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Research paper

Leaf trait coordination and variation of blue oak across topo-environmental scales

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Trees are arguably the most diverse and complex macro-organisms on Earth. The equally diverse functions of trees directly impact fluxes of carbon, water and energy from the land surface. A number of recent studies have shed light on the substantial within-species variability across plant traits, including aspects of leaf morphology and plant allocation of photosynthates to leaf biomass. Yet, within-tree variability in leaf traits due to microclimatic variations, leaf hydraulic coordination across traits at different physiological scales and variations in leaf traits over a growing season remain poorly studied. This knowledge gap is stymieing the fundamental understanding of what drives trait variation and covariation from tissues to trees to landscapes. Here, we present an extensive dataset measuring within-tree heterogeneity in leaf traits in California's blue oak (*Quercus douglasii*) across an edaphic gradient and over the course of a growing season at an oak–grass savanna in Southern CA, USA. We found a high level of within-tree crown leaf area:sapwood area variation that was not attributable to sample height or aspect. We also found a higher level of trait integration at the tree level, rather than branch level, suggesting that trees optimize water use at the organismal level. Despite the large variance in traits within a tree crown and across trees, we did not find strong evidence for adaptive plasticity or acclimation in leaf morphological traits (e.g., changes to phenotype which increased fitness) across temporal and spatial water availability gradients. Collectively, our results highlight strong variation in drought-related physiology, but limited evidence for adaptive trait plasticity over shorter time scales.

Keywords: hydraulic efficiency, leaf morphology, plant functional traits, *Quercus douglasii*, trait covariation, trait plasticity.

Introduction

Physiological and morphological plant traits influence functional responses to changes in resource availability. In water-limited systems, leaf morphological traits, such as leaf area:sapwood area (A_L:A_S), leaf size, leaf number and specific leaf area (SLA), are particularly important for determining the '*leaf hydraulic trait integration*' or covariation of key leaf traits that influence vegetation water demand, resistance to water deficits or even stress and carbon assimilation. For example, A_L:A_S determines the plant water demand of the leaves relative to the stem supply 'pipe', which is often assumed to be conserved along the entirety of a plant's branching architecture (Shinozaki et al. 1964, Mencuccini et al. 2019). The A_L:A_S has been shown to adjust to the changes in water availability across climate gradients within a species (Mencuccini and Grace 1994, DeLucia et al. 2000, Pinol and Sala 2000, Mencuccini and Bonosi 2001, Martinez-Vilalta et al. 2009, Rosas et al. 2019). This geographic adjustment could be driven by local adaptation, meaning genetically determined trait differences among populations. However, the pervasive adjustment in A_L:A_S is likely at least partially accomplished through environmentally driven plasticity that could be reversible (termed acclimation) or irreversible (e.g., developmental plasticity leading to divergent mature phenotypes) and which is typically assumed, but rarely shown, to be adaptive (i.e., trait change that increases fitness in a given environment (Nicotra and Davidson (2010)). The A_L:A_S is dependent in part on both SLA, or the amount of carbon a tree must invest per unit leaf area, and leaf size, both of which can affect the total leaf area and the numerator of A_L:A_S. Thus, plasticity in A_L:A_S can be regulated through integrated adjustments in either the SLA and/or leaf size, where both decreased SLA and decreased leaf size drive lower A_L:A_S and are associated with an increased leaf robustness to lower water availability within and across species (Poorter et al. 2009, Anderegg et al. 2021).

It is well established that, across species, leaf traits vary along an economic spectrum presumably to maximize the plant fitness (e.g., Reich 2014). However, this economic variation is more complicated within species (Anderegg et al. 2018) and within individual canopies (Lusk et al. 2008), and its direct relationship with allocation traits, such as A_L:A_S, is less well understood (Rowland et al. 2023). Although several studies have sampled within-tree variation in leaf morphological traits, such as A_L:A_S, the number of within-tree samples is generally limited due to tradeoffs in sampling because measurements are labor-intensive, especially for large sample sizes. As a result, previous efforts to understand within-species variation have aimed to sample across macro- rather than micro-gradients in climate (Anderegg et al. 2021) and inter- rather than intra-annually (Kerr et al. 2022). Further, within-tree and within-growing season variability in traits that impact leaf hydraulic integration, such as A_L:A_S, remain

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major physiological unknowns, and these have been identified as a priority research area for understanding the organismal phenotype and hydraulic function (Mencuccini et al. 2019).

Determining the hydraulic integration among traits and understanding the physiological scale at which integration emerges are both necessary for understanding the collective effects of trait variation on vegetation function. Two different hypotheses regarding such coordination lead to differing predictions of the scale at which we expect to see trait integration: (i) that strong branch-level coordination becomes weaker at the whole-tree level because traits are developmentally linked, but the tree crown consists of branches in a wide variety of environments; and (ii) that trait coordination is only manifested across large enough environmental gradients to cause large trait change, while within-site and within-crown variations are more stochastic. For example, in some studies, trait coordination across tissues was only detectable across climatically disparate sites (Anderegg et al. 2021). Thus, considering trait hydraulic integration at both the branch and individual levels is important in furthering our understanding of multiscale trait integration.

Within a tree crown, there is a mechanistic basis for expecting gradients in leaf morphology because variation of the leaf radiation load and the hydraulic path length from the roots to the leaves depend on the height of the tree, aspect within a tree crown, crown architecture and leaf clumping (Nikinmaa 1992, van der Sande et al. 2015). There is also precedent from other leaf economic trait studies. For example, it is well known that photosynthetic traits differ markedly in sun versus shade leaves within a tree crown (Dawson and Bliss 1993, Niinemets 2016) and change with leaf age over a leaf's lifetime (Menezes et al. 2022). However, there is also substantial evidence that the light environment, even at a particular depth in the crown or fixed aspect, is highly dynamic with brief, unpredictable periods of direct solar irradiance (termed sunflecks) (Chazdon and Pearcy 1991). For example, sunflecks have been shown to influence vegetation processes ranging from understory dynamics to tree and grassland physiology (Knapp and Smith 1987, Chazdon and Pearcy 1991, Buckley et al. 2023). Finally, in addition to a difficult-to-predict and dynamic canopy microenvironment, there are other plant physiological unknowns such as how xylem segmentation and conduit diameter could affect the variability between branches (Olson et al. 2020). Collectively, these factors influence the hyper-local microclimate, water demand and hydraulic stress experienced in a given tissue and may cause a breakdown of clear patterns in traits within a crown or strong hydraulic integration across traits at the branch level.

At the organismal level, the processes stimulating hydraulic trait variation and integration across traits extend beyond the leaves. For example, heterogeneity in water access due to fine-scale variations in soil type, rock water access (McCormick et al. 2021) or tree rooting depth could alter the timing and absolute levels of maximum water stress and could stimulate strong whole-tree integration between the rooting depth and A_L:A_S or other traits (Trugman et al. 2021). Across time, the strong seasonal variability in tissue growth with multiple environmental drivers and covariation across environmental drivers (Lupi et al. 2010) likely impacts the tree-level hydraulic trait integration. In each case, at the branch and tree levels, one would expect selection pressures for lower A_L:A_S, SLA and leaf size with decreased water availability. However, given the physiological and environmental complexities, it is not clear whether to expect stronger branch-level or whole-tree level hydraulic trait integration a priori.

Knowledge of possible adjustments across space and time in leaf morphology and hydraulic trait integration is critical not only for basic plant physiological understanding but may also be important for predicting demographic trends. For example, the ability of trees to adaptively adjust A_L:A_S throughout the growing season, either through leaf shedding or stem growth, could prevent lethal consequences of structural overshoot, whereby water abundance and rapid tree growth are followed by decreases in water availability, stronger water demand relative to supply and elevated hydraulic stress (Jump et al. 2017), such as would be in the case of an unanticipated dry end to a growing season. Thus, adaptive acclimation (reversable trait change that increases fitness) in A_L:A_S and its derivative traits could minimize increases in plant stress and decrease the plant mortality (e.g., Figure 1A).

Oak trees (*Quercus* spp.) are found on five continents and are the Northern Hemisphere's most ecologically and economically important angiosperm genus (Cavender-Bares 2019). Blue oak (*Quercus douglasii*) is an ecologically and culturally important deciduous oak species, which is dominant throughout much of California's oak woodland ecosystems that span the state at low elevations. Further, California's Mediterranean climate, with cool wet winters and warm dry summers, provides an ideal natural experiment to observe strong increases in water stress in blue oaks as the growing season progresses, allowing us to test the potential for adaptive plasticity in leaf size and number within a growing season. In this study, to understand leaf hydraulic integration and variation at fine spatial and temporal scales, we extensively sampled blue oaks at the Sedgwick Reserve in Southern CA, USA, at the dry range edge of blue oak extent (Figure 1B). We asked the following questions. (i) At what biological level of organization does leaf morphology vary most within blue oaks as a species? (ii) Do we observe clear hydraulic integration between leaf morphological traits within blue oaks, and at what biological scale(s)? (iii) Are there plastic responses in blue oaks leaf morphological traits within a growing season to mediate the stress impacts associated with decreasing water availability as the dry Mediterranean summer progresses? If we do observe clear plastic changes to phenotype, are they related to patterns of water stress and crown mortality?

Materials and methods

Site description

This study was conducted over the summer of 2021 at the University of California's Sedgwick Reserve (34°41′30″ N, 120°02′48″ W), which is located in the Santa Ynez Valley of Santa Barbara County, CA, USA (Figure 1B). Sedgwick Reserve is an oak–grass savanna dominated by blue oak trees. The site has a Mediterranean climate with a mean annual temperature of 16.7 °C and a mean annual precipitation (MAP) of 398 mm based on 30-year means (1981–2010) (Prism Climate Group, Oregon State University, 2011). Our study year was much drier than average, with a MAP of 197 mm for the 2021 water year.

Inventory measurements

To investigate the dominant scales of within-species variations, leaf hydraulic trait integration between A_L:A_S, SLA and

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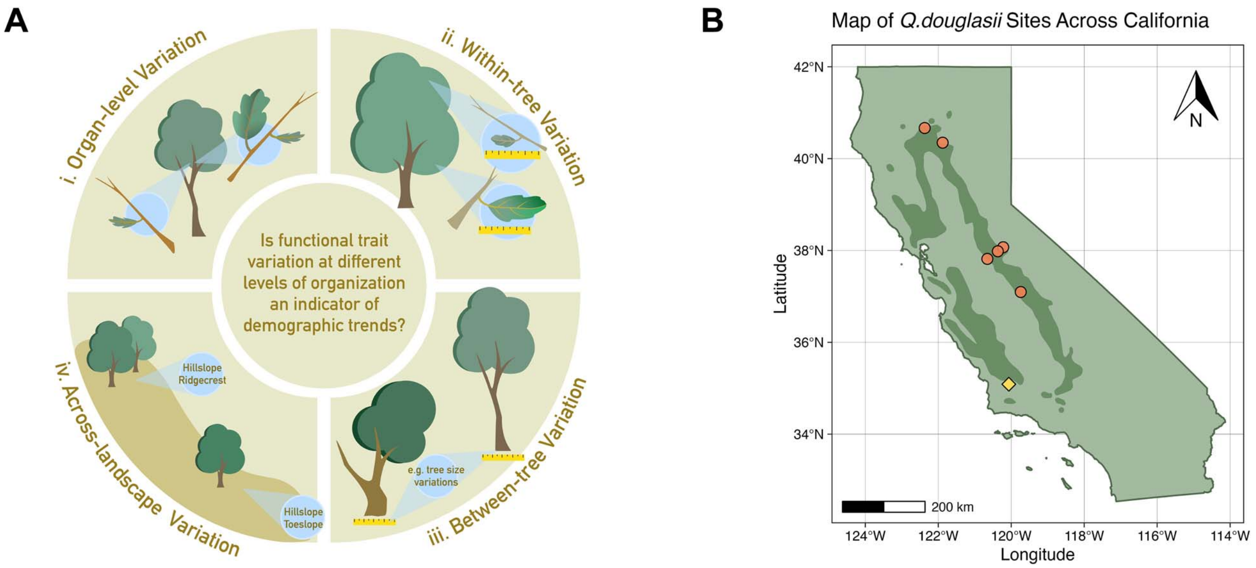

Figure 1. (A) Schematic of the biological levels of organization for leaf morphology addressed in this study, including variation in leaf morphology at a (i) fixed height or aspect within a tree, (ii) within-tree but cross height or aspect variation, (iii) between-tree variation (iv) and across-landscape variation such as variation between trees at areas of topographic water convergence or divergence. (B) Map of study sites across CA, USA. The diamond in (B) indicates the location of this study at Sedgwick Reserve and the circular points indicate CA-wide sites where blue oaks were sampled (see (Anderegg et al. 2023)). Darker shades indicate the current range of blue oaks in CA.

leaf size (e.g., Figure 1A) and subsequent impacts on the plant water status and fitness, we established four 50-m transects. Two were established on ridge crests, where we expected water divergence. Both ridge crest transects were paired with a similar transect at their respective toe slopes, where we expected water convergence. This experimental setup aimed to maximize the differences in local water availability and potential for subsequent phenotypic responses. One ridge–toe slope transect pair faced northwest and the other transect pair faced east. The number of trees per transect ranged from 8 to 21 (Table S1 available as Supplementary data at *Tree Physiology* Online). We visited the sites in the early (May/June) and late (August) summer of 2021 to quantify the progressive changes in plant water status and function during the summer dry down that is characteristic of Mediterranean climates. We took the following measurements for all living blue oak trees within 5 m of each side of our established transects: tree height (m), diameter at breast height (DBH, cm) and crown mortality (percent). Tree height was measured with a digital clinometer, DBH was measured with a DBH tape and crown mortality was visually estimated in increments of 10%. Summary statistics of all transect inventory and trait data are available in Table S1 available as Supplementary data at *Tree Physiology* Online.

Leaf trait measurements

For six to nine randomly selected trees per transect (depending on the relative density of blue oak trees, our study species, to coast live oak trees in the transect, *Quercus agrifolia*), we selected three terminal branches at two crown heights in each of the four cardinal directions cut at the prior year bud scar, and we included all current year stem and leaf tissues, for a maximum of 24 samples per tree per sampling period (or 48 over the field campaign). In cases where there were not enough branches to sample at a particular direction or height due to crown mortality or asymmetric crown shape, samples were taken at cardinal directions nearest to the one that was missing (e.g., collecting branchlets from the NW side of the tree when there is nothing to sample at the N side of tree) or omitted in the case of extensive crown mortality. The average number of samples per tree per measurement period (e.g., June and August) was 12.35 due to the prevalence of crown mortality (Figure S1 available as Supplementary data at *Tree Physiology* Online). We then measured the SLA, average leaf size, total number of leaves and A_L:A_S on current year terminal twigs (i.e., only tissue grown during the 2021 growing season) and all attached leaves using calipers, balances and ImageJ image analysis software (Table 1). The A_L:A_S and leaf size only were measured in May/June, and A_L:A_S, leaf size and SLA were all measured in August. In total, ~1100 samples were taken across the summer of 2021, representing one of the most comprehensive characterizations of within-tree variation in leaf traits in the literature to our knowledge. To complement our intensive Sedgwick Reserve-based leaf trait measurements, which is located at the dry range edge terminus of blue oak tree extent (Figure 1B), we compared our measurements with the range-wide within-species variation in leaf traits of blue oak distributed across CA (Figure 1B) (Anderegg et al. 2023).

Leaf water potential

As a diagnostic of maximum seasonal plant hydraulic stress during the 2021 growing season, we measured predawn water potentials before end-of-season leaf senescence in October 2021. Leaf water potentials were collected 2–3 h before dawn from several trees in each transect (see Table S1 available as Supplementary data at *Tree Physiology* Online) at the time of maximum tree water stress before winter precipitation occurred. A precipitation event part way through our measurements precluded us from collecting water potentials on all trees sampled for traits, as the predawn leaf water potentials post-rain would not give us an accurate diagnostic on maximum seasonal tree water stress for the 2021 growing season.

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Table 1. Measured leaf traits.

Trait	Definition
SLA	Dry mass SLA (cm^2 wet leaf area per g leaf dry mass)
A_L:A_S	Terminal branch leaf area to sapwood area ratio (cm^2 leaf area per mm^2 stem area underneath bark)
Leaf size	Average area of a single leaf based on all leaves on a terminal branch (cm^2 fresh leaf area)
Leaf number	Average number of leaves on current year's terminal twigs

To make water potential measurements, small branches were clipped from the crown of each tree. Individual leaves were then rapidly clipped at the petiole, wrapped in a slightly moistened paper towel to slow dehydration and immediately measured in a Scholander-style pressure chamber (model 1000, PMS Instruments, Corvallis, OR, USA). For each tree, we measured a minimum of three leaves and averaged across samples for all analyses.

Variance decomposition analysis

We performed variance decompositions on all trait measurements made at the Sedgwick Reserve to determine the dominant scale of trait variation (within-tree crowns, among individual trees within a transect/landscape position and between-transects/landscape positions). Our standard model for traits was a linear mixed-effects model with a fixed intercept term and was a random intercept for transect and tree nested within-transect. In this formulation, the random effect variance parameters represent the between-transect and between-tree within-transect, with the residual variance representing within-tree variance.

We also explored several other variations to understand the variance over time (for traits that were measured twice) and to understand the within-crown variation to height-related and aspect-related variations. These results are presented in the Supplementary data, given that we were not able to use the same model for all traits for these supplemental analyses. For our leaf size and A_L:A_S, we experimented with fitting linear mixed-effects models with a fixed intercept for sampling date and a random intercept for transect and tree nested within-transect (we did not have two sample dates for SLA). In this formulation, the marginal R^2 represents the variance due to date in the growing season, and the random effect variance parameters represent the between-transect and between-tree within-transect, with the residual variance representing within-tree variance. We also experimented with an additional random intercept for either height or aspect nested within tree. However, for SLA and leaf size, we were unable to get model convergence when including either height or aspect nested within-tree as a random intercept, and we found that neither height nor aspect could explain the A_L:A_S variance.

All analyses were performed in the R statistical environment (v.4.2.0; R Core Team, 2019). Mixed-effects models were fitted using the lme4 and lmerTest packages (Bates et al. 2015, Kuznetsova and Brockhoff 2017).

A_L:A_S relationship with tree structural attributes

We quantified the effect of vegetation structural attributes and trait–trait relationships on A_L:A_S. Specifically, we performed information theoretical-based model selection using linear mixed-effects models. The most complicated model included a global model with fixed intercept terms for DBH, crown mortality, sample aspect, sample height, SLA and leaf size and a random intercept for transect and tree nested within-transect, and we compared this model with all subsets of the global model using the dredge() function from the MuMIn package (v 1.47.5). We then used Akiake's Information Criterion corrected for small sample sizes (AICc) to select the best model. We used a separate global model to quantify the importance of the sampling period variance in A_L:A_S, with fixed intercept terms for DBH, crown mortality, sample aspect, sample height, leaf size and time and a random intercept for transect and tree nested within-transect (given that we only had SLA at the second period of measurement).

Trait–trait coordination

To examine the level of hydraulic trait integration across different physiological scales, we calculated Pearson's correlation coefficients for all pairs of traits at both the branch-level and the individual-averaged traits for both our Sedgwick Reserve trees and those collected state-wide (Anderegg et al. 2023). To avoid the impact that a larger sample size has on the correlation significance, we randomly resampled with replacement branch-level measurements to match the individual tree-averaged sample size over 1000 iterations and took the average correlation coefficient and significance. We visualized the correlation structure using the corrplot() function in the corrplot package (v 0.92). We then examined the difference between the trait–trait correlations in each pair of traits averaged to the individual-level versus branch-level trait correlations.

Analyses

We ran *t*-tests to determine the differences between means of trait measurements between the Sedgwick Reserve and CA-wide trees. In particular, *t*-tests were run on SLA, A_L:A_S and leaf size to determine whether or not significant differences in means existed across locations (with a $P = 0.05$ significance threshold). Additionally, Spearman's rho was calculated to determine the strength of the relationship between the observed crown mortality and predawn water potentials.

Results

Leaf morphology trait variance across biological levels of organization

We found that within-tree and within-transect variations dominated as the largest variances terms for all leaf morphological traits measured such that the combined variance component within-tree and tree-to-tree at the same topographic position comprised >80% of the total trait variation (Figure 2). The dominant scale of variation did differ across traits, however. For A_L:A_S, within-tree variation constituted the majority of the variation (58.6%) and between-transect variation constituted only 13.3% of the variation (Figure 2A).

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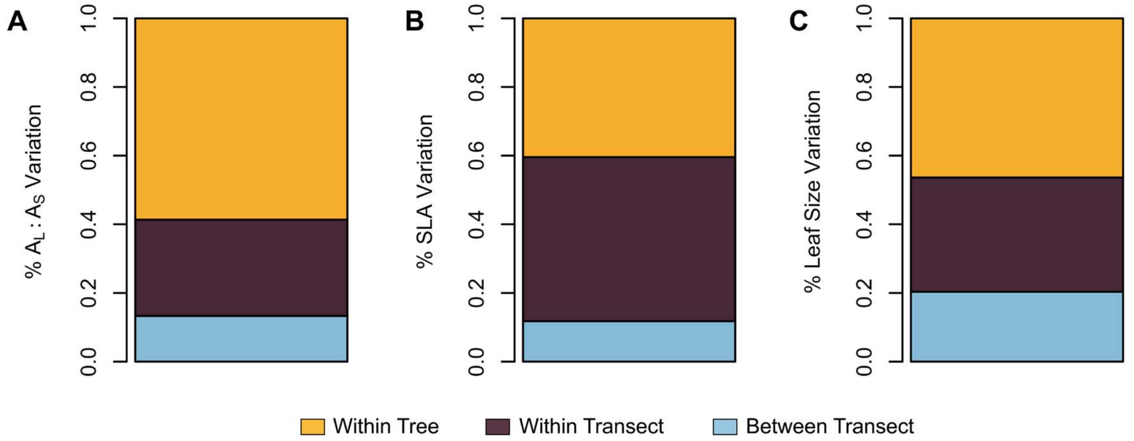

Figure 2. Variance decomposition of (A) leaf:sapwood area ($A_L:A_S$), (B) SLA and (C) leaf size measured for blue oak (*Q. douglasii*) across the topographic gradients in Sedgwick Reserve, CA, USA. Colored bars show proportion of total trait variance ('% trait variation').

For SLA, tree-to-tree variation within a transect constituted the largest trait variance component (47.8%) (Figure 2B). By contrast, for leaf size, within-crown variation constituted the largest trait variance component (46.4%), though variance was more evenly distributed across hierarchical scales compared with A_L:A_S and SLA (Figure 2C). For all traits measured, between-transect variation was the smallest variance component, ranging from 11.8 to 20.3% (Figure 2). For traits sampled twice (A_L:A_S and leaf size), between sampling times constituted 16.7% of variation for A_L:A_S, but these were was negligible for leaf size (Figure S2 available as Supplementary data at *Tree Physiology* Online). This difference between A_L:A_S and leaf size is consistent with the view that leaf expansion only occurs rapidly at the beginning of the growing season.

Counter to our expectations, neither the sample height nor the sample aspect within a given tree crown explained the variation in A_L:A_S. For variance decompositions that included either height nested within tree or aspect nested within tree as random intercepts and time as a fixed intercept, we found that within-height or within-aspect (within a given crown) constituted the majority of the variation (50.4 and 48.3%, respectively), followed by within-transect (25.4 and 23.7%, respectively), between sampling times (12.4 and 16.6%, respectively) and between-transects (11.3 and 11.4%, respectively). Interestingly, between-height within a crown or between-aspect within a crown constituted almost none of the within-tree variance (<0.01% for both) (Figure S3 available as Supplementary data at *Tree Physiology* Online).

There are several reasonable hypotheses related to tree water access and/or water demand that link the variation in leaf traits examined here to tree structural attributes such as tree size or crown mortality. However, we found no evidence that the tree size or crown mortality was associated with variations in A_L:A_S, SLA or leaf size (Figure 3) or changes in A_L:A_S over the course of the growing season (Figure S4 available as Supplementary data at *Tree Physiology* Online). Focusing on A_L:A_S, of the possible tree structural, physiological and environmental attributes tested, including DBH, crown mortality, sample aspect, sample height, SLA, leaf size and sample timing during growing season, the model that minimized AICc included only the SLA and leaf size (Table S2 available as Supplementary data at *Tree Physiology* Online). This result suggests significant hydraulic integration between traits and also indicates that DBH, crown mortality, sample aspect, sample height and sample timing do not significantly contribute to the observed variations in A_L:A_S.

Absolute trait ranges at the Sedgwick Reserve were large for all leaf morphological traits, and in some cases, were comparable to the entire range-wide trait variation for blue oak trees (Figure 4). For example, the mean and range of A_L:A_S sampled at the Sedgwick Reserve were not statistically different from that of blue oak A_L:A_S across the state of CA using a two-sample *t*-test and a $P = 0.05$ significance threshold (Figure 4A). The mean and ranges of both SLA and leaf size were significantly smaller ($P < 0.0001$) when compared with the global values of these traits in blue oaks (Figure 4B and C), with a relative decrease in trait medians of 18.73% for SLA and 47.85% for leaf size at the Sedgwick Reserve compared with CA-wide trees. The decreases in both the SLA and leaf size at the Sedgwick Reserve relative to the CA-wide populations are unsurprising because Sedgwick Reserve is at the dry range edge of blue oak extent (Figure 1B) and both reduced leaf size and low SLA are well-known plant adaptations to water stress (Gil-Pelegrín et al. 2017, Ramírez-Valiente et al. 2017). However, the fact that A_L:A_S was not proportionally reduced in response to site aridity deserves further consideration in the discussion.

Leaf hydraulic trait integration across biological levels of organization

We found significant covariation, or '*leaf hydraulic trait integration*', between A_L:A_S, SLA or leaf size (Figure 5 and Table S2 available as Supplementary data at *Tree Physiology* Online). Interestingly, trait integration was often stronger when averaged to the tree level compared with branch level, both for the Sedgwick Reserve blue oak population and CA-wide populations (Figure 5). Despite an average of 4× more samples per tree at Sedgwick Reserve compared with CA-wide trees, we saw much lower trait integration at the branch level compared with the tree level at Sedgwick Reserve (Figure 5A and B). By contrast, trait integration was comparable for the branch- and tree-averaged traits in the CA-wide populations, save for insignificant correlations between leaf number–leaf size and leaf number–SLA which were marginally significant ($P < 0.1$) for tree-averaged values (Figure 5C and D). Across all blue oak populations for

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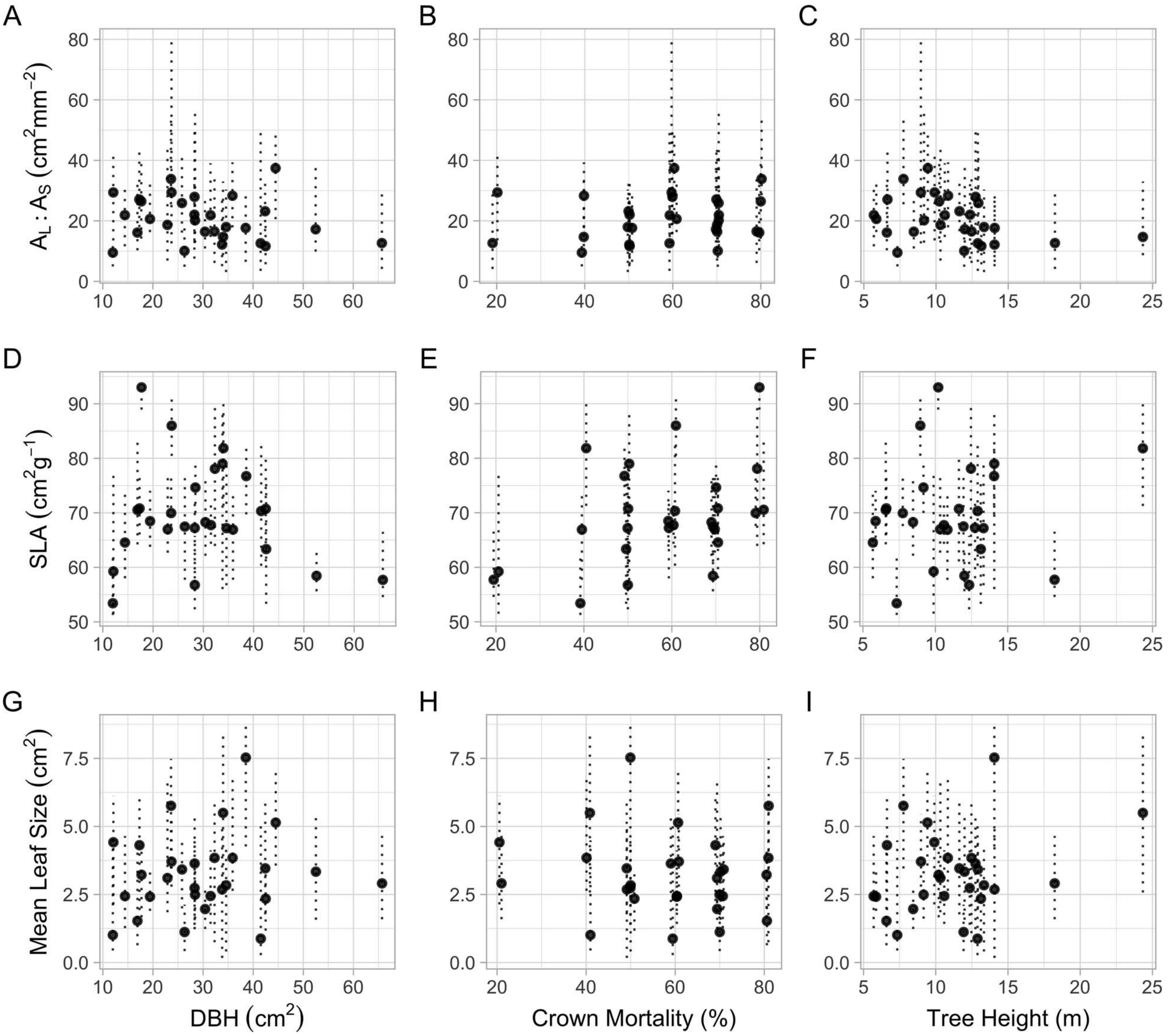

Figure 3. Within-tree trait range for (A–C) leaf:sapwood area ($A_L:A_S$), (D–F) SLA and (G–I) mean leaf size as a function of (A, D, G) tree DBH, (B, E, H) crown mortality and (C, F, I) tree height. Black dots represent individual trees in this study, and dashed lines represent the within-tree trait range.

tree-averaged measurements, we found a significant tradeoff between leaf size and leaf number, though the tradeoff and significance was stronger in the Sedgwick Reserve population compared with CA-wide trees. We also found significant positive correlations between leaf size–A_L:A_S and A_L:A_S–SLA (Figure 5B and D). These results are in agreement with traditional assumptions that higher water availability is associated with larger leaves, larger A_L:A_S and higher SLA.

Trait responses to tree stress

Previous studies have found a consistent relationship between predawn water potentials and tree mortality risk (Sapes and Sala 2021). At the Sedgwick Reserve, we found that crown mortality was significantly correlated with elevated tree hydraulic stress as measured through predawn water potentials at the end of the dry season (Spearman's rho = 0.749, $P = 0.02$) (Figure 6A), indicating that crown mortality is likely an early indicator of eventual mortality. However, we did not find any evidence for (presumably adaptive) adjustments in A_L:A_S to mitigate stress. For example, we found no statistical differences between the A_L:A_S of trees in the upper quartile of crown mortality (corresponding to >70% crown mortality) compared with the bottom quartile (corresponding to <50% crown mortality) (Figure 6B). Further, observed crown mortality was not related to the magnitude of seasonal shift (i.e., magnitude of plasticity) in A_L:A_S from June to August (Figure 6C). There was no statistically significant difference in the allocational changes between high and low crown mortality tree groups (Figure 6C) and there was no statistically significant change in A_L:A_S between sampling periods (Figure 6D).

Discussion

Our extensive dataset of >1100 trait measurements averaging 24.7 per tree (summed over two sampling periods) demonstrated that within-tree variation in A_L:A_S is the dominant scale of variation. Further, A_L:A_S variance at a single, topographically complex site is comparable to the total within-species A_L:A_S variance across the state of CA. Interestingly,

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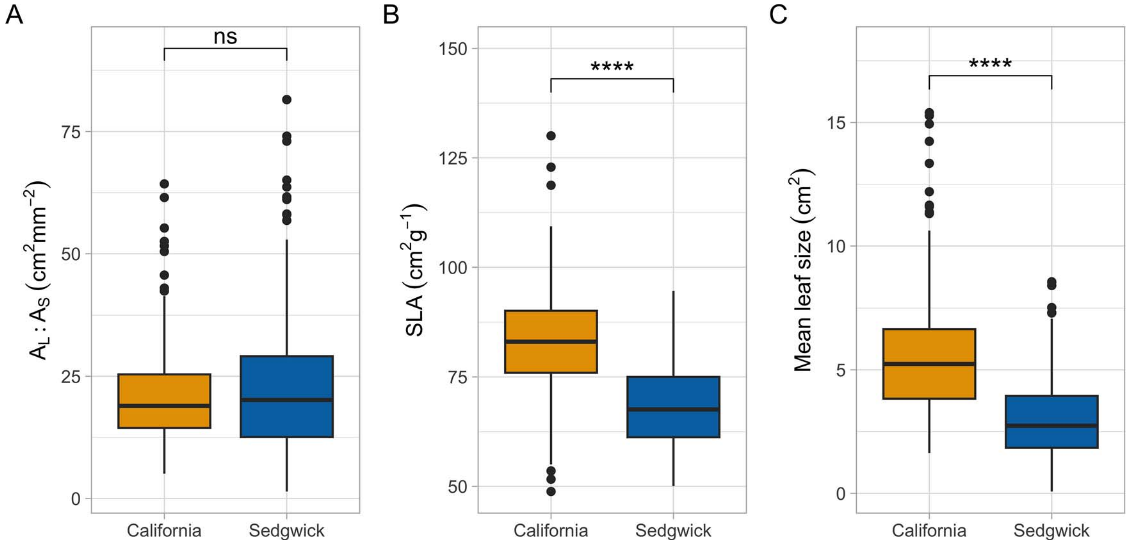

Figure 4. For some leaf traits, the trait range for blue oaks at Sedgwick Reserve is comparable to the global trait range in CA. Comparisons of blue oak leaf morphological traits across CA and at Sedgwick Reserve for (A) leaf:sapwood area (A_L:A_S), (B) SLA and (C) mean leaf size. For the box–whiskers plots, black lines indicate the median trait value, boxes denote the interquartile range (IQR), whiskers denote 1.5 times the IQR and black dots indicate outliers. Significance stars were calculated using a two-sample *t*-test with *P*-values at the 0.05 level and indicate the level of significance for differences between means (ns for non-significant and ∗∗∗∗ for $P < 0.0001$).

leaf hydraulic trait integration or the correlations between A_L:A_S, SLA and leaf size were stronger at the tree-averaged level compared with the branch level. Despite the large variance in A_L:A_S with our study site, we did not find evidence for acclimation in A_L:A_S across topographic gradients, gradients in long-term stress or as the dry season progressed in the blue oak population at Sedgwick Reserve.

A_L:A_S variance and plant water use

Several studies have documented within-species adjustments in A_L:A_S across geographic gradients in climate (Martinez-Vilalta et al. 2009, Anderegg et al. 2021) and linked the adjustments in this ratio mechanistically to the changes in water availability (Trugman et al. 2019). Despite strong evidence for adaptive variation across space at broad scales, we found that hillslope position-related variation in A_L:A_S, as might be predicted from topographically mediated differences in water availability, accounted for <14% of trait variance. This may be due to the extreme aridity of Sedgwick Reserve. The MAP is ∼400 mm, thus there is likely little overland or lateral subsurface flow in most years at the field site. In this case, topographic position would not necessarily be predictive of water supply. Instead, we found that variation among trees within a transect dwarfed topographic differences, and variation within individual crowns dwarfed the tree-to-tree variation.

The fact that within-crown variation is the dominant scale of variation for A_L:A_S is intriguing and also present beyond the *Quercus* genus (e.g., *Acacia* and *Eucalyptus*, see Anderegg et al. 2021). Further, this variation in A_L:A_S was poorly explained by crown position, either based on the hydraulic pathlength or radiation load within a tree crown associated with branch height or aspect (respectively) (Figure S2A and B and Table S2 available as Supplementary data at *Tree Physiology* Online). The lack of systematic variation within a crown merits additional discussion and hypotheses. However, first, we must acknowledge that accurately sampling enough within-crown variance and detailing the environmental conditions within a tree to is a difficult task, so it is possible that there are height and/or pathlength controls that are significant but not detected due to our sampling methodology. It is also possible that our model system, which is a blue oak open-canopy savanna, might display different dominant scales of trait variation than trees in a closed-canopy forest where light variation with tree height is more systematic. With these acknowledgements, we offer two alternative, physiologically focused hypotheses as to how within-crown variance influences plant water use strategy. (H1) Water use is regulated at the branch level and A_L:A_S adjustments are stimulated by hyper-local microclimatic effects that are the product of the precise arrangement of leaves within a tree crown and sunfleck distribution (rather than systematic variations in hydraulic path length or radiation). (H2) Trees are optimizing for whole-plant rather than branch-level water use, and the variation of A_L:A_S within a tree crown is more stochastic, independent of microclimate (whether sunfleck/leaf angle driven or hydraulic path length/aspect driven).

In H2, the drivers of allocation for any specific branch are less coordinated, which can be attributed to a variety of factors such as: (i) variability of hormone signaling and regulation at fine scales, (ii) differential impacts of xylem segmentation or anatomy between branches, (iii) the stochasticity of herbivory and physical damage or (iv) the randomness of microenvironmental drivers of leaf versus stem development and expansion. Importantly, the whole tree crown is diffusely coordinated to impact the whole-plant water use, given that organismal-level function is the critical factor in tree fitness. Our results show that there is less leaf hydraulic trait integration at the branch-level compared with tree-averaged traits (Figure 5), which points toward H2, that blue oak trees optimize the whole-plant water use as a more viable hypothesis to consider moving forward. Importantly, the proportion of within-crown variance captured in this study with 12.35 samples per tree per time period is comparable to the estimates from the range-wide study of blue oaks using four

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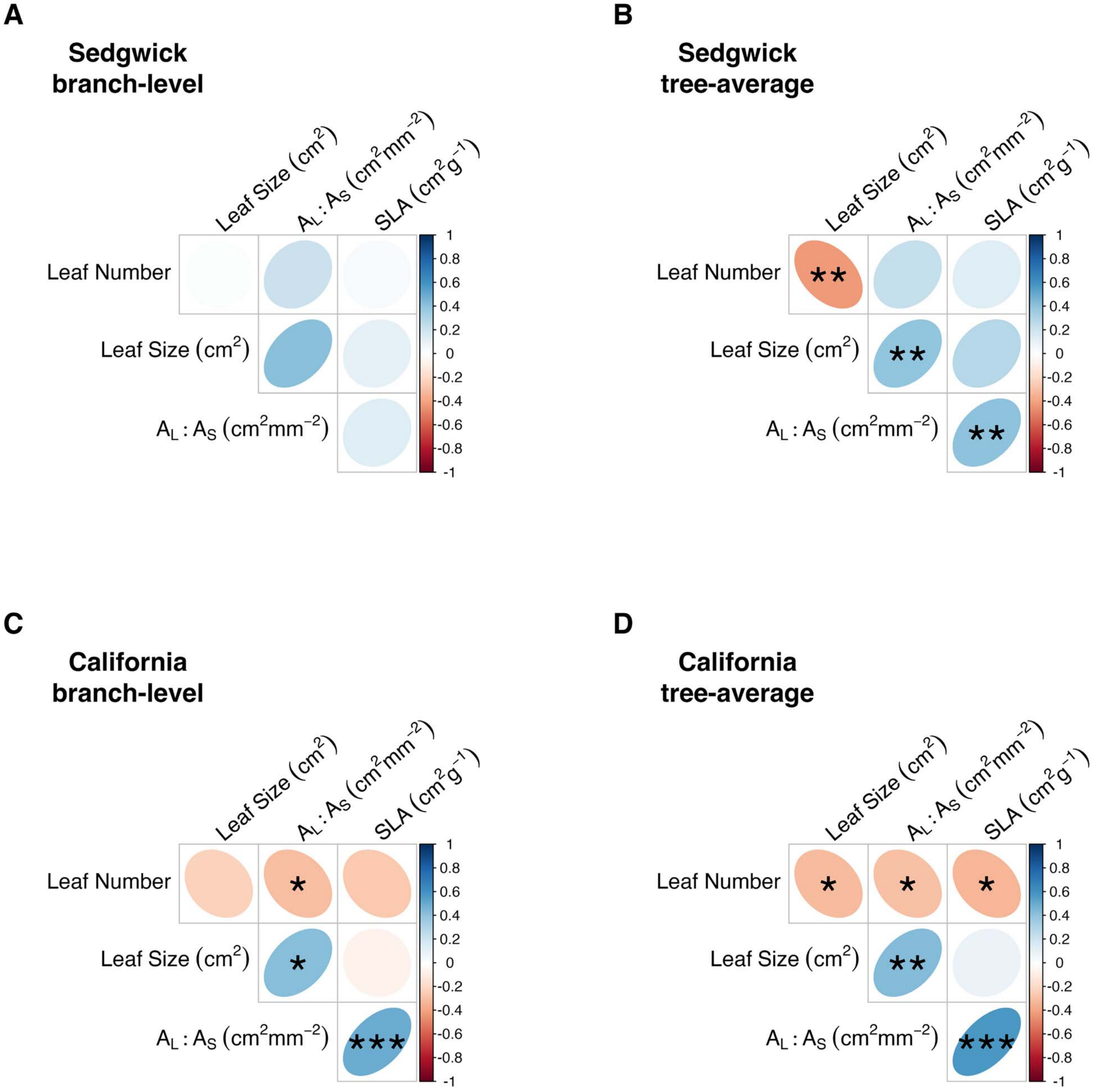

Figure 5. Branch- (A, C) and tree-averaged (B, D) trait–trait correlations in Sedgwick Reserve blue oak population (A and B) and across several blue oak populations CA-wide (C and D). Ellipses and color show direction and strength of the correlation, and points show statistically significant relationships at the $P < 0.1$, $P < 0.05$ and $P < 0.01$.

to five samples per tree (Anderegg et al. 2023) and of another study in eight Australian angiosperms where three samples per tree were used (Anderegg et al. 2021), suggesting that three samples per tree is a sufficient protocol moving forward and that within-tree heterogeneity is likely the norm across species.

Trait integration across scales

In addition to finding less trait integration at the branch level (see discussion around H2), we found less trait integration at the Sedgwick Reserve relative to the CA-wide populations (Figure 5B and D). Trait integration patterns are particularly apparent for the correlations between leaf size–A_L:A_S and SLA–A_L:A_S. It is possible that the decreased trait integration at the Sedgwick Reserve is due to the smaller trait space spanned for leaf size and SLA compared with the trait space covered in the CA-wide populations (Figure 4B and C). However, it is intriguing that the SLA and leaf size were restricted at the Sedgwick Reserve compared with populations across CA, likely in response to site aridity, but the A_L:A_S at Sedgwick Reserve is not (Figures 1B and 4). This suggests some other potential compensating physiological mechanism(s) may be at play that allow for the sustained variation in A_L:A_S in dry sites. For example, A_L:A_S is in part related to SLA and leaf size (which are integral in determining total leaf area), but sapwood cross sectional area and xylem conductance (related to xylem conduit diameter) are also integral in the leaf and stem hydraulic integration (Anfodillo and Olson 2021), so it is possible that adjustments in xylem physiology are an important compensating factor governing the plant phenotypic responses at the Sedgwick Reserve.

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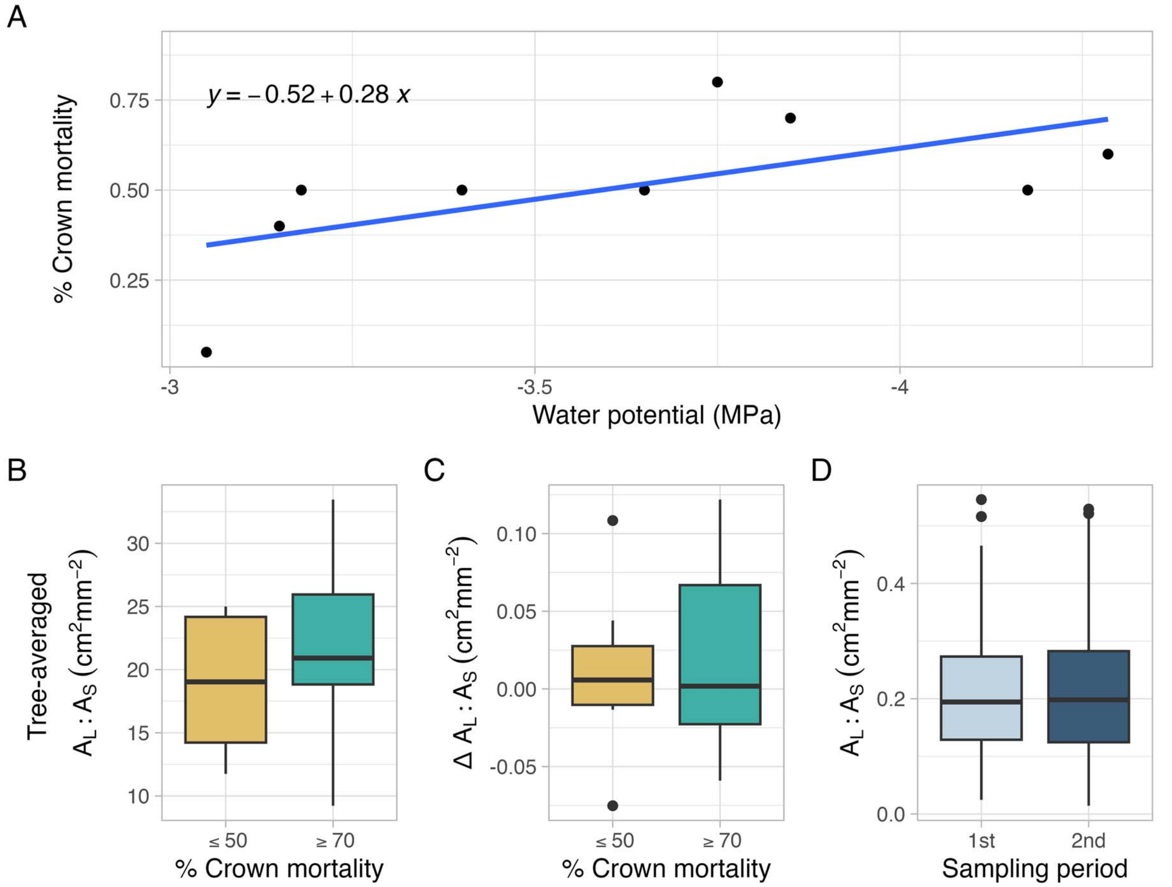

Figure 6. Elevated crown mortality corresponds with increased hydraulic stress, but there is no evidence for adaptive adjustment in A_L:A_S in the most stressed, higher crown mortality trees. (A) Percent crown mortality compared with end-of-season predawn leaf water potentials. (B) Absolute A_L:A_S in low (<50%) and high (>70%) crown mortality trees. (C) Changes in tree-averaged A_L:A_S (August minus June values) for low (<50%) and high (>70%) crown mortality trees. The 25th percentile for crown mortality corresponded with 50% crown loss and the 75th percentile corresponded with 70% crown loss. (D) Absolute A_L:A_S in our May/June (first) and August (second) field campaigns. For (A), the solid line indicates the linear best fit line over the data. For the box–whiskers plots (B–D), horizontal lines indicate the median trait value, boxes denote the interquartile range (IQR), whiskers denote 1.5 multiplied by the IQR and black dots indicate outliers.

The decreased trait integration at the Sedgwick Reserve compared to the global blue oak population (Figure 5) may also be due to physiological limitations resulting from the cumulative stress impacts of over a decade of relatively dry conditions. Our sampling year was much drier than average for the area (MAP was half of average). Further, there have been a number of dry years over the past decade at the Sedgwick Reserve. Increased stress on hydraulic and carbon systems, both in our measurement year and in previous years, could have substantially impacted the growth and carbon partitioning between stem and leaves.

It is tempting to hypothesize that there were compensating effects between crown mortality and A_L:A_S at the Sedgwick Reserve, given the extensive crown mortality observed in many trees (Figure S1 and Table S1 available as Supplementary data at *Tree Physiology* Online and Figure 6). Crown mortality decreases the total crown area, reducing water demand at the tree level without requiring a reduction in A_L:A_S. However, we found no evidence for crown mortality explaining any observed variation in A_L:A_S across space or over the measurement period (Figures 3 and 6 and Figure S4 and Table S2 available as Supplementary data at *Tree Physiology* Online), so it seems unlikely that crown shedding was adaptive in response to drought conditions, as has been found in some instances (Trugman et al. 2018).

Acclimation in A_L:A_S and performance

We did not find a link between A_L:A_S and crown dieback across trees with different levels of crown mortality or over time with increasing water stress over the summer (Figure 6, Table S2 available as Supplementary data at *Tree Physiology* Online). This is not necessarily surprising because we measured a small set of leaf morphological traits that control the integrated plant phenotype, and trait integration, or equifinality among multiple trait combinations, likely confounds demographic predictions based on a single trait alone (Trugman 2022). Our results highlight the importance of future research measuring multiple functional traits along orthogonal ecological axes (Anderegg 2023). We also did not find evidence for leaf shedding or increases in sapwood area either to decrease water demand or increase transport area over the course of the dry season in the most stressed (or less stressed) trees despite MAP reaching only 50% of average (197 mm vs the locational average of 398 mm). In fact, the median change in A_L:A_S was positive (but not statistically

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significant) as the summer (dry season) progressed, indicating that trees added leaves (Figure 6C and D). Our null result with respect to adaptive acclimation in blue oak A_L:A_S over the course of a growing season in response to water limitation helps inform the timescale over which blue oak trees adjust A_L:A_S in response to water availability. These results motivate similar investigations across multiple biomes and species, particularly in closed canopy forests where gradients in light may be more important in driving the plant phenotypic variation and integration compared with the open oak savannas.

Conclusions

We found a high level of within-tree crown A_L:A_S variation not attributable to sample height (which impacts or is impacted by hydraulic path length) or sample aspect (which influences radiation load and microclimate) in blue oak trees located in an open canopy oak–grass savanna. Further, the lack of branch-level integration in leaf morphological traits relative to tree-averaged traits suggests that trees are optimizing water use at the integrated organismal level rather than at the branch level in response to microclimatic variation within a tree crown. However, the lack of a clear link between A_L:A_S and crown mortality suggests that multiple other trait axes are responsible for regulating the plant water supply and demand and thus regulating stress. Our work highlights outstanding questions about scaling within-tree processes, particularly in blue oak trees, to the organismal-level phenotypic responses to water stress.

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Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

Conflict of interest

None declared.

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Data availability statement

The data and code that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

A.T.T., A.W. and L.D.L.A. conceived and designed the experiment. A.W. and A.T.T. carried out the fieldwork and analyses with input from L.D.L.A. and T.E.D. A.T.T. and A.W. prepared the manuscript with contributions from all co-authors. All authors read and approved the manuscript.

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