plots within each block (a total of 120 individuals). Soil at the experimental site is in the Clarendon Formation (Chromosol), characterized by sandy loam with low organic matter content (0.7%), and moderate to low fertility. Each block consisted of a rainout shelter with aluminum frame 12 m long x 8 m wide x 8 m tall with a PVC sprinkler system. The shelter had transparent roof and side curtains that closed during rain events but left a 1m gap at the bottom to maintain air flow. See Drake et al. (2017) for more details. Each block contained two treatment plots, and each plot was 6 m x 8 m and bounded by a vertical barrier buried to 1.2 m depth to limit sub-soil water movement. On 29th May 2015, each plot was planted with a single clonal replicate of each genotype in random order at 1 m x 1 m spacing and surrounded by a single row of border plants to minimize edge effects. All of the plots received uniform irrigation for five months until watering treatments began on 15th November 2015.

The watering treatments were wet and dry, designed to receive irrigation approximately equal to MAP at the wet and dry edges of the subspecies distribution, interpolated using the ANUClimate 1.0 model of the Ecosystem Modelling and Scaling Infrastructure (Hutchinson et al., 2014). For the wet treatment, irrigation was scheduled to replicate daily precipitation of 1971. This year had the frequency, duration, and size of daily precipitation typical for years with annual total precipitation similar to average MAP at the wettest site during 1960 – 2014. Irrigation for the dry treatment had similar scheduled frequency but reduced daily precipitation amount to approximate MAP of the driest site. Soil moisture was monitored every 15 min for the duration of the experiment using soil moisture probes (Campbell Scientific, Townsville, QLD Australia) buried at depths of 30, 55 and 80 cm. On 20th February 2016, roughly three months after the treatments began, we ceased irrigation in the dry treatment because soil moisture differences between the treatments were negligible (Fig. 2). After cessation of irrigation, volumetric soil water content diverged between treatments and remained higher in the wet treatment (Fig. 2).

Leaf samples for trait measurements were collected in 2016 from every individual of all genotypes and treatments (120 individuals total). Fully expanded leaves were collected from the terminal end of branches exposed to the sun, over a three-day period at end of February (summer; day of year = 53), beginning of May (autumn; DOY = 122), middle of
July (winter; DOY = 197), and beginning of November 2016 (spring; DOY = 311). The leaves were collected 2-6 hours after sunrise, stored in cool, dark, and moist conditions for 30 minutes, and transported to a nearby laboratory for measurements of leaf traits.

Measurements of leaf metabolic traits and performance proxies

Leaf traits measured were LMA (g m⁻²), concentrations of N and P (Nmass and Pmass; mg g⁻¹), mass-based respiration rate in the dark at 25 °C (Rmass; nmol O₂ g⁻¹ s⁻¹), and Arrhenius activation energy as an index of Rmass temperature response (Eₐ; kJ mol⁻¹). We henceforth refer to them as leaf metabolic traits to distinguish from other related traits such as life span.

Respiration rate was measured as oxygen (O₂) uptake rate using a Q2 system (Astec-Global, Netherlands) on 50 mm² disks of detached leaves following the method of Scafaro et al. (2017) that allowed for automated measurements in controlled temperatures in the dark (see Supporting Information Methods S1). Respiration rate was measured on the four disks from a single leaf at four temperatures (15, 20, 25, and 30 °C), expressed on mass basis using dry mass of the disks, and fitted to the Arrhenius equation to estimate normalization constant (r₀ in nmol O₂ g⁻¹ dry mass s⁻¹) and activation energy (Eₐ). We report the respiration rate at 25 °C as Rmass. LMA was calculated from the leaf disks using dry mass of each disc after drying at 60 °C for at least three days. Nmass and Pmass were measured on a separate section of the same leaf used in the respiration measurements using the Kjeldahl digestion method. The leaf sections were oven-dried, ground, hot-digested in acid-peroxide, and analyzed calorimetrically using a flow injection system (QuikChem 8500; Lachat Instruments, Loveland, CO, USA).

For every individual tree, aboveground biomass (AGB) was estimated using height and basal diameter of the main stem measured monthly, and AGB was used to estimate relative growth rate (RGR) of aboveground biomass. AGB was estimated using an allometric equation for juvenile E. camaldulensis (Ounban et al., 2016): \( AGB = 0.033(DH^{0.959}) \), where \( D \) is stem diameter (cm), and \( H \) is plant height (m). Aboveground RGR was estimated from AGB with the classical approach, as average daily rate of the period between the monthly measurements (Hunt, 1982): \( RGR = \frac{\ln(AGB_2) - \ln(AGB_1)}{(t_2 - t_1)}. \)

Climate of genotype provenance

Climate of genotype provenance was characterized using individual and combined bioclimatic variables (Table 1). Bioclimatic variables were taken from WorldClim (downloaded from http://worldclim.org/version2) and represent annual and seasonal trends.
For individual climate variables, we used MAT and MAP taken from *WorldClim* and AI. AI was calculated as annual potential evapotranspiration divided by MAP. Annual potential evapotranspiration was taken from CGIAR-CSI (Zomer et al., 2007; Zomer et al., 2008), and was yearly mean of 1950-2000 modeled from *WorldClim* for a 30 arc sec grid containing the locations of genotype provenance (downloaded from [https://cgiarcsi.community/data/global-aridity-and-pet-database/](https://cgiarcsi.community/data/global-aridity-and-pet-database/)). For combined variables, we used principle component analysis to convert all 19 Bioclimatic variables, AI, plus longitude and latitude into three principle components (PC1, PC2, and PC3) that explained 92% of the variance in the climate variables. We also characterized climate dissimilarity between genotype provenance and experimental site by calculating a Euclidian distance (ED) on the three-dimensional PC space between the climates of provenance and the climate of the experimental site. See Supporting Information Methods S2 for the list of *WorldClim* variables and variable contributions to principle components.

**Statistical analysis**

Trait variation was analyzed using linear mixed effects model to quantify the relative contributions of genotypic variation and phenotypic plasticity. The mixed effects model was constructed with a trait as the dependent variable and all factors as random effects. The factors included were genotype, watering treatment, sampling date (as day of year), all possible two-way and three-way interactions among them, and experimental factors of replicate blocks and clonal individuals. We included the experimental factors in the model to account for the study design. See Supporting Information Methods S3 for model details. The model was used to partition the total variance in the dependent variable (a trait) among the factors. Variances ($\sigma^2$) associated with genotype – including two- and three-way interactions with treatment and sampling date – were defined as components of genotypic variation, and variances associated with treatment and season independent of genotype were defined as components of phenotypic plasticity. They were defined apart from variances associated with the rest of the parameters, plot, individual, and residual. The residual included four- and five-way interactions between all of the source and experimental factors, and reflected the variation from biological and methodological sources such as sampling scheme, measurement error, and physiological changes at the time scale of minutes to days.

The significance of the factors was examined with likelihood ratio tests comparing a
model with all of the factors against another without the factor of interest. The effect size ($r^2$) of the factor of interest was calculated with the method of Nakagawa and Schielzeth (2013) and Nakagawa et al. (2017). The relationship between trait value and climate of genotype provenance was determined by comparing models with and without climate as a factor. The models compared were similar to the model above but with climate variable as a fixed factor replacing genotype, and contained watering treatment, sampling date, plot, individual, and two- and three-way interactions between climate (where appropriate), treatment, and sampling date. Relationships between traits were quantified in terms of standardized major axis slopes and associated correlation coefficients.

Analyses were done in R (R Core Team, 2018) with R studio (RStudio Team, 2016); using packages dplyr (Wickham et al., 2017) and lubridate (Grolemund & Wickham, 2011) for general data preparation; lme4 (Bates et al., 2015), lmerTest (Kuznetsova et al., 2017), MuMIn (Bartoń, 2018) for analysis using mixed effects models; smatr (Warton et al., 2012) for standardized major axis slope fitting; and ggplot2 (Wickham, 2009) for visualization.

Results

Genotypic variation was a considerable component of the variation in AGB but only a minor fraction of the variation in leaf metabolic traits and RGR, even when genotypic variation in plastic responses (G x E) was accounted for (Fig. 3). This refuted the hypothesis that genotypic variation would be a substantial source of leaf metabolic trait variation among the 10 E. camaldulensis genotypes with geographically diverse provenances. Trait variation was attributed to the source factors genotype, watering treatment, sampling date (DOY), all possible two-way and three-way interactions among them, and experimental factors of clonal individuals and replicate plots (Table 2). Sources that were genotypic (genotype and its interactions with watering treatments and DOY) accounted for 31% of the variation in AGB but 14% of variation in LMA, 18% in $N_{mass}$, 7% in $P_{mass}$, 3% in $R_{mass}$, <1% in $E_A$, and 2% in RGR (variances for all model parameters are listed in Table 2). For genotypic variation to be substantial, within-genotype variation must have differed among the 10 provenances and have been considerably smaller than total variation across genotypes, assuming that measurement error affected all genotypes similarly. However, most genotypes had within-genotype trait variation as large as the total (experiment-wise) variation across genotypes (Fig. 4). Within-genotype variation included plastic responses to watering treatment and DOY, as well as experimental factors such as clonal replicates and measurement error. The
genotypic differences in AGB may have reflected the small genotypic variation in RGR or

genotypic variations in unmeasured traits such as tissue longevity. Initial size of the trees can
play a role as well, but clonal cuttings were selected for similarity in size before planting. Nevertheless, genotypic variation was more substantial and evident in AGB than in leaf metabolic traits.

For all leaf metabolic traits, the dominant source of variation was phenotypic plasticity in response to watering treatment and DOY (Fig. 3). Phenotypic plasticity to DOY, but not to watering treatment, had genotypic component (Table 2), partially supporting the hypothesis that genotypes differ in plastic responses. In our statistical model, the genotypic component of plasticity was represented by the interaction terms involving genotype (G x E). The interaction between genotype and DOY accounted for a relatively large fraction of the variation for LMA, N\text{mass}, and P\text{mass}, and a small fraction of the variation in AGB (Fig. 3, top; statistical significance is shown in Table 2). For LMA and P\text{mass}, the interaction term accounted for a greater fraction of variation than genotype alone (Fig. 3, top), suggesting that trait plasticity along sampling dates differed considerably among genotypes. Phenotypic plasticity to watering treatment did not vary among genotypes for all traits measured (Table 2). Three-way genotypic interaction term (genotype x watering treatment x DOY) explained minor trait variation for all leaf traits measured and performance proxies (Table 2). This suggests that although genotypes differ in the plastic response to DOY in LMA, N\text{mass}, and P\text{mass}, the genotypic variation in plastic response is only weakly related to growth and vegetative performance. A substantial fraction of variation in leaf metabolic trait values remained unexplained by genotypic variation or phenotypic plasticity (Fig. 3, bottom).

As genotypic sources contributed to a minor fraction of the variation in leaf trait and RGR values, leaf trait and RGR values could only be weakly related to the climate of genotype provenance. Indeed, we found little evidence of relationships between climate and leaf traits or performance proxies, refuting the hypothesis that genotypic variation would be related to climate of provenance even though genotypes were sourced from a wide range of climates. In comparing models with and without climate as a factor, none of leaf traits and performance proxies showed a significant relationship with any climate variable, except that genotypic variation in N\text{mass} was weakly related to PC3 representing seasonality ($p = 0.02$, $r^2 = 0.04$). The model comparison accounted for the effects of watering treatment, sampling date, plot, and clonal replicates. It also accounted for two- and three-way interactions between climate, treatment, and sampling date. Therefore, genotypic variation in phenotypic plasticity (G x E) was unrelated to climate of genotype provenance. We also tested the
relationship between the variables measured and climate of genotype provenance in a
bivariate comparison that did not consider watering treatment, sampling date, nor
experimental design. We found only weak correlations that disappeared in some DOY (see
Supporting Information Notes S1).

All genotypes showed large phenotypic plasticity in all leaf traits and performance
proxies (Fig. 5), and plasticity across DOY was a greater source than watering treatment (Fig.
3, top). DOY alone explained 48% of variation in LMA, 17% in N\text{mass}, 19% in P\text{mass}, 20% in
R\text{mass}, 17% in E\text{A}, 34% in AGB, and 81% in RGR. The plastic response across sampling dates
were somewhat cyclical for LMA, N\text{mass}, and P\text{mass} (Fig. 5), but not for R\text{mass}, E\text{A}, and RGR
that declined throughout the year, suggesting that the response was both seasonal and
developmental. By contrast, plastic response was smaller with watering treatment (Fig. 5,
Table 2). LMA was higher, and N\text{mass}, P\text{mass}, R\text{mass}, and AGB were lower under dry treatment,
but watering treatment was a minor and non-significant source of variation in E\text{A} and RGR
(Table 2). To assess drought stress, we examined stomatal conductance in relation to soil
water content in two genotypes from the wettest and driest sites, but found a marginal
relationship only during summer (see Supporting Information Notes S2).

Leaf trait-trait relationships across genotypes were consistent with leaf economic
spectrum and were maintained under plastic response to watering treatments and sampling
dates (Fig. 6). Trait-trait correlations were consistent with the leaf economic spectrum, with
each relationship being significant to our data overall at $p < 0.01$ ($r^2 = 0.14, 0.19, 0.13,$ and
0.39 respectively). Both watering treatment and sampling date affected leaf trait values, but
the changes in leaf trait values did not lead to major deviations in trait-trait correlations of
leaf economic spectrum (Fig. 6), supporting the hypothesis that intraspecific leaf trait-trait
relationships reflect the general patterns of the leaf economic spectrum.

Trait-trait relationships responded differently to watering treatment and sampling date
(Fig. 6). A change in a trait-trait relationship – resulting from plastic responses – can be
characterized as either a shift along the overall trend line or changes in the trend line itself (in
slope or elevation). The former suggests that a plastic response in one trait is proportional to,
and predictable from, a plastic response in another trait, while the latter suggests that trait
plastic responses are idiosyncratic. Under the two watering treatments, trait values mostly
shifted along the overall slope (Fig. 6, left column). For example, the values of LMA and
N\text{mass} shifted along the overall trend line ($p < 0.01$), proportionally increasing in LMA as
N\text{mass} decreased, without a significant change in the elevation ($p = 0.13$) or the slope ($p =
0.9$). An exception was P\text{mass}, which decreased in greater proportion relative to N\text{mass},
changing the slope of the relationship ($p = 0.01$), but the magnitude of change in slope was minor. By contrast, sampling date had a greater impact on the slope (and thus the elevation) of the trend lines of all pairwise relationships except for $P_{mass}$ vs $N_{mass}$ (at $p < 0.01$; Fig. 6, right column). Of the four sampling dates, winter (DOY 197) or spring (DOY 311) often deviated from the rest. Spring had distinct slope for $N_{mass}$ vs LMA ($p < 0.01$), and winter had distinct slope for $R_{mass}$ vs LMA ($p < 0.01$) and for $R_{mass}$ vs $N_{mass}$ ($p < 0.01$). In addition, though soil water content changed with sampling date (Fig. 2), plastic responses to sampling date did not follow plastic responses to watering treatment (Fig. 6). For example, soil water content increased from autumn to winter, as much as watering treatment did within winter, but plastic responses across autumn to winter resulted in different slopes for all trait combinations (Fig. 6, left column). Taken together, these results show that watering treatment had similar proportional effects on each leaf trait, but sampling date affected each leaf trait disproportionately.

Leaf traits were largely unrelated to RGR (Fig. 7), refuting the hypothesis that growth rate would be higher and increases when plastic responses occur towards certain trait combinations. As Figure 5 shows, RGR declined substantially along sampling dates, diverging from the cyclic trends in leaf traits LMA, $N_{mass}$, and $P_{mass}$. To account for this decline in testing if RGR was higher for individual trees with certain leaf trait combination, we standardized all leaf traits, RGR, and AGB within a sampling date into z-score (signed number of standard deviation away from the mean). We included AGB in the analysis since AGB may reflect accumulated differences in RGR as stated previously. The z-scores of RGR and AGB were compared with leaf trait combinations in principle component analysis, which is multivariate and statistically analogous to standardized major axis regression used to analyze trait-trait relationships (Warton et al., 2006). As expected from the leaf economic spectrum, LMA was negatively related to $N_{mass}$, $P_{mass}$, and $R_{mass}$, but AGB and RGR were both orthogonal to the leaf traits (Fig. 7), showing little to no relationship between a combination of leaf traits and growth rate.

**Discussion**

*E. camaldulensis* is widely distributed in Australia across a broad range of climates, and we expected adaptive divergence among the genotypes and thus substantial genotypic variation in leaf metabolic traits associated with the climate of provenance. Previous studies have shown genotypic variation in growth rate of *E. camaldulensis* from diverse provenances planted in a common garden (Criddle et al., 2000), leaf dry mass and N concentration...
differences among genotypes of a grass species *Dactylis glomerata* (Albert et al., 2010), and a relationship between needle lifespan and local climate within several species of gymnosperm trees (Reich et al., 2014). Thus, we hypothesized 1) substantial genotypic variation in leaf trait variation related to climate, 2) considerable genotypic differences in plastic response of leaf metabolic traits, 3) trait-trait relationships consistent with leaf economic spectrum, and 4) relationships between leaf metabolic traits and growth rate. However, we found very little evidence to support the hypotheses except for hypothesis three, challenging our premise that the genotypes of *E. camaldulensis* is genetically diverse, well adapted to the climate of their provenance, and possess leaf trait plasticity that increases performance in their home environment. We discuss the elements of the premise below.

Genetic diversity may be small in *E. camaldulensis* at the subspecies level. *Eucalyptus camaldulensis* prefers riparian habitats, and genotypes at different sites may experience soil moisture or microclimates that are less diverse than the climate data suggest. At the species level, *E. camaldulensis* populations differ enough in morphology and genetic material examined using DNA profiling techniques for classification into seven subspecies (Pryor & Byrne, 1969; Butcher et al., 2008; McDonald et al., 2009). Australia-wide diversity in the genetic material of the species has been linked to environmental variables that included geography and climate (Butcher et al., 2008; Dillon et al., 2014). However, based on fossil records, current distribution patterns, and distribution patterns of vertebrates, Butcher et al. (2008) argued that genotypic variation in *E. camaldulensis* species arose out of range expansion and contraction that reduced gene flow among the subspecies, and that species-wide variability is mostly a result of genetic drift and less of selection. All of the genotypes in our study belong to the subspecies *camaldulensis* with a distribution range containing most of Murray-Darling basin of southeastern Australia. Genetic drift can reduce genotypic variation in a population when gene flow is low (Star & Spencer, 2013).

However, our results suggest that genetic diversity in subspecies *camaldulensis* can be substantial as reflected in the genotypic variation in AGB. Genotypic variation explained 31% of variance in AGB but 20% or less of variance in leaf traits (Fig. 3). Leaf metabolic traits may not have had enough time to reflect genetic variation (Germino et al., 2019). In contrast to leaf traits, AGB may be more indicative of genetic diversity because AGB is an outcome that aggregates genotypic variations in not only leaf traits but also other unmeasured traits that affect biomass accumulation, such as hydraulic traits, root characteristics, and tissue turnover rates. AGB compounds the effects genotypic variations over longer timescales also, and small genotypic variation in above ground RGR can lead to large differences in
AGB. While this is possible, both RGR and AGB were mostly unrelated to leaf traits within a
sampling date (Fig. 7), and unlike AGB, RGR showed very little genotypic variation overall.

The lack of strong relationship between leaf traits and performance proxies suggests
that genotypic variation in AGB may reflect genetic diversity in some unmeasured traits
rather than leaf metabolic traits. In a common garden under drought experiment, seedlings of
different *E. camaldulensis* subspecies showed differential response appropriate to climate of
provenance, such as greater allocation of dry matter to roots in subspecies from semi-arid
climates than from humid tropics (Gibson *et al.*, 1995). Genotypic variation in several
photosynthetic traits was clearly related to climate of provenance among genotypes collected
Australia-wide (Dillon *et al.*, 2018), genotypes from seven provenances across Australia
differed in respiratory response to experimental changes in soil salt content and pH (Marcar
*et al.*, 2002), and genotypes exposed to elevated atmospheric CO₂ differed in photosynthetic
nitrogen use efficiency and carbon allocation to roots for possibly greater nutrient uptake
(Aspinwall *et al.*, 2018). These studies suggest that genetic diversity in *E. camaldulensis* is
adaptive, but for select traits that depend on growing conditions. However, genotypic
variation in leaf traits and performance proxies were unrelated to climate of genotype
provenance in this study. The genotypes of *E. camaldulensis* subspecies camaldulensis may
well be diverse and well adapted to their home environment, but climate is likely a weak if
not unimportant driver of the genetic diversity.

Our results support the view that intraspecific trait variation weakly follows global
trends across climate gradients (as in shallower slope compared to global trends) because
traits vary less within than across species and global trends across species are driven by
species turnover in addition to intraspecific variation (Ackerly & Cornwell, 2007). Our
premise, that climate gradients drove genotypic variation in leaf functional traits, was based
on trends seen in global data on naturally growing leaves collected in the field. Wright *et al.*
(2004) found that species-level values of LMA increased with MAT and decreased with
MAP among, and Atkin *et al.* (2015) reported that temperature-normalized R\textsubscript{mass} decreased
with aridity index and increasing MAT. We found that these global trends are reflected
within species weakly if at all, and other studies have found that intraspecific variation is a
minor component of community wide trait variation. Intraspecific variation, from combined
effects of genotypic variation and phenotypic plasticity, on average contributed less than a
quarter of community wide variation in LMA across a gradient of water availability in
California (Cornwell & Ackerly, 2009), and about half of community wide variation in LMA
and N content per leaf area across Australian rangelands (Dong *et al.*, 2017).
Aridity was a key climate variable in selecting genotype provenances, and watering treatments were designed to replicate wettest and driest MAP of the subspecies geographical distribution. Watering treatment caused small plastic responses, but the effect was mostly uniform across genotypes. However, watering treatment did not affect RGR (Table 2) and stomatal conductance (Supporting Information Notes S2), suggesting that the plants in the dry treatment were unstressed, even though *E. camaldulensis* prefers riparian habitats and may be sensitive to changes in soil moisture. The plastic responses were proportional among the traits and did not vary with genotype and thus climate of genotype provenance, and watering treatment may have to be severe to elicit genotypic differences in the plastic responses. These responses may instead be indirect effects of watering treatment, through soil decomposition and nutrient availability rather than physiology. Soil decomposition generally declines under dry conditions, reducing nutrient availability (Orchard & Cook, 1983; Davidson *et al.*, 1998). Soil nutrient availability strongly affects plant strategies for nutrient acquisition, and lower nutrient availability tends to shift allocation to roots in favor of leaves and decreasing tissue nutrient concentration (Chapin, 1980; Aerts & Chapin, 2000). Root traits in turn are coordinated with leaf traits (Tjoelker *et al.*, 2005; Reich, 2014), and global surveys across species show correlation between leaf traits and measures of soil nutrient availability, with LMA decreasing with soil dryness, and leaf N and P concentrations decreasing with soil N and P content (Ordonez *et al.*, 2009; Poorter *et al.*, 2009).

Sampling date was the greatest source of variation in leaf traits and performance proxies, likely reflecting both cyclic changes in physiology with season and aperiodic changes with development as individual plants grow in size. We attempted to separate the effect of size and season using size and seasonal temperature corrected trait values, and found that plastic responses to changes in size were much less than the response to seasonal changes in temperature (see Supporting Information Notes S3). However, Size corrected trait values reduced the variability from sampling date in trait-trait relationships. Trait changes with size within species has been observed (Cornelissen, 1999; Cavaleri *et al.*, 2010), but are rare when trait values of different species are compared with maximum size (Price *et al.*, 2014). Trait responses to seasonal changes in temperature have also been observed (Reich *et al.*, 2016), but some of the patterns we observed contradicted the previous studies. Measured at a common temperature, R\text{mass} was positively related to seasonal changes in temperature (Supporting Information Notes S3) even though studies of acclimation in respiration rate often find a negative relationship (Atkin & Tjoelker, 2003; Reich *et al.*, 2016). Though the cause is unclear, our results show that phenotypic plasticity along sampling date is a critical
driver of intraspecific variation in leaf metabolic traits at this stage of tree growth.

In this study, phenotypic plasticity, rather than genotypic variation, was the greater source of variation in key leaf traits (LMA, N and P concentrations, mass-based respiration rate, and temperature response of respiration) in *E. camaldulensis*, despite considerable genotypic variation reflected in aboveground biomass. Genotypic variation was unrelated to climate of genotype provenance, and leaf traits measured were unrelated to performance proxies. This suggests that leaf traits are low in heritability because intraspecific variation in leaf metabolic traits mostly reflect phenotypic plasticity rather than genetic diversity among genotypes, and leaf metabolic traits may be unrelated to performance at this stage of tree growth. Phenotypic plasticity is an important component when examining intraspecific variation in leaf metabolic traits for this species, while climate of genotype provenance appears less relevant.

Acknowledgements

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Author Contributions

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The following Supporting Information is available for this article:

**Methods S1.** Description of automated measurement of leaf respiration rate.

**Methods S2.** Principal component analysis of WorldClim variables.

**Methods S3.** Definitions of the terms included in linear mixed effects model.

**Notes S1.** Simple correlation between traits and climate variables.

**Notes S2.** Stomatal conductance under watering treatment.

**Notes S3.** Effects of size and season on trait values.

**Table S1.** List of variable name and abbreviation used to characterize the climate of genotype provenance.

**Figure S1.** Contributions of each climate variables to first three principle components.

**Figure S2.** Climate of genotype provenance represented in first two principle components.

**Figure S3.** Relationship between trait value and aboveground biomass and seasonal change in temperature.
Tables

Table 1. Location and climate of provenances of the 10 genotypes and the common garden (Richmond, NSW Australia), showing longitude (Lon, Northing), latitude (Lat, Easting), altitude (Alt), mean annual temperature (MAT), mean annual precipitation (MAP), and aridity index (AI, calculated as MAP / potential evaporation), first three principle components (PC1, PC2, PC3) as aggregate measures of climate after dimensionality reduction of WorldClim Bioclimatic variables, and climate dissimilarity calculated as Euclidean distance (ED) between the climate of provenances and Richmond on the three-dimensional principal component space.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Lon (°E)</th>
<th>Lat (°N)</th>
<th>Alt (m)</th>
<th>MAT (°C)</th>
<th>MAP (mm)</th>
<th>AI</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
<th>ED</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>141.52</td>
<td>-35.57</td>
<td>83</td>
<td>15.6</td>
<td>362</td>
<td>0.26</td>
<td>-1.29</td>
<td>-3.39</td>
<td>0.25</td>
<td>9.29</td>
</tr>
<tr>
<td>2</td>
<td>143.11</td>
<td>-36.52</td>
<td>149</td>
<td>14.8</td>
<td>450</td>
<td>0.35</td>
<td>0.90</td>
<td>-2.57</td>
<td>-0.07</td>
<td>7.89</td>
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<td>3</td>
<td>148.06</td>
<td>-35.04</td>
<td>396</td>
<td>14.3</td>
<td>813</td>
<td>0.61</td>
<td>3.71</td>
<td>2.19</td>
<td>-3.03</td>
<td>7.17</td>
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<td>4</td>
<td>141.43</td>
<td>-37.02</td>
<td>178</td>
<td>13.8</td>
<td>603</td>
<td>0.49</td>
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<td>2.45</td>
<td>7.65</td>
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<tr>
<td>5</td>
<td>147.11</td>
<td>-31.33</td>
<td>164</td>
<td>18.7</td>
<td>468</td>
<td>0.31</td>
<td>-3.66</td>
<td>2.20</td>
<td>0.76</td>
<td>7.86</td>
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<td>6</td>
<td>144.51</td>
<td>-34.31</td>
<td>81</td>
<td>17.2</td>
<td>357</td>
<td>0.25</td>
<td>-2.71</td>
<td>-1.16</td>
<td>-1.28</td>
<td>9.40</td>
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<tr>
<td>7</td>
<td>146.47</td>
<td>-36.36</td>
<td>175</td>
<td>14.5</td>
<td>693</td>
<td>0.55</td>
<td>3.39</td>
<td>0.43</td>
<td>-1.56</td>
<td>6.44</td>
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<tr>
<td>8</td>
<td>146.53</td>
<td>-36.06</td>
<td>154</td>
<td>15.1</td>
<td>611</td>
<td>0.47</td>
<td>1.78</td>
<td>-0.03</td>
<td>-1.61</td>
<td>6.92</td>
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<tr>
<td>9</td>
<td>147.09</td>
<td>-33.06</td>
<td>192</td>
<td>17.6</td>
<td>477</td>
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<td>2.20</td>
<td>-0.46</td>
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<tr>
<td>10</td>
<td>143.39</td>
<td>-31.28</td>
<td>77</td>
<td>19.5</td>
<td>262</td>
<td>0.16</td>
<td>-6.43</td>
<td>0.12</td>
<td>0.59</td>
<td>10.96</td>
</tr>
<tr>
<td>Richmond</td>
<td>150.75</td>
<td>-33.61</td>
<td>20</td>
<td>17.2</td>
<td>897</td>
<td>0.61</td>
<td>3.35</td>
<td>3.76</td>
<td>3.96</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Table 2. Overall mean, variance attributed to each factor, and total variance for each trait.
The values were taken from mixed effects model, with all factors as random effects. The
factors were genotype (G), watering treatment (W), sampling date (day of year, D), and their
interactions. The model also included experimental factors of replicate plot and clonal
individual as random factors to control for experimental design. Bolded values indicate
significance of the factors, based on model selection method using likelihood ratio test and
AIC. The values of aboveground biomass (AGB) were natural log transformed for the
analysis. Back transformed, mean AGB is 729 g. The units were g m\(^{-2}\) for leaf mass per area
(LMA), mg g\(^{-1}\) for leaf N (N\(_{\text{mass}}\)) and P concentrations (P\(_{\text{mass}}\)), nmol O\(_2\) g\(^{-1}\) s\(^{-1}\) for leaf dark
respiration rate measured at 25 °C (R\(_{\text{mass}}\)), kJ mol\(^{-1}\) for activation energy (E\(_A\)) as a proxy of
temperature response of R\(_{\text{mass}}\), mg g\(^{-1}\) day\(^{-1}\) for relative growth rate of aboveground biomass
(RGR), and g for AGB (before log transformation).

<table>
<thead>
<tr>
<th>Factor</th>
<th>LMA</th>
<th>N(_{\text{mass}})</th>
<th>P(_{\text{mass}})</th>
<th>R(_{\text{mass}})</th>
<th>E(_A)</th>
<th>RGR</th>
<th>AGB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>103</td>
<td>26</td>
<td>1.4</td>
<td>21</td>
<td>67</td>
<td>9.0</td>
<td>6.6</td>
</tr>
<tr>
<td>Genotype</td>
<td>39</td>
<td>3.8</td>
<td>0</td>
<td>1.3</td>
<td>0</td>
<td>1.1</td>
<td>0.43</td>
</tr>
<tr>
<td>Watering</td>
<td>24</td>
<td>2.7</td>
<td>0.030</td>
<td>1.5</td>
<td>1.2</td>
<td>0.22</td>
<td>0.12</td>
</tr>
<tr>
<td>Day of year</td>
<td>290</td>
<td>6.1</td>
<td>0.043</td>
<td>9.6</td>
<td>74</td>
<td>75</td>
<td>0.48</td>
</tr>
<tr>
<td>G x W</td>
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<td>0.68</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td>0.21</td>
<td>0</td>
</tr>
<tr>
<td>G x D</td>
<td>49</td>
<td>2.0</td>
<td>0.007</td>
<td>0.1</td>
<td>0</td>
<td>0.34</td>
<td>0.014</td>
</tr>
<tr>
<td>W x D</td>
<td>7.1</td>
<td>0.74</td>
<td>0.010</td>
<td>0</td>
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<td>0.42</td>
<td>0.007</td>
</tr>
<tr>
<td>G x W x D</td>
<td>0</td>
<td>0</td>
<td>0.008</td>
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<tr>
<td>Plot</td>
<td>25</td>
<td>4.2</td>
<td>0.018</td>
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<td>Clone</td>
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<tr>
<td>Residual</td>
<td>140</td>
<td>15</td>
<td>0.094</td>
<td>32</td>
<td>310</td>
<td>14</td>
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<tr>
<td>Total</td>
<td>607</td>
<td>36.5</td>
<td>0.226</td>
<td>48.2</td>
<td>429</td>
<td>91.9</td>
<td>1.41</td>
</tr>
</tbody>
</table>
Figure legends

**Figure 1.** Graphical representation of the hypothesis. Grey boxplot represents within genotype variation in trait value, and black arrows indicate the direction and magnitude of phenotypic plasticity in response to watering treatment and across sampling dates. The inset shows bivariate trait relationship predicted by leaf economic spectrum, and the plasticity response along the spectrum.

**Figure 2.** Dry treatment decreased soil water fraction. Volumetric soil water fraction was measured throughout study period (2016) for wet and dry watering treatments at three depths of 30, 55 and 80 cm. The soil water fraction was lower in dry treatment especially in winter (day of year = 197) and spring (DOY = 311). The shaded areas indicate sampling periods.

**Figure 3.** Genotypic sources constituted roughly a third of variance in aboveground biomass (AGB) but at most a fifth in leaf traits and relative growth rate of aboveground biomass (RGR). Leaf traits were leaf mass per area (LMA), concentrations of N (N\text{mass}) and P (P\text{mass}), leaf dark respiration rate measured at 25 °C (R\text{mass}), and activation energy (E\text{A}) as a proxy of temperature response of R\text{mass}. The variation in trait values was attributed to different sources using mixed effects models for each trait with all sources included as random factors. The sources examined were genotype, watering treatment, sampling date (DOY), their interactions, and the experimental factors of plots and clonal replicates. For simplicity, the sources were grouped into genotype, interactions with genotype, DOY, watering treatment, and other (top plot). In the bottom plot, the other sources were further partitioned in to water and DOY interaction, replicate plots, clonal individuals, and residual. AGB was log transformed.

**Figure 4.** Leaf trait values and relative growth rate (RGR) varied within genotype much more
than among genotypes. Within genotype trait values include data from both watering
treatments and all seasons. The distribution of trait values is shown as violin plot for each and
all genotypes. Violin plot combines kernel density plot and boxplot. Kernel density estimate
is drawn as shaded area, showing a non-parametric estimate of probability density. Boxplot
shows median, inter-quartile range, 95% confidence interval of the median, and outliers. The
genotypes are ordered on ascending aboveground biomass (AGB). Leaf traits were leaf mass
per area (LMA), concentrations of N (N_{mass}) and P (P_{mass}), leaf dark respiration rate measured
at 25 °C (R_{mass}), and activation energy (E_A) as a proxy of temperature response of R_{mass}.

**Figure 5.** Changes in trait value with sampling date (day of year) were somewhat cyclic for
leaf mass per area (LMA), and concentrations of N (N_{mass}) and P (P_{mass}), but not for leaf dark
respiration rate measured at 25 °C (R_{mass}), and activation energy (E_A) as a proxy of
temperature response of R_{mass}, aboveground biomass (AGB), and relative growthrate of AGB
(RGR). Mostly leaf trait values and AGB decreased slightly under dry watering treatment,
but E_A and RGR did not (Table 2). The colored lines show arithmetic mean within genotype,
and black lines show arithmetic mean among genotypes. Sampling dates were day of year
(DOY) 53, 122, 197, 311, and correspond to summer, autumn, winter, and spring.

**Figure 6.** Plastic response to watering treatment (left column) generally followed leaf
economic spectrum, but plastic response along sampling date did not (right column). Leaf
traits examined were leaf mass per area (LMA), concentrations of N (N_{mass}) and P (P_{mass}), and
leaf dark respiration rate measured at 25 °C (R_{mass}). The lines are drawn with standardized
major axis regression, and are colored if either slope or the intercept differs significantly
among the groups. The axes are in log scale. The ellipses are drawn at 95% confidence level
under multivariate normal distribution. Sampling dates (day of year; DOY) 53, 122, 197, and
correspond to summer, autumn, winter, and spring, and the colors for DOY qualitatively shows increasing aridity, from navy to red, based on soil water content shown on Fig. 2.

Figure 7. Leaf traits were largely unrelated to relative growth rate (RGR) and aboveground biomass (AGB). Each individual tree (circles) are projected onto a plane defined by principle component dimension one and two. The arrows represent the relationship between traits considered and the direction and the quality of their representation in the principle component plane. The traits considered were RGR, AGB, leaf mass per area (LMA), concentration of N (N) and P (P), and mass based leaf dark respiration measured at 25 °C (R). All traits considered were natural log transformed to reduce the influence of extreme values, and standardized within a sampling date into z-score (signed number of standard deviation away from the mean).
Figure 1. Graphical representation of the hypothesis. Grey boxplot represents within genotype variation in trait value, and black arrows indicate the direction and magnitude of phenotypic plasticity in response to watering treatment and across sampling dates. The inset shows bivariate trait relationship predicted by leaf economic spectrum, and the plasticity response along the spectrum.
Figure 2. Dry treatment decreased soil water fraction. Volumetric soil water fraction was measured throughout study period (2016) for wet and dry watering treatments at three depths of 30, 55 and 80 cm in each plot. Lines show mean soil water fraction for each treatment and depth. Yellow ribbon spans daily minimum and maximum temperature (°C) taken at nearby weather station (data taken from Australian Bureau of Meteorology). The soil water fraction was lower in dry treatment especially in winter (day of year = 197) and spring (DOY = 311). The shaded areas indicate sampling periods.
Figure 3. Genotypic sources constituted roughly a third of variance in aboveground biomass (AGB) but at most a fifth in leaf traits and relative growth rate of aboveground biomass (RGR). Leaf traits were leaf mass per area (LMA), concentrations of N (N_{mass}) and P (P_{mass}), leaf dark respiration rate measured at 25 °C (R_{mass}), and activation energy (E_A) as a proxy of temperature response of R_{mass}. The variation in trait values was attributed to different sources using mixed effects models for each trait with all sources included as random factors. The sources examined were genotype, watering treatment, sampling date (DOY), their interactions, and the experimental factor of replicate block (see Table 2 for factors included and variance, \sigma^2, explaine
by each factors). For simplicity, the sources were grouped into genotype, interactions with
genotype, DOY, watering treatment, and other (top plot). In the bottom plot, the other sources
were further partitioned in to water and DOY interaction, replicate blocks, and residual. AGB
was log transformed.
Figure 4. Trait values varied within genotype much more than among genotypes. Within genotype trait values include data from both watering treatments and all seasons. The
distribution of trait values is shown as violin plot for each and all genotypes. Violin plot combines kernel density plot and boxplot. Kernel density estimate is drawn as shaded area, showing a non-parametric estimate of probability density. Boxplot shows median, inter-quartile range, 95% confidence interval of the median, and outliers. The genotypes are ordered on ascending AGB.
Figure 5. Changes in trait value with sampling date (day of year) were somewhat cyclic for LMA, N$_{mass}$, and P$_{mass}$, but not for others, and mostly trait values decreased slightly under dry watering treatment. The colored lines show arithmetic mean within genotype, and black lines show arithmetic mean among genotypes. Sampling dates were DOY 53, 122, 197, 311, and correspond to summer, autumn, winter, and spring.
**Figure 6.** Plastic response to watering treatment (left column) generally followed leaf economic spectrum, but plastic response along sampling date did not (right column). The lines are drawn with standardized major axis regression, and are colored if either slope or the intercept differs significantly among the groups. The axes are in log scale. The ellipses are drawn at 95% confidence level under multivariate normal distribution. Sampling dates (DOY) 53, 122, 197, and 311, correspond to summer, autumn, winter, and spring, and the colors for DOY qualitatively shows increasing aridity, from navy to red, based on soil water content shown on Fig. 2.
Figure 7. Leaf traits were largely unrelated to RGR and AGB. Each individual tree (circles) are projected on to a plane defined by principle component dimension one and two. The arrows represent the relationship between traits considered and the direction and the quality of their representation in the principle component plane. The traits considered were RGR, AGB, LMA, N_{mass} (N), P_{mass} (P), and R_{mass} (R). All traits considered were natural log transformed to reduce the influence of extreme values, and standardized within a sampling date into z-score (signed number of standard deviation away from the mean).
New Phytologist Supporting information

Article title: Linking intraspecific trait variation to phenotypic plasticity and genotypic variation in *Eucalyptus camaldulensis*

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We explored the variation in leaf dark respiration rate among leaves within an individual canopy. Leaf dark respiration rates were measured on one leaf every two leaf nodes along two branches for a total of eight leaves per individual tree. The measurements were taken at 25 °C and expressed on leaf area and dry mass basis. The branches were in the top 1/3 of an individual tree, and 3 to 6 individuals were sampled for two genotypes from provenances with the coldest and hottest mean annual temperature (genotypes 3 and 10 in Table 1). Respiration rates were measured in February and again in July 2016. Respiration rate on leaf area and dry mass basis declined along leaf nodes toward the base of the branch (both at $p < 0.01$; Fig. S1), but similarly for both genotypes regardless of season or watering treatment (slope of decline did not change with genotype; $p = 0.11$ for area basis, 0.67 for dry mass basis). The decrease was more pronounced in summer (February) than in winter (July) for mass based respiration rate ($p < 0.01$), but were similar for area based rate ($p = 0.11$). For both mass- and area-based rates, the coefficient of variation did decline along the nodes toward the base in control watering treatment for both genotypes in summer ($p < 0.02$), but it did not change under all other cases ($p > 0.27$; $p > 0.18$ for interactions).
Methods S2

We used the method of Scafaro et al. (2017). Four leaf disks of 50 mm² each were collected from middle lamina of a single leaf using an 8-mm diameter cork-borer. Each disk was placed into a 2 mL vial, containing 100 μL of water to prevent leaf disk desiccation, and sealed with a gas-tight cap containing a gas-phase oxygen-sensitive metal-salt fluorophore. The disks were allowed to equilibrate to the measurement conditions for 30 mins and then repeatedly measured every 3 mins over the subsequent 60 min period using a Q2 system (Astec-Global, Netherlands). The measurements tracked the decline in O₂ concentration as % of air (100%) and N₂ (0%) standards. The measurements were used to calculate leaf respiration rate in the dark \( R_{\text{dark}} \) (mol O₂ disk⁻¹ s⁻¹) as \( R_{\text{dark}} = \left( \frac{P_O S}{RT} \right) \), where \( P_O \) is partial pressure of O₂ in kPa (set at 20.95% of atmospheric pressure), \( V \) is volume of the sample vial (1.9 cm³), \( S \) is the slope of decline in O₂ concentration relative to the air and N₂ standards (in % of air standard per second), \( R \) is the universal gas constant 8314 (cm³ kPa K⁻¹ mol⁻¹), and \( T \) is the measuring temperature in Kelvin.
Methods S3

Climate of genotype provenance was characterized using Bioclimatic variables were taken from WorldClim (Fick and Hijmans 2017), aridity index (AI; annual potential evapotranspiration divided by mean annual precipitation), longitude, and latitude (Table S1). They were reduced to 3 principle components, and the contribution of top 15 variable to each principle component (PC1, PC2, PC3) is shown in Fig. S2. Three principle components explained 92% of total variance in climate variables, but no single variable defined the climate of provenance. Twelve variables, including MAT, MAP, and AI, contributed more than expected (4.5 % if all variables contributed equally) to the first principle component dimension (PC1) with none contributing more than 8%. PC1 explained 51% of the total variance. Nine variables, including temperature and precipitation of the wettest and driest quarters, contributed more than expected to the second dimension (PC2), and PC2 explained 26% of the total variance. The third dimension (PC3) explained 16%, and eight variables including seasonality measures of temperature and precipitation, contributed to PC3. The analysis of climate variables showed that the climate of the experimental site (Richmond) is at the edge of the distribution of the climates of genotype provenance defined by the first two dimensions (Fig. S3).
Methods S4

We used the model of the form

\[ y_{ijklm} = a_i + b_{ij} + c_{ik} + d_{il} + (bc)_{ijk} + (bd)_{ijl} + (bcd)_{ijkl} + (cd)_{kl} + g_{im} + \varepsilon_{ijklm}, \]

where observation \( y_{ijklm} \) is of trait \( i \), from genotype \( j \), watering treatment \( k \), sampling date \( l \), and plot \( m \). The parameter \( a_i \) is the experiment-wise mean for trait \( i \) (where \( i = 1 \ldots 7 \)), and \( b_{ij}, c_{ik}, d_{il}, \) and \( g_{im} \) are the deviations from the mean for genotype \( j \) (\( j = 1 \ldots 10 \)), treatment \( k \) (\( k = 1 \) or 2), sampling date \( l \) (\( l = 1 \ldots 4 \)), and replicate block \( m \) (\( m = 1 \ldots 6 \)) respectively. The parameters \((bc)_{ijk}, (bd)_{ijl}, \) and \((bcd)_{ijkl}\) are interaction terms involving genotype, \((cd)_{kl}\) is an interaction between treatment and sampling date, and \( \varepsilon_{ijklm} \) is the residual. We included replicate block in the model to account for the study design. All of the model parameters except \( a_i \) are assumed to be random with \( \mathcal{N}(0, \sigma^2) \). Variances \( (\sigma^2) \) associated with parameters involving genotype – \( b_{ij}, (bc)_{ijk}, (bd)_{ijl}, \) and \((bcd)_{ijkl}\) – were defined as components of genotypic variation, and variances associated with treatment and season – \( c_{ijk}, d_{ijkl}, \) and \((cd)_{kl}\) – were defined as components of phenotypic plasticity. The residual \( \varepsilon_{ijklm} \) includes other three- and four-way interactions involving plot. Values of AGB were natural log transformed before analysis. The following R code was used to fit the model and check assumptions:

```r
# model factors
eq <- "~ (1|geno) + (1|treat) + (1|doy) + (1|plot) + (1|geno:doy) + (1|geno:treat) + (1|treat:doy) + (1|geno:treat:doy)"

# function to fit model
varpart <- function(y, equation = eq, DF = dat) {
  require(lme4)
}

# specify model components
f <- as.formula(paste(y, equation))

# fit the model with lmer function and print the summary
m <- do.call("lmer", list(f, quote(DF)))

# get variance
out <- get_var(m)
```
out$trait <- y

return(out)
}

# fit model to all traits
all <- lapply(list("LMA", "RM", "EA", "N", "P", "RGR", "log(AGB)"), varpart)

# function to plot residuals
qq <- function(m) {
  par(mfrow = c(1, 2))
  plot(predict(m), residuals(m), main = m@call[['formula']][[2]])
  qqnorm(residuals(m))
  qqline(residuals(m))
  par(mfrow = c(1,1))
}

# plot residuals for random effects
qqeach <- function(m) {
  require(ggpubr)
  a <- plot(m, doy~residuals(.))
  b <- plot(m, geno~resid(.))
  c <- plot(m, treat~resid(.))
  d <- plot(m, plot~resid(.))
  y <-m@call[['formula']][[2]]
  gridExtra::grid.arrange(a, b, c, d, nrow = 2, top = text_grob(y))
  print(plot(m, as.formula(paste0(y, " ~ fitted(.) | geno:treat")), abline = c(0,1)))
  print(plot(m, as.formula(paste0(y, " ~ fitted(.) | geno:doy")) , abline = c(0,1)))
  print(plot(m, as.formula(paste0(y, " ~ fitted(.) | doy:treat")) , abline = c(0,1)))
  print(plot(m, as.formula(paste0(y, " ~ fitted(.) | geno:treat:doy")) , abline = c(0,1)))
}

This model was overfitted as indicated by some factors having zero or near zero variance
(main text Table 2). Based on model selection with likelihood ratio test and AIC (main text),
we further reduced the number of factors in the model, and the results are reported in Table S2. These results differ from Table 2 only very slightly and did not affect our interpretation nor conclusion because the further reduced model essentially re-partitioned the near zero variance attributed to now excluded factors.

We used untransformed values for traits other than AGB (including RGR) for ease of interpretation. It is difficult to interpret the variance of natural log (or log) transformed variable, and estimated variance cannot be easily back transformed. This resulted in slight violation of the assumption of normality. Thus, we repeated the model analysis using natural log transformed trait values, and the results were very similar whether transformed or untransformed values were used. The results are shown in Fig. S6, the same format of Fig. 3.
The correlation between a climate variable and a trait was examined with Pearson’s correlation coefficient. In this analysis, no trait except mass based leaf dark respiration rate measured at 25 °C (Rmass) was correlated with mean annual temperature (MAT), mean annual precipitation (MAP), or aridity index (AI) at \( p < 0.05 \). Rmass was correlated with MAT, MAP, AI, and aggregated climate variables (PC1 and PC2), but only weakly at Pearson’s \( r = 0.1 \) or -0.1. Rmass was positively correlated with the MAT of provenance in this study, in contrast to the previous finding that Rmass was negatively correlated with MAT when comparing species from multiple biomes growing in the field (Atkin et al., 2015). Some traits were weakly correlated to aggregate variables for climate: leaf mass per area (LMA) was correlated with PC2 and climate dissimilarity (ED), leaf N concentration (Nmass) with aggregated climate variable (PC3), and leaf P concentration (Pmass) with ED, but the correlations were weak with \( r \) below 0.2. The variables activation energy (EA), relative growth rate (RGR) and aboveground biomass (AGB) showed no significant correlation with any climate variables. When compared within sampling date, LMA, Nmass, Pmass, and Rmass were more strongly correlated with climate (at most \( r = 0.3 \) or -0.3), but the correlation in one sampling date disappeared in the rest, suggesting that it was at best ephemeral if not spurious. Neither leaf metabolic traits nor performance proxies showed consistent correlations with climate variables across seasons. The variables EA, AGB, and RGR showed no significant correlation with climate in any season except for RGR with PC3 in spring (\( r = 0.2 \)).
Notes S2

To examine the direct effect on physiology, we examined the response of stomata to soil water content to test if the watering treatment caused stress. Stomatal conductance was measured in the field using Licor-6400 (Licor 6400XT, Licor Biosciences, Nebraska USA) on two genotypes from the wettest and driest MAP sites (genotypes 3 and 10), during February (summer), May (autumn), and July (winter). Stomatal conductance was compared against soil water fraction (measured at 80 cm depth averaged over 10 days prior to measurement of stomatal conductance) in a linear model with genotype, treatment, and measurement date as additional factors. Stomatal conductance increased 0.63 mol H₂O m⁻² s⁻¹ for very 10 % increase in the soil water fraction only in dry treatment during February regardless of genotype (p < 0.01; Fig. S4). Otherwise, stomatal conductance was unrelated to soil water fraction regardless of genotype, treatment, or measurement date (p = 0.17) but was higher in wet treatment (p < 0.01; Fig. S4). These results suggest that plants were generally unstressed by reduced soil water fraction but was affected by watering treatments through unknown secondary factors.
Notes S3

We examined the effects of size by testing if trait values were related to aboveground biomass (AGB) using mixed effects modelling with AGB as fixed effect and plot and clonal individuals as random effects. All traits were related to AGB ($p = 0.05$ for leaf P concentration, $P_{\text{mass}}, p < 0.01$ for rest; Fig. S5, left column), but the relationship accounted for differing portion of the variation in each trait. The variation in trait value explained by AGB ($r^2$) was 0.09 for leaf mass per area (LMA) and as small as 0.01 for $P_{\text{mass}}$. Both LMA and leaf N concentration ($N_{\text{mass}}$) increased with AGB, while $P_{\text{mass}}$, mass based leaf dark respiration measured at 25 °C ($R_{\text{mass}}$), $E_A$ and relative growth rate (RGR) decreased. We then used the relationship to correct for the effect of size on trait values, and examined if the size-corrected values would change trait variation with sampling date, the fraction of variance explained, and trait to trait relationships. To test if traits were related to AGB, both trait values ($y$) and AGB were natural log transformed such that the model had the form $\ln(y) \sim a + b \ln(\text{AGB})$. We used the coefficient $b$ to account for the relationship in untransformed trait values as $y / \text{AGB}^b$ (this size corrected trait value showed no relationship with AGB as expected). We plotted the size corrected trait value against sampling date (day of year; DOY) similar to Fig. 5 in main text for comparison, but the two figures had similar trend with DOY. We also used the size corrected trait value as the independent variable and repeated the variance partitioning procedure, and the results were similar to Fig. 3 in main text, with substantial fraction still attributed to DOY. Size corrected trait values reduced the variability from sampling date in trait to trait relationships. With uncorrected trait values, sampling date changed both slope and elevation of the relationship between LMA and $N_{\text{mass}}$ and $R_{\text{mass}}$ (Fig. 6 in main text), but with size corrected values, sampling date changed elevation but not slope of the relationship between LMA and $N_{\text{mass}}$ ($p < 0.01$ for elevation, $p = 0.08$ for slope), and between $N_{\text{mass}}$ and $R_{\text{mass}}$ ($p < 0.01$ for elevation, $p = 0.15$ for slope).

We also examined the effect of cyclic seasonality on trait values by testing if traits varied with seasonal change in temperature. For all traits, variation in trait values were related to daily minimum temperature averaged over five nights prior to sampling ($p < 0.01$; Fig. S5, right column). The results were similar when daily maximum (averaged over five days prior) was used instead, and we report only those of daily minimum. LMA decreased with the minimum temperature (with $r^2 = 0.37$), but $N_{\text{mass}}$ (0.04), $P_{\text{mass}}$ (0.16), $R_{\text{mass}}$ (0.11), $E_A$ (0.02), and RGR (0.59) all increased with temperature. Similar to the above analysis on the effects of size, we used model coefficients to correct for the effects of the minimum temperature (the temperature corrected trait values showed no correlation with the minimum
When plotted against DOY, the temperature corrected trait values had similar trends with DOY but was much less pronounced. Repeating the variance partitioning procedure, temperature corrected trait values had much less but not zero fraction of variance attributed to DOY, especially for LMA, N_{mass}, and E_{A}. As expected, temperature corrected values reduced the variability in LMA and P_{mass} with sampling date, but sampling date still had different slope in the relationship between temperature corrected values of LMA and N_{mass}, LMA and R_{mass}, and N_{mass} and R_{mass} (all at p < 0.01). Our attempt to separate the effect of size on trait value should be taken with caution since it essentially tries to disentangle cyclic patterns from underlying trends in time series with only four time points. It is also uncertain if seasonal changes in temperature were responsible, since similar results can be obtained with any variable such as light intensity that change seasonally with temperature.
Table S1. List of variable name and abbreviation used to characterize the climate of genotype provenance. First 19 are Bioclimatic variables taken from WorldClim.

<table>
<thead>
<tr>
<th>Name</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual mean temperature</td>
<td>MAT</td>
</tr>
<tr>
<td>Mean diurnal range (mean difference in min and max daily temperature)</td>
<td>DiurT</td>
</tr>
<tr>
<td>Isothermality</td>
<td>isotherm</td>
</tr>
<tr>
<td>Temperature seasonality (standard deviation * 100)</td>
<td>Tseasonality</td>
</tr>
<tr>
<td>Max temperature of warmest month</td>
<td>maxTWM</td>
</tr>
<tr>
<td>Min temperature of coldest month</td>
<td>minTCM</td>
</tr>
<tr>
<td>Temperature annual range</td>
<td>annTrange</td>
</tr>
<tr>
<td>Mean temperature of wettest quarter</td>
<td>TwetQ</td>
</tr>
<tr>
<td>Mean temperature of driest quarter</td>
<td>TdryQ</td>
</tr>
<tr>
<td>Mean temperature of warmest quarter</td>
<td>TWQ</td>
</tr>
<tr>
<td>Mean temperature of coldest quarter</td>
<td>TCQ</td>
</tr>
<tr>
<td>Annual precipitation</td>
<td>MAP</td>
</tr>
<tr>
<td>Precipitation of wettest month</td>
<td>PwetM</td>
</tr>
<tr>
<td>Precipitation of driest month</td>
<td>PdryM</td>
</tr>
<tr>
<td>Precipitation seasonality</td>
<td>Pseasonaltiy</td>
</tr>
<tr>
<td>Precipitation of wettest quarter</td>
<td>PwetQ</td>
</tr>
<tr>
<td>Precipitation of driest quarter</td>
<td>PdryQ</td>
</tr>
<tr>
<td>Precipitation of warmest quarter</td>
<td>PWQ</td>
</tr>
<tr>
<td>Precipitation of coldest quarter</td>
<td>PCQ</td>
</tr>
<tr>
<td>Aridity index</td>
<td>AI</td>
</tr>
<tr>
<td>Latitude</td>
<td>lat</td>
</tr>
<tr>
<td>Longitude</td>
<td>lon</td>
</tr>
</tbody>
</table>
The values were taken from mixed effects model, with select factors. The factors considered were genotype (G), watering treatment (W), sampling date (day of year, D), their two-way interactions (G x W, G x D, W x D), three-way interaction involving genotype (G x W x D), and experimental factors of replicate block. The factors were selected into the model based on likelihood ratio tests and AIC comparing a model with all of the factors against another without the factor of interest. NAs indicate factors excluded from the model. Some factors were included despite having low significance because it’s interaction term was significant.

The values of aboveground biomass (AGB) were natural log transformed for the analysis. Back transformed, mean AGB is 729 g. The units were g m\(^{-2}\) for leaf mass per area (LMA), mg g\(^{-1}\) for leaf N (N\(_{\text{mass}}\)) and P concentrations (P\(_{\text{mass}}\)), nmol O\(_2\) g\(^{-1}\) s\(^{-1}\) for leaf dark respiration rate measured at 25 °C (R\(_{\text{mass}}\)), kJ mol\(^{-1}\) for activation energy (E\(_A\)) as a proxy of temperature response of R\(_{\text{mass}}\), mg g\(^{-1}\) day\(^{-1}\) for relative growth rate of aboveground biomass (RGR), and g for AGB (before log transformation).

<table>
<thead>
<tr>
<th>Factor</th>
<th>LMA</th>
<th>N(_{\text{mass}})</th>
<th>P(_{\text{mass}})</th>
<th>R(_{\text{mass}})</th>
<th>E(_A)</th>
<th>RGR</th>
<th>AGB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>103</td>
<td>26</td>
<td>1.4</td>
<td>21</td>
<td>67</td>
<td>9.0</td>
<td>6.6</td>
</tr>
<tr>
<td>Genotype</td>
<td>41</td>
<td>4.3</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>1.2</td>
<td>0.41</td>
</tr>
<tr>
<td>Watering</td>
<td>26</td>
<td>3.0</td>
<td>0.030</td>
<td>1.5</td>
<td>NA</td>
<td>NA</td>
<td>0.12</td>
</tr>
<tr>
<td>Day of year</td>
<td>296</td>
<td>6.5</td>
<td>0.042</td>
<td>9.5</td>
<td>72</td>
<td>75</td>
<td>0.49</td>
</tr>
<tr>
<td>G x W</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>G x D</td>
<td>47</td>
<td>1.8</td>
<td>0.010</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>W x D</td>
<td>NA</td>
<td>NA</td>
<td>0.010</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>G x W x D</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Block</td>
<td>26</td>
<td>4.3</td>
<td>0.018</td>
<td>3.7</td>
<td>42</td>
<td>1.2</td>
<td>0.09</td>
</tr>
<tr>
<td>Residual</td>
<td>172</td>
<td>17</td>
<td>0.113</td>
<td>33</td>
<td>307</td>
<td>14</td>
<td>0.25</td>
</tr>
<tr>
<td>Total</td>
<td>608</td>
<td>36.6</td>
<td>0.225</td>
<td>47.4</td>
<td>421</td>
<td>91.3</td>
<td>1.38</td>
</tr>
</tbody>
</table>
Figure S1. Variation in leaf dark respiration rates of leaves at different nodes along branches within the canopy of an individual tree. Respiration rate on leaf area (R$_{\text{area}}$) and dry mass (R$_{\text{mass}}$) bases declined along leaf nodes toward the base of the branch.
Figure S2. Contributions of each climate variables to first three principle components. The red dotted line indicates expected contribution if all variables contributed equally (4.5%)
Figure S3. Climate of genotype provenance represented in first two principle components. Climate variables included were latitude, longitude, aridity index, and all 19 Bioclimatic variables taken from WorldClim. The numbers correspond to genotypes, and Richmond is the site of the common garden. Colors (squared cosine) indicate how well the climate of provenance is represented by the two principle components. Straight line distance (Euclidian distance; ED) between Richmond and the climate of genotype provenance reflects dissimilarity of climates, and ED was calculated for each provenance.
Figure S4. Stomatal conductance was unrelated to soil water fraction in in genotypes from Providence with driest and wettest MAP, except in dry treatment during February. In May and July, stomatal conductance was higher in wet treatment (p < 0.01), but was unrelated to soil water fraction (p = 0.17). Regression line for dry treatment in February has the slope of 0.63 mol H₂O m⁻² s⁻¹ for every 0.1 increase in the soil water fraction (r² = 0.65).
Figure S5. Relationship between trait value and aboveground biomass (right column) and seasonal change in temperature (left column). The lines are drawn for relationships with $p < 0.05$. DOY 53, 122, 197, and 311, correspond to summer, autumn, winter, and spring. For the right column, both axes are on log scale, and for left column y-axis is on log scale.
Figure S6. Fig. 3 in main text redrawn with results of analysis using natural log transformed values, with similar results that genotypic sources constituted roughly a third of variance in aboveground biomass (AGB) but at most a fifth in leaf traits and relative growth rate of aboveground biomass (RGR). Leaf traits were leaf mass per area (LMA), concentrations of N (N\text{mass}) and P (P\text{mass}), leaf dark respiration rate measured at 25 °C (R\text{mass}), and activation energy (E\text{A}) as a proxy of temperature response of R\text{mass}. The variation in trait values was attributed to different sources using mixed effects models for each trait with all sources included as random factors. The sources examined were genotype, watering treatment, sampling date (DOY), their interactions, and the experimental factor of replicate block (see Table 2 for factors included and variance, \sigma^2, explained by each factors). For simplicity, the
sources were grouped into genotype, interactions with genotype, DOY, watering treatment, and other (top plot). In the bottom plot, the other sources were further partitioned into water and DOY interaction, replicate blocks, and residual.