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SPECIAL ISSUE ON INTRASPECIFIC VARIATION IN PLANT FUNCTIONAL TRAITS

Leaf traits and performance vary with plant age and water availability in *Artemisia californica*

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• **Background and Aims** Leaf functional traits are strongly tied to growth strategies and ecological processes across species, but few efforts have linked intraspecific trait variation to performance across ontogenetic and environmental gradients. Plants are believed to shift towards more resource-conservative traits in stressful environments and as they age. However, uncertainty as to how intraspecific trait variation aligns with plant age and performance in the context of environmental variation may limit our ability to use traits to infer ecological processes at larger scales.
• **Methods** We measured leaf physiological and morphological traits, canopy volume and flowering effort for *Artemisia californica* (California sagebrush), a dominant shrub species in the coastal sage scrub community, under conditions of 50, 100 and 150 % ambient precipitation for 3 years.
• **Key Results** Plant age was a stronger driver of variation in traits and performance than water availability. Older plants demonstrated trait values consistent with a more conservative resource-use strategy, and trait values were less sensitive to drought. Several trait correlations were consistent across years and treatments; for example, plants with high photosynthetic rates tended to have high stomatal conductance, leaf nitrogen concentration and light-use efficiency. However, the trade-off between leaf construction and leaf nitrogen evident in older plants was absent for first-year plants. While few traits correlated with plant growth and flowering effort, we observed a positive correlation between leaf mass per area and performance in some groups of older plants.
• **Conclusions** Overall, our results suggest that trait sensitivity to the environment is most visible during earlier stages of development, after which intraspecific trait variation and relationships may stabilize. While plant age plays a major role in intraspecific trait variation and sensitivity (and thus trait-based inferences), the direct influence of environment on growth and fecundity is just as critical to predicting plant performance in a changing environment.

Key words: *Artemisia californica*, intraspecific trait variation, drought, photosynthesis, water-use efficiency, plant age.

INTRODUCTION

Trait differences across species have long been used to understand how species, populations and communities function in different environments (Gleason, 1926; Harper, 1967). However, the use of species means can mask important intraspecific variation (Violle *et al.*, 2012; Siefert *et al.*, 2015) arising from genetic, environmental and ontogenetic sources (Albert *et al.*, 2011). By creating differential fitness responses to abiotic and biotic stressors, intraspecific trait variation can influence the response of populations and species to environmental change (Darwin, 1859), with cascading impacts on community and ecosystem functions (Miner *et al.*, 2005; Bolnick *et al.*, 2011). While quantifying intraspecific trait variation for every species is implausible, characterizing the nature of intraspecific variation along critical axes of environmental and developmental variation is essential, particularly for studies of trait evolution and species-level patterns (Albert *et al.*, 2011; Bolnick *et al.*, 2011).

Environmental variation can result in substantial trait variation as plant growth strategies shift from stress tolerance at low resource availability to rapid growth at high resource availability. For example, interspecific trait variation in response to soil moisture gradients has been well characterized, with dominant plants in arid environments possessing tough tissues, high root mass fraction and high water-use efficiency (Chapin *et al.*, 1993; Díaz *et al.*, 1999; Wright *et al.*, 2001; Reich *et al.*, 2003; Poorter and Markesteijn, 2008; Cornwell and Ackerly, 2009), although patterns may depend on the temporal and spatial scale examined (Sandel *et al.*, 2010; Moles *et al.*, 2014). Similar intraspecific patterns have been documented along environmental gradients and when individuals are exposed to drought (Heschel *et al.*, 2002; Valladares and Sánchez-Gómez, 2006; Jung *et al.*, 2014; Larson and Funk, 2016; Eziz *et al.*, 2017; Winkler *et al.*, 2018), although the nature of environment-induced shifts clearly vary. For example, while long-lived perennials may move further towards resource conservation, annual species with drought escape strategies may be pushed towards rapid growth, high rates of resource acquisition and early flowering under water limitation (Kooyers, 2015; Volaire, 2018). Collectively, these studies suggest that environmental

factors are a major driver of intraspecific trait variation for a range of species.

Most studies of trait variation compare individuals or species at a single ontogenetic stage. However, plants experience different abiotic and biotic stressors as they age and change in size, which should lead to different growth and allocation patterns (Grubb, 1977; Coleman *et al.*, 1994; Barton and Koricheva, 2010; Lasky *et al.*, 2015). Although many trait-based inferences rely on traits collected from adults, seedlings may have different attributes aligned with establishment and rapid growth, as greater plant size may decrease consequences of or exposure to stress. For example, larger seedlings may better withstand damage from herbivores, and seedlings with deeper root systems may have greater access to soil water to avoid or delay drought. The few studies that have examined how traits vary with age found that younger individuals have trait values aligned with resource acquisition, while older individuals have more conservative traits (Mason *et al.*, 2013; Spasojevic *et al.*, 2014; Damián *et al.*, 2018; Dayrell *et al.*, 2018). The relationship between these traits and plant performance can also vary with plant age. Traits such as leaf mass per area (LMA), water-use efficiency (WUE) and seed mass tend to be more strongly linked to growth and mortality in seedlings compared with adult plants (Casper *et al.*, 2005; Gibert *et al.*, 2016; Harrison and LaForgia, 2019). Thus, while previous work suggests that plant age and size play a major role in intraspecific trait variation, more studies are needed to determine how specific traits can be used to predict plant performance across multiple developmental stages.

Finally, environmental stress and plant age may interact in potentially important ways to influence intraspecific trait variation. If seedlings and juveniles are more focused on establishment and growth, this can come at the expense of defence and stress tolerance (Markesteijn and Poorter, 2009; Lum and Barton, 2020), resulting in variable trait–environment relationships across different demographics of the population (Lusk and Warton, 2007). For example, Cavender-Bares and Bazzaz (2000) found that young oak seedlings were more sensitive to drought than mature individuals. Furthermore, trait plasticity in response to environmental variation may depend on plant age, with additional implications for fitness. Specifically, higher trait plasticity in seedlings (e.g. Niinemets, 2004) may buffer them from fluctuating environmental conditions. Linking intraspecific trait variation to plant performance across age groups and environmental variation will help establish how traits can be used to more accurately predict the response of species and communities to climate change (Kimball *et al.*, 2016).

In this study, we ask three questions. First, how do leaf traits change with water availability and plant age? We expect a shift towards more resource-conservative traits in low water environments and in older individuals; specifically, tough leaf construction, high WUE and low leaf nitrogen concentration and rates of carbon assimilation. Secondly, are traits and performance less impacted by water availability in older individuals? A resource-conservation strategy employed by older individuals may lead to low variation in trait values and performance across water treatments when compared with young individuals. We also expect to see more drought sensitivity (low leaf water potential) in younger plants. Finally, do water availability

and plant age impact how traits relate to each other and to performance? Based on previous work, we expect that trait relationships will be stronger in young plants (Casper *et al.*, 2005; Gibert *et al.*, 2016; Harrison and LaForgia, 2019), but it is possible that trait correlations are consistent across years, with trait means shifting towards resource conservation in older plants. It is also possible that different traits drive performance under dry and wet conditions (Heschel *et al.*, 2004).

MATERIALS AND METHODS

Study species and site

Artemisia californica (California sagebrush) is a dominant, facultatively drought-deciduous shrub in coastal sage scrub systems. Coastal sage scrub is a Mediterranean-climate ecosystem found along the coast of California (USA) and is characterized by a diverse mix of shrubs and herbaceous plants (Cleland *et al.*, 2016). Coastal sage scrub, and *A. californica* in particular, is an excellent system for studying how traits drive plant responses to water availability for several reasons, including strong and variable abiotic stress in the region; *A. californica* ages quickly, moving to adulthood by year two; and documented plasticity in *A. californica* leaves through seasonal dimorphism associated with drought response (Westman, 1981). For the experiment, we obtained 5-month old locally sourced seedlings of *A. californica* from Tree of Life Nursery (San Juan Capistrano, CA, USA).

We investigated plant traits and performance in this system by establishing a rainfall manipulation experiment at the University of California South Coast Research and Extension Center in Irvine, CA, USA (33°41′N, 117°43′W; 125 m elevation, 330 mm average annual precipitation). Precipitation in Mediterranean-climate ecosystems can vary strongly inter- and intra-annually. Over the last 20 years, annual precipitation in this region has ranged from 60 to 700 mm. In normal years, plants often receive >50 mm of rain per month throughout the wet season. However, in drought years, precipitation can be much more intermittent. For example, in 2004, the bulk of precipitation was received in 1 month (February), with all other months receiving <20 mm (data from National Weather Service, Santa Ana, CA, USA). During our experiment, annual precipitation was below the 330 mm historic average in all three growing seasons: 110, 301 and 207 mm for the 2013–14, 2014–15 and 2015–16 growing seasons, respectively. Annual average temperature was fairly consistent across years, ranging from a low of 17.6 °C in 2013 to a high of 18.9 °C in 2014.

Experimental design

We established our study site in a fallow field which was irrigated and disked to reduce weed growth and fenced to discourage herbivory. To manipulate rainfall, we constructed nine rain-out shelters [3 m (w) × 7.3 m (l) × 2.6 m (h)] with retractable clear plastic covers. Covers were raised during major rainfall events (>0.25 mm) to exclude precipitation. To implement rainfall treatments, we added water with a sprinkler irrigation system installed in each structure (sprinkler height 1.5 m; flow rate 14 mm h^{-1}; >75 % distribution uniformity). We installed

soil moisture sensors at a depth of 15 cm (one EC-5 sensor per structure; Decagon Devices, Inc., Pullman, WA, USA) to monitor soil moisture status. To avoid interference with root systems when sensors were replaced over the 3 year experiment, soil moisture sensors were installed in the corner of each structure, 1.8 m away from one edge of the structure and 0.15 m away from the other.

We analysed soil texture, pH and nutrient concentrations for three samples per shelter. Soil pits were dug in each structure during May 2016 and one sample was collected from three depths (15–25, 65–75 and 115–125 cm) and pooled. Prior to analysis, soils were put through a 2 mm sieve to remove rocks and organic matter. Soil pH was determined on 20 mg of air-dried soil in deionized water. A second sample of soil was dried at 60 °C for 48 h and analysed for sediment grain size using a hydrometer (H-B Instrument Co., ASTM Soil Hydrometer 152H) following a slightly modified protocol of Kalra and Maynard (1991). A third sample of air-dried soil was sent to the UC Davis analytical lab for detemination of nitrogen (NO_3), phosphorus (Olsen P) and potassium concentration. Soil data are presented in Supplementary data Table S1.

In each shelter, we established 8–1 m^2 plots containing one plant of *A. californica* and one each of seven other species common to coastal sage scrub (each planted 30 cm apart and randomly located within plots). Seedlings were transplanted in December 2013. We immediately replaced any seedlings that died in the first month. Plots were weeded biweekly throughout the first growing season and as needed to reduce external competition for the remainder of the experiment.

After a 1 month establishment period in which all plants received approx. 20 mm of precipitation, we began watering treatments in January 2014. Each structure was randomly assigned to one of three watering treatments (3 treatments × 3 shelters × 8 plots = 72 total plots). Treatments included ambient precipitation (100 % of average January–April precipitation; 220 mm target), high precipitation (150 % of January–April average; 330 mm target) and low precipitation (50 % of January–April average; 110 mm target). Water was applied from January to April each year to achieve these targets (typically 2–3 times per week). Soil moisture was consistently different across water treatments in the 3 year experiment (Supplementary data Fig. S1). Average soil moisture for the growing season (January–May) was: 50 % (Y1, 18.3 %; Y2, 17.4 %; Y3, 19.5 %); 100 % (Y1, 19.8 %; Y2, 20.1 %; Y3, 20.9) and 150 % (Y1, 25.7 %; Y2, 22.7 %; Y3, 23.7 %). Covers were retracted to allow natural rainfall from May to December of each year (i.e. the dry season). Except for a large summer storm in September 2015 (53 mm of rainfall, Supplementary data Fig. S1), rainfall into the uncovered plots was negligible during summer months. At the end of the first growing season, we had 24, 21 and 22 individuals in the 50, 100 and 150 % plots, respectively (i.e. 67 of 72 planted individuals). We experienced some additional mortality during the third year, bringing the total number of plants in the 100 % plots to 17.

Sampling

We sampled carbon assimilation and water-use traits that are known to be affected by variation in water availability. Traits and reproductive data were sampled each year for 3 years (2014, 2015 and 2016). Photosynthetic data were collected on one recently mature leaf per plant. Between 08.00 and 13.00 h local time, we measured leaf photosynthetic capacity (A_{mass}, nmol g^{-1} s^{-1}), transpiration rate (E, mol m^{-2} s^{-1}), stomatal conductance (g, mol m^{-2} s^{-1}) and quantum efficiency of photosystem II (Φ_{PSII}) with a LI-6400 portable photosynthesis system (LI-COR, Lincoln, NE, USA). Water-use efficiency was calculated as A/E. Environmental conditions included saturating light levels at 2000 µmol photon m^{-2} s^{-1}, CO_2 concentration at 400 µL L^{-1}, block temperature at 25 °C and chamber flow rate between 300 and 500 µmol s^{-1} to maintain a relative humidity between 40 and 60 %. The Φ_{PSII} was calculated as $(F_m' - F_s)/F_m'$, where F_s is the fluorescence yield of a light-adapted leaf and F_m' is the maximal fluorescence during a saturating light flash. When necessary, photosynthetic rates were temperature corrected using standard equations (von Caemmerer, 2000) and area corrected to account for leaves that were too small to fill the chamber. We destructively sampled one recently mature leaf per plant to measure mid-day (09.00 to 13.00 h local time) leaf water potential (Ψ_{leaf}, MPa) at least 1 d after any irrigation activity (Model 1000 pressure chamber, PMS instruments, Albany, OR, USA). Additional leaves were collected and scanned for leaf area, dried at 60 °C for 3 d, weighed to calculate LMA (g m^{-2}), ground and analysed for leaf nitrogen analysis (% leaf N by mass; N_{mass}) using a Costech 4010 Elemental Combustion System (Costech Analytical Technologies, Valencia, Ca, USA).

At the end of each growing season (April–May), we assessed canopy volume (height × canopy area). Given the difficulty of counting small, abundant inflorescences along *A. californica* flowering stalks, we measured total flowering stalk length as a metric of flowering effort. The number of seeds per flower and flower density per stalk did not vary greatly between *A. californica* plants in a similar experiment (G. Vose, unpubl. data). All individuals flowered every year, and flowering measures coincided with peak flowering, which varied from year to year (June–July 2014, August–September 2015 and August–September 2016).

Statistical analysis

All analyses were conducted in R (R version 3.3.1). To evaluate our how leaf traits change with water availability and plant age, we performed two analyses. We first used perMANOVA [permutation-based multivariate analysis of variance (MANOVA)] to examine the effect of water treatment and age on multivariate trait space (A_{mass}, g, Φ_{PSII}, WUE, leaf N and LMA). We used the full set of scaled metrics as the response and water treatment, year and their interaction as explanatory variables (999 permutations, Euclidean distances; vegan package). We then conducted a repeated-measures ANOVA for each trait or performance response metric, with year, water treatment and their interaction as fixed effects and individual plant as a random effect (nlme package). When traits were not normally distributed, any outliers greater than two standard deviations from the mean were tagged. If their removal improved the distribution, they were removed. Data were also log transformed as necessary to meet assumptions of normality. Following Moran

(2003), instead of conducting sequential Bonferroni corrections for multiple statistical tests, all *P*-values are reported.

To assess differences in intraspecific trait variation across years, we used a plasticity index (PI_v) described by Valladares *et al.* (2006). The index ranges from zero (no plasticity) to one (maximum plasticity) and is the difference between the minimum and maximum value of the treatment means of a trait divided by the maximum value. Plasticity indices were evaluated across years with a one-way ANOVA. To assess how water availability and plant age impact relationships among traits and performance, we performed two analyses. First, a principal component analysis (PCA) was used to identify trade-offs among traits (performance metrics were excluded) using the psych package in R. Analyses were conducted on standardized trait means and repeated for each year to understand broad changes in trait trade-offs over time. Axes were varimax rotated and constrained to the first four components to improve axis interpretability. To further interpret which traits were correlated with each other and with performance metrics across treatments and within years, we calculated Pearson's correlation coefficients.

RESULTS

Multivariate trait space responded significantly to year and the interaction between water treatment and year (water $P = 0.38$, $R^2 = 0.02$; year $P < 0.001$, $R^2 = 0.26$; water × year $P < 0.001$, $R^2 = 0.05$). These patterns aligned with those observed for individual metrics. Although performance variables (canopy volume and flowering effort) showed significant rainfall responses, only water potential (trended lower in dry conditions) was significantly impacted by rainfall across years (Fig. 1; Supplementary data Table S2). In contrast, several traits and performance variables were generally affected by plant age. Older plants had higher LMA, lower leaf N concentration and lower photosynthetic function (A_{mass}, g and Φ_{PSII}) – consistent with a more conservative resource-use strategy (Fig. 1; Supplementary data Table S2). Only WUE was fully independent of plant age. As expected, older plants were larger and allocated more to reproduction than younger plants.

For several traits, the effect of rainfall depended on year (significant treatment by year interactions). In most cases, these results were consistent with the idea that older plants are less impacted by variation in water treatment. Water potential, a measure of plant stress, varied across water treatments in Year 1, but not in later years (Fig. 1). Plants growing in high water treatments had greater leaf N concentration, stomatal conductance and canopy volume in Years 1 and 2, but these differences disappeared in third-year plants. Plasticity indices for photosynthetic rate and water-use traits (stomatal conductance, leaf water potential and WUE) were higher in younger plants, but these patterns did not hold when analysed across all traits. PI_v ranged from 0.04 to 0.63 depending on the trait and year (Supplementary data Table S3), but there was no difference in PI_v across years ($F = 2.15$, $P = 0.138$), even when canopy volume and reproductive allocation were excluded from the analysis ($F = 2.88$, $P = 0.082$).

Functional trade-offs captured by our PCA were consistent across the 3 years (Supplementary data Fig. S2). The first two PCA axes described relationships among carbon assimilation and leaf economic traits (A_{mass}, g, N and LMA), and explained roughly 50 % of the variation among individuals each year (Supplementary data Fig. S2, Table S4). In each year, another 40 % of the variation was explained by water- and light-use efficiency (third and fourth axes).

Several bivariate trait correlations were consistent across years and water treatments (Figs 2 and 3). Plants with high photosynthetic rates tended to have high stomatal conductance, leaf N concentration and light-use efficiency, and there were consistent negative correlations between stomatal conductance and WUE, and between leaf N concentration and LMA. In many of the cases, relationships among traits were similar across treatments and years, even while the absolute values of traits shifted with age (e.g. Fig. 2A). In other cases, trait correlations differed between young (Year 1) and older (Years 2 and 3) individuals. For example, the economic trade-off between leaf construction and leaf N concentration observed across all individuals was strongest in older plants but non-significant in first-year plants (Figs 2B and 3). In contrast, plants with high LMA were more stressed (lower water potential) in the low water treatment when young, but this pattern reversed (Year 2) or was not present (Year 3) in older plants (Figs 2C and 3).

Despite the relatively large number of individual plants examined each year ($n = 62–67$), few traits were significantly correlated with canopy volume or flowering effort, our two performance metrics (Fig. 3). We observed an increasing positive correlation with LMA and performance as plants aged, although this was only statistically significant in some age by treatment combinations. Light-use efficiency was positively correlated with canopy volume and flowering effort in Year 2, and photosynthetic rate was positively correlated with canopy volume in Year 3. Plants under greater water stress (more negative water potentials in the low water treatment) had lower flowering effort, but this was only statistically significant in Year 1. Canopy volume and flowering effort were positively correlated in only four of the nine age by treatment combinations.

DISCUSSION

Intraspecific trait variation strongly impacts ecological and evolutionary processes (Bolnick *et al.*, 2011; Violle *et al.*, 2012; Hausch *et al.*, 2018), but it is not well characterized along important environmental and ontogenetic axes for many plant species. Our first objective was to understand how key functional traits pertaining to resource use and acquisition change with water availability and plant age. As expected, we found a shift towards more resource-conservative traits with drought and in older plants, but the effect was more consistent for age. Older individuals had higher LMA, lower leaf N concentration and reduced photosynthetic function compared with younger plants. For many of these traits, the largest difference in function occurred between Years 1 and 2, suggesting that trait values change most as species move out of the establishment phase. Thus, our results support previous work showing that older individuals have more conservative traits (Mason *et al.*, 2013; Spasojevic *et al.*, 2014; Damián *et al.*, 2018; Dayrell *et al.*, 2018) and reinforce the idea that abiotic and biotic stressors differentially impact growth and allocation patterns as plants age. Our results also demonstrate that plant age is an important source
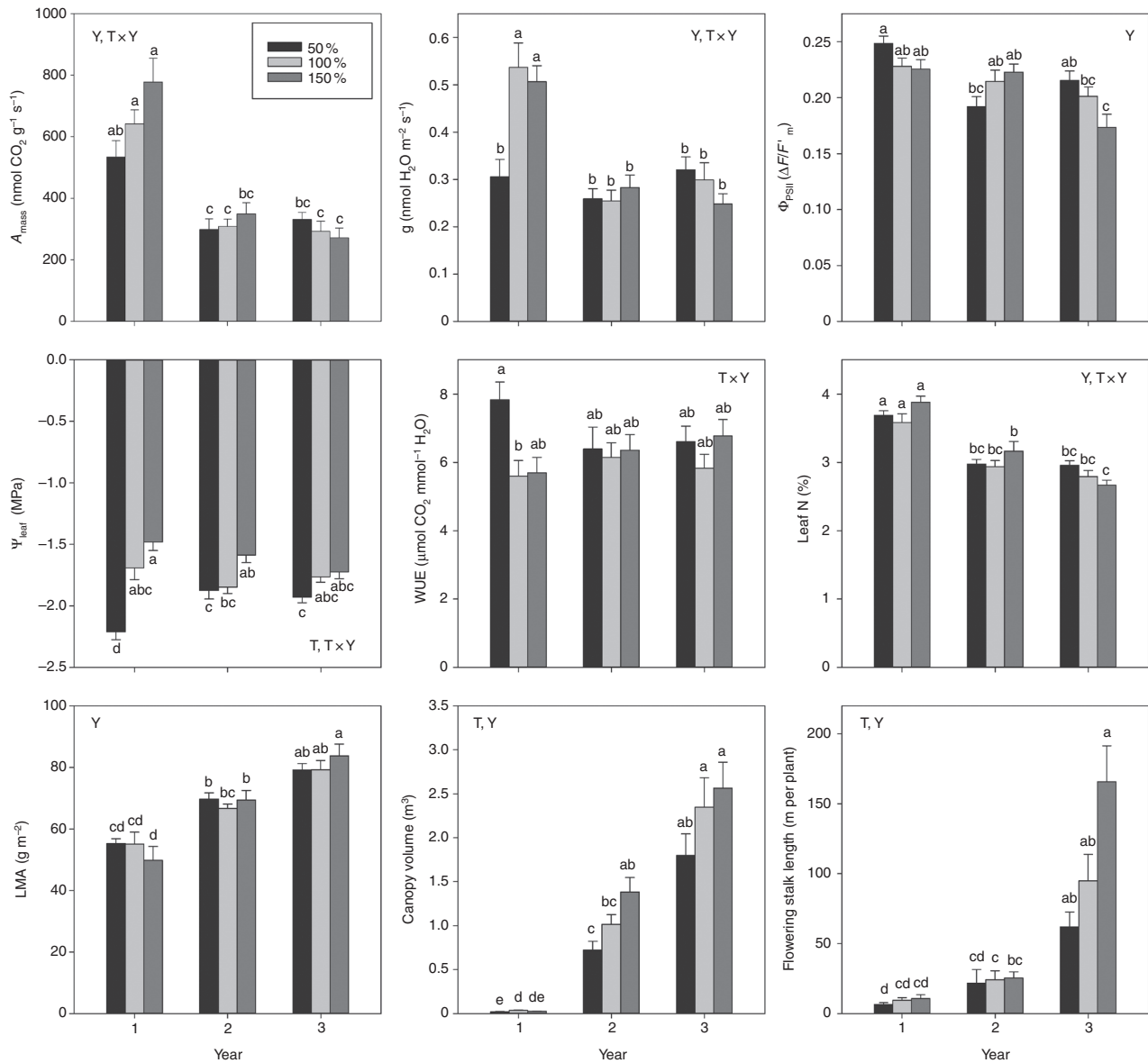
Fɪɢ. 1. Trait means and standard errors for *A. californica* grown in three watering treatments (50, 100 and 150 % ambient precipitation) across 3 years. Significant effects of treatment (T), year (Y) and their interaction (T × Y) at *P* < 0.05 are shown, based on repeated-measures ANOVA. Group means with the same lower case letter are not significantly different from each other at *P* < 0.05. Trait abbreviations are provided in Table 1.

of intraspecific trait variation that should be considered when making trait-based inferences.

Our prediction of higher resource conservation in water-stressed plants was partially supported. Drought resulted in higher stress (lower leaf water potential) across years and increased WUE for first-year plants. Remarkably, photosynthetic function was not consistently affected by water treatment. Significant treatment by year interactions suggest that leaf physiology was more sensitive to variation in water availability in the first year (e.g. A_{mass} and *g*) than in older plants. This may indicate that our low water treatment (50 % ambient precipitation) was not low enough to trigger trait plasticity in drought-deciduous species once established. Older plants may be more drought tolerant due to a shift towards tougher tissues and lower rates of water loss (low stomatal conductance);

however, older plants with established root systems may also have greater access to soil water. California is characterized by strong interannual variation in precipitation, and a year with 50 % ambient precipitation is not uncommon (California Irrigation Management Information System, Irvine, CA, USA). Thus, it is likely that *A. californica* adults are well adapted to deal with rainfall deficits, and may show weak or moderate trait variation in response to normal (approx. 50–150 % ambient) interannual variation in precipitation or under drier, future climate scenarios (Cook *et al.*, 2015). Because we measured traits on the same individuals over time, differences between young and old plants may have been influenced by variable environmental conditions across years. For example, the range of soil moisture between low- and high-water treatments varied across the 3 years, although this was mainly driven by very high soil
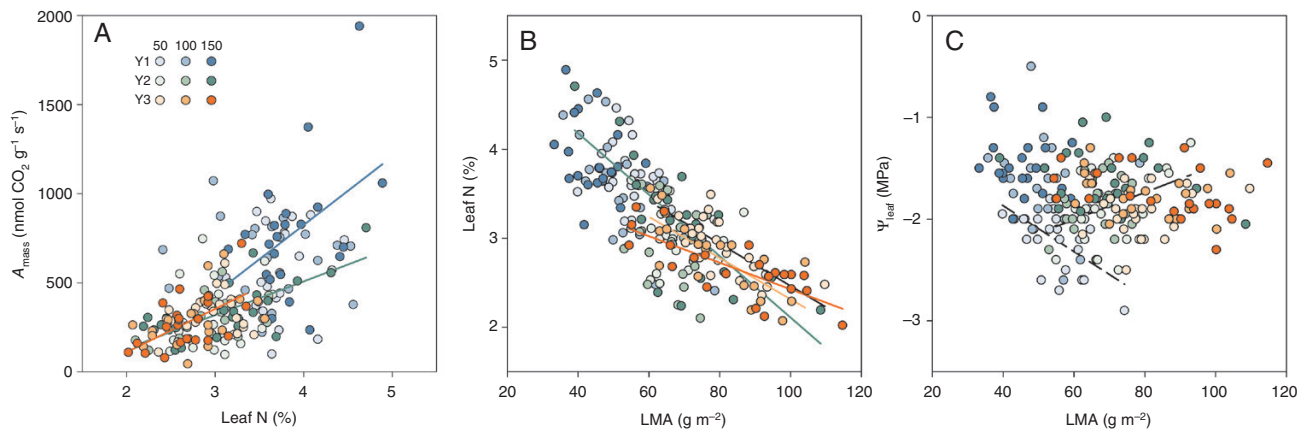
FIG. 2. Bivariate plots for (A) leaf N concentration and photosynthetic rate, (B) leaf N concentration and leaf mass per area (LMA) and (C) LMA and leaf water potential. Colours denote different ages: 1 (blue), 2 (green) and 3 (orange) years. Water treatments are represented by different shades: 50 % (light), 100 % (medium), 150 % (dark). We observed consistent relationships among several traits (such as A_{mass}, leaf N and LMA) across individuals, but young and older plants occupied different points along these axes (A and B). The relationship between LMA and leaf water potential varied across groups, but plants in the 50 % water treatment generally displayed higher water potential at low LMA (C). Coloured lines indicate significant relationships for 100 and 150 % treatments. For clarity, significant relationships for 50 % treatments are represented by black lines (short dash, Year 1; long dash, Year 2; solid, Year 3).

moisture in the 150 % treatment during the first year. Planting a new cohort of individuals each year would have removed this influence of year; but this approach limits our understanding of how the same individuals change over time.

Our second objective was to determine if traits and performance are differentially impacted by drought as plants age. Our expectation that young plants demonstrate more trait variation across the water gradient was largely supported. First- and second-year plants exhibited greater sensitivity to variation in water availability (as indicated by leaf water potential), and conditions of high water availability led to decreases in WUE and increases in stomatal conductance and canopy volume in these age groups. Despite having the lowest leaf water potentials, first-year plants in the low-water treatment nevertheless displayed higher photosynthetic function than second- and third-year plants in most water treatments. This supports the notion that young plants may have more plastic, growth-oriented strategies which benefit from greater resource availability. While greater plasticity may benefit young seedlings in a variable environment (Niinemets, 2004), high intraspecific trait variation in this age group could also arise from larger differences in size among plants. Traits and biomass allocation can be more strongly affected by plant size than plant age (Coleman *et al.*, 1994; Wright and McConnaughay, 2002; Lusk and Warton, 2007), but our data suggest that variation in size was consistent across age groups (the coefficient of variation for canopy volume was 55.4, 63.2 and 77.6 % for Years 1, 2 and 3, respectively). More studies that follow individual plants over time and in different environments, such as ours, can help determine which traits drive responses to environmental variation over a plant's lifetime.

Finally, we explored how traits were linked to each other and to performance across age groups and water treatments. We expected to see strong correlations between traits and our performance metrics (canopy volume and flowering effort), particularly for first-year plants (Casper *et al.*, 2005; Gibert *et al.*, 2016; Harrison and LaForgia, 2019). In many systems, seedlings are vulnerable to a range of abiotic and biotic stresses, and seed and seedling traits are critical to

establishment, growth and survival (Larson *et al.*, 2015). As woody plants age and increase in size, a larger percentage of biomass is allocated to support tissues (Poorter *et al.*, 2012), so it is possible that we would not expect to see strong relationships between leaf traits and performance in older plants. In contrast to our predictions, we found few correlations between traits and performance in any year or water treatment. Notably, first-year plants with high LMA were more stressed in our low water treatment, leading to lower flowering effort. However, this pattern reversed in Year 2; plants with high LMA had higher canopy volume and flowering effort in the low-water treatment. With this one exception, our results do not suggest that different traits drive performance under dry and wet conditions, or across age groups. This contrasts with some studies that find context-dependent trait–performance relationships (e.g. Sandquist and Ehleringer, 1997; Donovan *et al.*, 2009). For example, Heschel *et al.* (2004) found that WUE was adaptive during drought, while decreased root biomass allocation was beneficial under wet conditions.

Multivariate axes of trait variation and correlations among traits (where they existed) were similar across years and water treatments, and were largely consistent with the trade-off between resource acquisition and conservation reflected in the leaf economic spectrum (Wright *et al.*, 2004). These data support the idea that, while trait values may change with age, trade-offs among resource acquisition, resource allocation and maintenance costs exist across an individual's lifetime. In fact, these trade-offs may be determined at a very early age. Larson *et al.* (2020) found that trait variation associated with growth/economic strategies existed in seedlings that were just 4 d old. Consistent trait relationships will simplify efforts to use traits to model large-scale processes; however, some studies find limited agreement between seedling and adult traits (Harrison and LaForgia, 2019).

The influence of physiological and morphological traits on fitness is complex because traits influence reproductive fitness through performance measures such as growth rate and plant size (Farris and Lechowicz, 1990; Geber and Griffen, 2003). Here, we found consistent positive correlations between canopy
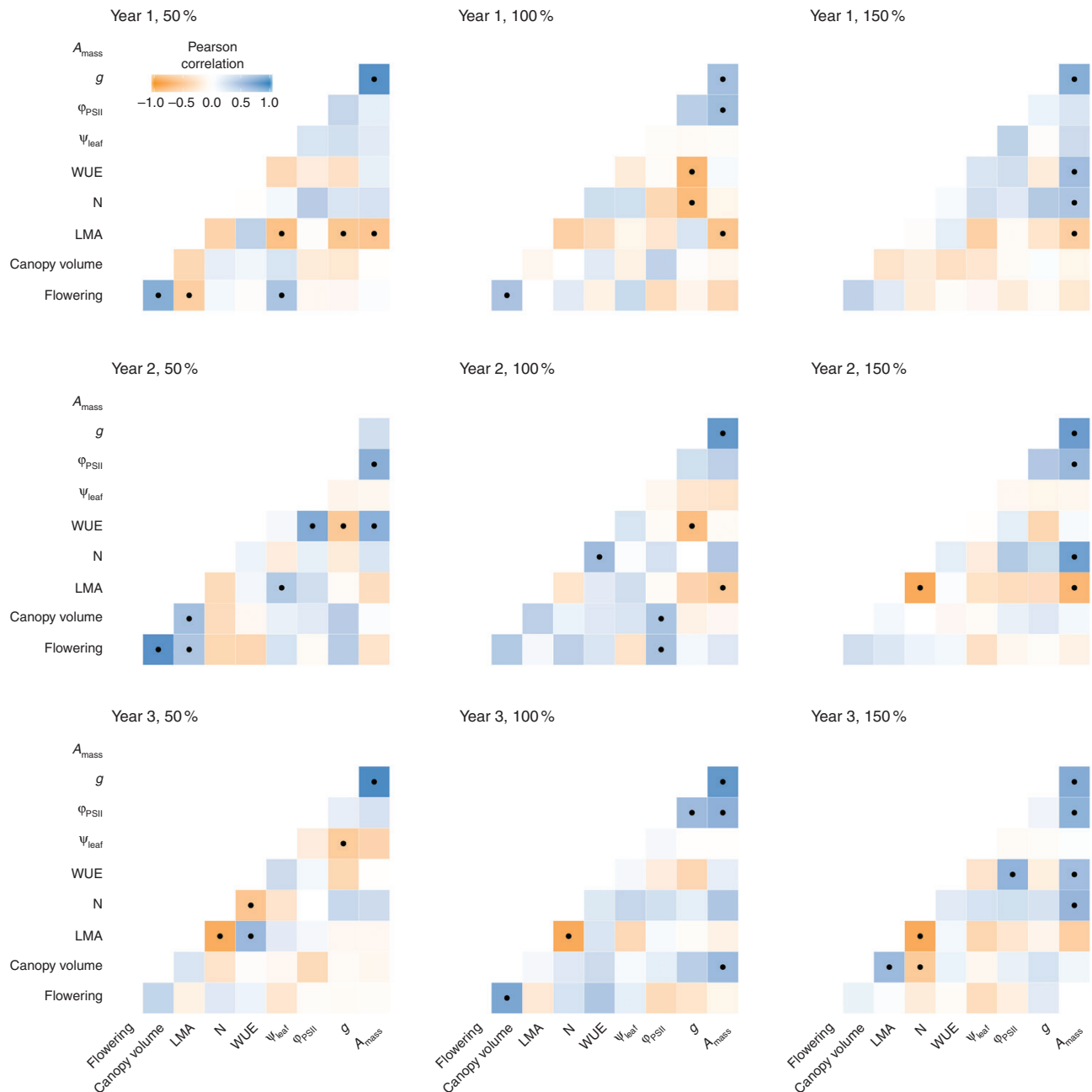
FIG. 3. Pearson correlation coefficients for *A. californica* leaf traits across water treatments in each of the 3 years. Blue and orange squares indicate positive and negative correlations, respectively. Significant correlations at $P < 0.05$ are shown with a dot in the square. Trait abbreviations are provided in Table 1.

volume and reproductive allocation, although these were only statistically significant for some year and treatment combinations, suggesting that larger plants have more resources available for reproduction. Morphological and physiological traits may influence growth through several functional pathways; thus, the contribution of any one trait to plant performance cannot be examined in isolation from other traits. As we gain a better understanding of how traits interact to influence growth, partitioning the effects of individual traits on plant performance via tools such as structural equation modelling will be possible; however, few experimental designs include the large sample sizes needed for structural equation models.

Why were there so few correlations among traits and performance? Trait variation is sensitive to where a population is positioned on an environmental gradient (Albert *et al.*, 2010); thus, if our precipitation treatments (50–150 % ambient) fell within the optimal range for *A. californica*, it is possible that we sampled a limited range of trait values, resulting in weak or insignificant correlations. Additionally, different combinations of traits may promote equal fitness (Marks and Lechowicz, 2006), resulting in weak correlations between individual traits and performance. For example, populations of *Encelia farinosa*, a desert shrub, from a dry and wet site displayed different trait values for photosynthetic rate, leaf area and growth, but these different trait combinations

TABLE 1. *Traits measured in the study along with their abbreviation, function and observed relationships with increasing age and water availability*

Trait	Abbreviation	Function	Age	Water
Mass-based photosynthetic rate	A_{mass}	Carbon assimilation, Leaf construction	–	
Stomatal conductance	g	Carbon assimilation	–	+ (Y1)
Efficiency of photosystem II	Φ_{PSII}	Light-use efficiency	–	+ (Y3)
Mid-day water potential	Ψ_{leaf}	Stress		– (Y1, 2)
Water-use efficiency	WUE	Water-use efficiency		– (Y1)
Leaf nitrogen concentration	Leaf N	Carbon assimilation, Water-use efficiency	–	
Leaf mass per area	LMA	Leaf construction	+	
Canopy volume		Growth	+	+ (Y1, 2)
Flowering effort		Fitness	+	+

Effects of water availability were only statistically significant in certain years; these are denoted with Y1–3. For a more detailed treatment of observed effects, see Fig. 1.

resulted in a similar flowering effort (Sandquist and Ehleringer, 1997). Finally, a more complete picture may emerge when additional traits are considered, especially root traits, which may be important in this semi-arid system (Nguyen *et al.*, 2017).

Our study demonstrates that plant age and environmental gradients create a sizable amount of intraspecific trait variation, which complicates the use of species means to model ecological processes (e.g. Laughlin *et al.*, 2018). Previous studies have found that intraspecific trait variation can be nearly as large as interspecific variation in communities and across taxa (Mason *et al.*, 2013; Siefert *et al.*, 2015). However, our study suggests that variation may be constrained to certain ontogenetic stages and environments. In particular, our data suggest that much of the trait variation across different age cohorts is captured during the establishment phase and that age is a stronger driver of intraspecific trait variation than water availability. In the absence of such data, ecologists should randomly select, or select in a standardized way, individual organs, plants and populations that best suit the questions being addressed (Pérez-Harguindeguy *et al.*, 2013).

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Table S1: soil properties in the experimental plots at the South Coast Research and Extension Center. Table S2: repeated-measures ANOVA results with treatment, year and their interaction as fixed effects and individual plant as a random effect. Table S3: plasticity indices (PI_v) for each trait in each year and averaged across years. Table S4: standardized loadings for four relative components from PCA with all traits. Figure S1: daily average soil volumetric water content for the three water treatments over three growing seasons. Figure S2: PCA of leaf traits in Year 1.

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