

Chapman University

Chapman University Digital Commons

Biology, Chemistry, and Environmental Sciences
Faculty Articles and Research

Science and Technology Faculty Articles and
Research

2-5-2020

Ecological Strategies Begin at Germination: Traits, Plasticity, and Survival in the First Four Days of Plant Life

Julie E. Larson

Brian L. Anacker

Sara Wanous

Jennifer L. Funk

Follow this and additional works at: https://digitalcommons.chapman.edu/sees_articles



Part of the [Botany Commons](#), [Other Plant Sciences Commons](#), [Plant Biology Commons](#), and the [Plant Breeding and Genetics Commons](#)

Ecological Strategies Begin at Germination: Traits, Plasticity, and Survival in the First Four Days of Plant Life

Comments

This is the accepted version of the following article:

Larson, J.E., Anacker, B.L., Wanous, S. & Funk, J.L. (2020) Ecological strategies begin at germination: traits, plasticity, and survival in the first four days of plant life. *Functional Ecology*.

which has been published in final form at <https://doi.org/10.1111/1365-2435.13543>. This article may be used for non-commercial purposes in accordance with [Wiley Terms and Conditions for Self-Archiving](#).

Copyright

British Ecological Society

Functional Ecology

Ecological strategies begin at germination: traits, plasticity, and survival in the first four days of plant life

Julie E. Larson¹†*, Brian L. Anacker^{2,3}, Sara Wanous¹, Jennifer L. Funk¹

¹Schmid College of Science & Technology, Chapman University, Orange CA, USA 92866

²City of Boulder Open Space and Mountain Parks, Boulder CO, USA 80301

³Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO, USA 80309

†Current address: Department of Ecology and Evolutionary Biology and Institute of Arctic and Alpine Research, University of Colorado, Boulder, CO, USA 80309

*Corresponding author: julie.e.larson@colorado.edu

Acknowledgements

We thank M. Bertirotti, N. Burtis, K. Louie, N. Mirza, M. Nguyen, K. Nguyen, M. Rosenfield for research assistance, the Suding lab for helpful comments, and D. Winkler, N. Kraft, and Irvine Ranch Conservancy for supplying seed. This work was funded by NSF-IOS 1256827.

Author Contributions

JL and JF conceived the ideas and designed methodology; JL, SW, and BA collected and analyzed the data; JL led writing of the manuscript. All authors contributed to drafts and gave final approval for publication.

Data Accessibility

Data available from Zenodo: <http://doi.org/10.5281/zenodo.3581459>

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/1365-2435.13543](https://doi.org/10.1111/1365-2435.13543)

This article is protected by copyright. All rights reserved

JULIE LARSON (Orcid ID : 0000-0001-7968-916X)

DR JENNIFER L FUNK (Orcid ID : 0000-0002-1916-5513)

Article type : Research Article

Section: Plant Growth and Development

Editor: Dr Jennifer Baltzer

Abstract

1. We commonly use trait variation to characterize plant function within and among species and understand how vegetation responds to the environment. Seedling emergence is an especially vulnerable window affecting population and community dynamics, yet trait-based frameworks often bypass this earliest stage of plant life. Here we assess whether traits vary in ecologically-meaningful ways when seedlings are just days old. How do shared evolutionary history and environmental conditions shape trait expression, and can traits explain which seedlings endure drought?
2. We measured seedling traits in the first four days of life for 16 annual plant species under two water treatments, exploring trait tradeoffs, species-level plasticity, and the ability of traits to predict duration of survival under drought.
3. Nearly half of traits showed the imprint of evolutionary history (i.e., significant phylogenetic signal), often reflecting differences between grasses and forbs, two groups separated by a deep evolutionary split. Water availability altered trait expression in most cases, though species-level plastic responses also reflected evolutionary history.
4. On average, new seedlings exhibited substantial trait variation structured as multiple tradeoffs like those found in mature plants. Some species invested in thick roots and

shoots while others invested in more efficient tissues. Separately, some invested in tougher roots and others in deeper roots. We also observed tradeoffs related to growth rates (fast or slow) and biomass allocation (above or belowground). Drought survival time was correlated most strongly with seed mass, root construction and allocation traits, and phylogeny (grasses versus forbs).

5. *Synthesis.* Our results show that seed and seedling trait variation among annual species is substantial, and that a few attributes could capture major dimensions of ecological strategies during emergence. With seedling survival times ranging two-fold among annuals (from 7.5 to 14.5 days), these strategies could mitigate recruitment responses to more frequent or longer dry spells. Multivariate trait and plasticity strategies should be further explored in studies designed to assess trait-fitness linkages during recruitment.

Key words: annual life history, functional trait, drought, recruitment, seed mass, seedlings, trait spectrum, plant economics

Introduction

Functional traits influence plant performance in the context of limiting abiotic and biotic factors, and have become a tool to track or predict changes in plant abundance over large scales (e.g., across communities, regions, or years; Garnier, Navas & Grigulis 2016; Funk *et al.* 2017). However, trait-based frameworks lag in their application to finer-scale, demographic processes that directly influence community trajectories (e.g., Laughlin *et al.* 2018). Seedling recruitment can depend on abiotic and/or biotic variation at very small scales (e.g., meters or weeks), yet be a major demographic driver of community dynamics and species distributions (e.g., Stampfli & Zeiter 2008; Lebrija-Trejos *et al.* 2016). Here we explore the extent and implications of variation in a suite of traits during a critical but less commonly studied window of recruitment: the first few days following germination.

While several processes can contribute to a good or bad recruitment year (seed production, germination, survival), a growing body of evidence suggests that survival after seedling emergence can be a defining period (Muscarella *et al.* 2013; Larson *et al.* 2015). This period is especially critical for seed-dependent annual plants, and in areas with extreme weather events or periodic climates that inflict hazards (Rebollo *et al.* 2001; Uselman *et al.* 2015). If germination-triggering precipitation events are increasingly accompanied by prolonged or more frequent droughts (as expected in many regions, Cook *et al.* 2014), risks and consequences of failed seedling emergence will increase. Most trait-based inferences for ‘seedlings,’ while informative, come from plants that are weeks, months, or a year(s) old (particularly in woody plants, Gibert *et al.* 2016, which form a significant basis for current understanding). When new seedlings can reach just centimeters for resources in any direction, will differences in tissue construction, allocation, or growth have a measurable impact on survival?

Studies suggest that inter- and intraspecific variation in seedling morphology in the days after germination can be substantial (Poorter, Remkes & Lambers 1990; Evans & Etherington 1991) and influence recruitment (Larson *et al.* 2015; Leger, Atwater & James 2019). Still, few studies have explicitly linked early seedling traits to drought response despite well-developed theory (Kitajima & Myers 2008; Comita & Engelbrecht 2014; Saatkamp *et al.* 2019). With ties to many aspects of performance, seed mass has become a focal trait to represent plant regeneration strategies (small-seeded species grow faster, survive less, colonize better; Moles & Westoby 2004; Kitajima & Myers 2008). Larger seeds support seedling persistence under

drought via greater internal resource provisioning and slower metabolism (e.g., Leishman & Westoby 1994; Lebrija-Trejos *et al.* 2016; Harrison & LaForgia 2019). However, dense, thick leaf tissues that can maintain hydraulic conductance under extreme water deficit may also promote drought ‘tolerance’ (e.g., Kursar *et al.* 2009). Still other traits can enhance drought ‘avoidance’ by allowing plants continued access to water under drying conditions (e.g., via deeper or longer roots, though evidence is mixed, Comita & Engelbrecht 2014). Separately, a drought ‘escape’ strategy could enable a plant to complete its life cycle more quickly and evade stress, e.g., via rapid emergence (Leger, Atwater & James 2019) or growth fueled by thin, efficient tissues (i.e. more absorptive surface area for a given mass investment). When seedlings are so small that soil drying rate could easily outpace growth rates, the fittest seedlings may be those that can *tolerate* dry conditions for the longest period. However, we lack quantitative tests of whether different drought response strategies are detectable and impact fitness in the first days of plant life.

Trait expression can be a product of species’ shared evolutionary history, but also of processes leading to intraspecific trait variation (e.g., maternal and epigenetic effects, local adaptation, or plasticity [a focus of this study]). The relative influence of these factors on trait expression has implications for how we approach trait-based inferences. From an ecological perspective, shared ancestry could provide a useful proxy for species’ developmental strategies. For example, evidence suggests that monocots and dicots evolved different germination and dormancy traits (e.g., Willis *et al.* 2014), which could carry into seedling development given the functional connection between seed and seedling stages (Donohue *et al.* 2010). However, evidence for plant trait or recruitment patterns based on coarser taxonomic groupings, such the grass-forb dichotomy, are mixed (e.g., Reich *et al.* 2003; Uselman *et al.* 2015). Even when species exhibit structured trait differences on average, several factors can lead to varied stress responses *within* species (e.g., Leger, Atwater & James 2019). Drought, for example, can induce plastic shifts towards slower growth, thicker tissues, and greater root allocation, but with substantial variability (or opposite responses) across species (e.g., Evans & Etherington 1991; Larson & Funk 2016; Freschet *et al.* 2018). If seedling traits respond to the microenvironment within days of germination, plastic shifts (here, assessed at the species-level) could have consequence for recruitment under stress (Reader *et al.* 1993).

We characterized trait variation during the first days of seedling life for 16 annual grass

and forb species at two water levels and linked this variation to survival under drought. We hypothesize that (H1) trait variation is product of both shared evolutionary history and experienced environmental conditions, with species exhibiting plastic responses to drought associated with different adaptive syndromes (e.g., reduced growth and sturdier tissues, or rapid, deep growth to access water). We also hypothesize that (H2) interspecific trait relationships reveal dimensions of ecological strategy aligning with established tradeoffs (e.g., growth, tissue construction, and allocation tradeoffs). Finally, we hypothesize that (H3) trait variation and its underlying sources (shared evolutionary history and environmental conditions) have implications for seedling drought survival.

Materials and methods

Species selection

We measured seed and seedling traits in 16 annual C3 grass and forb species occurring in coastal sage scrub systems of southern California, USA (Supporting Information, Table S1). These systems experience a Mediterranean climate with cool, wet winters and hot, dry summers (330mm annual precipitation). Over the last decade, California has experienced an historic period of recurring droughts (Griffin & Anchukaitis 2014) and is expected to experience more extreme drying in the next century (Cook *et al.* 2014). Simultaneously, land use change and exotic species introductions have facilitated the transition of mixed scrublands to annual-dominated grasslands. Study species represent a range of exotic and native annuals which share a strong demographic dependence on recruitment. Seeds were collected locally or sourced from regional growers (Table S1), allowing us to use a wider range of species, but preventing us from evaluating some localized drivers of trait variation (e.g., maternal effects).

Trait & plasticity measurements

For each species, we recorded time to germination and collected several seedling traits under two water potentials to estimate species trait values and plasticity (16 species x 2 water potentials [wet, dry] x 4 replicates = 128 total replicates). Note that we assess species-level plasticity – trait variation among individuals from a source population across different environments (as opposed to phenotypic plasticity, which is evaluated at the genotype level) (Valladares, Sanchez-Gomez & Zavala 2006). For each replicate, thirty seeds were germinated on filter paper (Whatman No. 1) soaked in a polyethylene glycol 8000 solution. Two

Accepted Article

concentrations were created to achieve water potentials of -0.025 MPa and -0.25 MPa for ‘wet’ and ‘dry’ water treatments, respectively (following Hardegee & Emmerich 1990). Our ‘wet’ treatment represents typical field capacity of mineral soils (around -0.03MPa), and our ‘dry’ treatment is an order of magnitude drier but still above species’ base water potentials (determined in earlier trials). Petri dishes were sealed, re-saturated with fresh solution every other day to maintain consistent concentrations, and kept in a growth chamber at 18°C (consistent with average high temperatures during peak field germination) with a 12hr light cycle (55 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$; below 200 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$ is typical at ground-level in Mediterranean systems, Dobarro, Valladares & Peco 2010). Positions within the chamber were shuffled daily.

We checked petri dishes daily, recording seeds as germinated once the radicle reached 2mm. We ceased germination trials when no further germination occurred for three consecutive days; at this point, most seedlots had reached $\geq 70\%$ germination, when further germination would not have substantially altered calculated parameters. As soon as at least three seeds per replicate germinated, three seedlings were selected at random and measured for initial radicle length and shoot length (coleoptile for grasses, hypocotyl for forbs). The same seedlings developed for four days (dates tracked for each individual) before final traits were collected. We chose four days because this was the earliest point by which all species had fully-emerged cotyledons (forbs) or leaves (grasses). We removed any damaged or irregular seedlings, leaving one to three healthy seedlings to be pooled for each replicate trait measure. We separated the ‘root’ (i.e. radicle), ‘shoot’ (i.e. coleoptile or hypocotyl), and ‘leaf’ (i.e. photosynthetic cotyledon in forbs or first leaf in grasses). We scanned roots and shoots for length and diameter with WinRHIZO software (Regent Instruments, Quebec, CA), then scanned and analyzed leaves for area (Image J software). Roots, shoots, and leaves were placed in moist towels within plastic bags and refrigerated overnight to ensure full hydration prior to fresh weight collection, then dried (>2 days at 60°C) prior to collecting dry mass.

We estimated thirteen seed and seedling traits of four types:

- **Two seed traits.** *Seed mass* was the average per seed dry weight (>2 days at 80°C) estimated from three replicate pools of 50 seeds. *Time to germination* was the estimated number of days for 25% of seeds to germinate – meant to assess whether rapid development begins with rapid germination. We calculated daily cumulative germination

percentages scaled by the maximum germination percentage observed in any replicate per species. We then used interpolation to estimate the time at which 25% of germinable seeds had germinated. We used the 25th percentile because some species failed to reach 50% germination in the 'dry' treatment (but times for 25th and 50th percentiles were correlated ($R^2=91\%$)). Although we did not assess seed dormancy (an important alternative strategy for drought avoidance), 13 of 16 species reached 70% germination or higher (Table S2), suggesting that dormancy occurs but was not a dominant strategy here.

• **Two seedling growth traits.** *Root elongation rate* and *shoot elongation rate* were calculated according the relative growth rate formula: relative elongation rate = $\ln(X_2 - X_1) / (t_2 - t_1)$, where X_1 and X_2 are lengths at times t_1 (day 0) and t_2 (day 4), respectively.

• **Two allocation traits.** These traits indicate the relative allocation of tissues/energy to roots vs. shoots. *Root mass ratio* was calculated as the ratio of root dry biomass relative to total plant dry biomass. We also estimated the *ratio of root length to shoot length* at the final measurement.

• **Seven tissue construction traits.** These traits indicate different approaches to building leaves and roots. *Root diameter* and *shoot diameter* were estimated as the average diameter (mm) across the final root or shoot. *Specific root length* was the ratio of root length to dry mass, and *specific leaf area* was the ratio of area to dry mass in either photosynthetic cotyledons (forbs) or first leaf (grasses). Finally, we estimated *root, shoot, and leaf dry matter content* as the ratio of dry to fresh biomass in each structure.

Plasticity indices for each trait were calculated as in Valladares et al. (2006, PIV index = (maximum mean - minimum mean) / maximum mean) with the sign changed (+/-) to indicate the direction of water response in trait values of each species.

Survival

We conducted a common garden dry-down experiment to assess whether traits or their plasticity have implications for seedling survival under drought. All species were germinated in petri dishes at two water potentials (-0.025 MPa and -0.25 MPa) as described for trait measurements. After four days, seven seedlings per species and treatment were transplanted into individual pots (5.7cm x 5.7cm x 7.6cm, 185mL) containing a 2:1 mixture of sand:field-collected soil. Soil moisture was brought to 18% volumetric water content (VWC) at the time of planting with no additional watering during the experiment (value chosen based on field data indicating

18%-20% VWC in dry to average years, unpublished data, Irvine, CA). We monitored soil moisture in 12 extra pots containing soil but no seedlings (1-2 pots per staggered transplanting dates; ThetaProbe, Delta-T Devices). Pots were kept indoors under grow lights (approximately $80 \mu\text{mol photon m}^{-2}\text{s}^{-1}$; 22°C).

We monitored seedlings daily and quantified days until death as the response variable. Seedlings showing signs of mortality in the first two days (presumably due to transplant shock) were excluded and replanted. Seedlings were scored according to the criteria of Engelbrecht & Kursar (2003), and considered dead when all aboveground parts lost color and showed no elasticity. To verify seedling death, we subsequently watered each pot to saturation and monitored for revival (occurrence: 1 of 224 seedlings). To verify that water limitation caused mortality, we also planted one extra pot per species and treatment that was watered daily (survival: 32 of 32 seedlings). Although survival can be highly variable, within-species standard errors were relatively low in this controlled environment (Table S2), and 7 replicates (or 14, when treatments were pooled) were sufficient to capture variation.

Analysis

To determine phylogenetic non-independence among species, we assessed each trait (species averages) and plasticity index for phylogenetic signal. To create a phylogeny, we made a backbone tree for all 16 species and an outgroup (*Magnolia grandiflora*) using the Phylomatic tree of angiosperm families (phylodiversity.net/phylomatic/) (`branching` package in R). We then acquired genetic sequences from GenBank (including ITS [600bp], *rbcL* [600 bp], *matK* [1500bp] and *trnT-trnL* [700bp]) and aligned with the MUSCLE program (www.ebi.ac.uk/Tools/msa/muscle/). We used the genetic data to refine any polytomies of our backbone tree (RaxML inference based on max. likelihood, CIPRES v.3.3 [phylo.org]) and estimate branch lengths (BEAST v1.8.4, bayesian tree estimation) (Stamatakis 2006).

We used our phylogeny to calculate Blomberg's *K* (`phytools` package in R), an estimate of phylogenetic signal. To visualize patterns, we also plotted values of survival plus four traits and their plasticity indices across the phylogeny (traits selected to represent different PCA axes [see below]; figures for other traits and plasticity indices in Fig. S1). The *K* test results and visual inspection made it clear that many traits and plastic responses differed most between grasses and forbs. To account for this generality and assess its role alongside environmental factors, we included growth form (grass or forb) as a factor in subsequent models exploring the

Accepted Article

role of water in trait variation. For each trait, we specified a linear mixed model with water treatment, growth form, and their interaction as fixed effects and species as a random effect, and tested significance of fixed effects using type III F-tests with Satterthwaite-approximated degrees of freedom (`lmer` package in R; trait data transformed to meet assumptions, Table S3). We confirmed that our use of growth form in this way was sufficiently rigorous to account for phylogenetic non-independence by independently fitting a set of statistical models that directly account for phylogeny (Appendix S1).

To identify major trait tradeoffs across species, we used principal components analysis (PCA; `psych` package in R) with species mean trait values (seed mass plus 12 seed/seedling traits averaged across treatments). Because water availability affected most traits, we also considered its effect on trait tradeoffs. To quantify the proportion of variation in trait space explained by water availability, we used redundancy analysis with species mean trait values for each water treatment (12 traits only, excluding seed mass) (`vegan` package in R). We also qualitatively compared results of two separate PCAs with ‘dry’ or ‘wet’ traits only. Traits were scaled and examined for linearity prior to each analysis, and PC axes were varimax-rotated (i.e. rotated components – RC’s) and constrained to the first 3-4 components to improve axis interpretability (i.e. loading of traits onto single axes). Pearson correlation coefficients between traits aided interpretation.

To explore links between traits and survival, we regressed each axis from the averaged trait PCA against survival time (averaged across water treatments) in a multiple regression model. Bivariate relationships between traits and survival time aided interpretation. All analyses were completed in R (R Core Team 2018).

Results

H1: Phylogeny and water availability

We found substantial seedling trait variation among annuals, with mean traits varying from 2- to 5-fold across species (means and standard errors in Table S2, Fig. S1A). Five of thirteen traits had significant phylogenetic signals, all related to seedling tissue construction or biomass allocation (Table 1, Fig. S1A). In four out of five cases, signals reflected significant differences between grasses and forbs, with the biggest splits occurring belowground: greater allocation to tougher roots by grasses (i.e., greater root dry matter content and root mass ratio,

Fig. 1D & F), but deeper roots in forbs (higher root:shoot length ratio). Root and shoot growth rates did not show phylogenetic signal (e.g., Fig. 1E), and while one leaf trait showed a signal (leaf dry matter content), it was not strongly associated with the grass-forb dichotomy (Fig. 1C). Although grasses tended to have larger seeds than forbs on average (two-tailed t-test, $p=0.036$), we did not detect a significant phylogenetic signal for seed mass or time to germination.

Six of twelve plasticity indices had a significant phylogenetic signal (Table 1, Fig S1B), reflecting adjustments to root allocation (root to shoot length, root mass ratio; Fig. 1K), as well as tissue construction (specific root length, specific leaf area, shoot and leaf dry matter content, Fig. 1H). All six plasticity indices also reflected differences between grasses and forbs, along with four additional plastic responses (e.g., Fig. 1J; Table 1). However, survival time differences between 'wet' and 'dry' seedlings were mostly minor with no links to phylogeny (Fig. 1G). Plastic responses are characterized in greater depth below.

Most traits were significantly affected by the water treatment following germination (see treatment effects in Fig. 2 and Table S3). The most consistent 'dry' responses were lengthened time to germination and tougher tissue construction (higher root/shoot/leaf dry matter content), followed by slowed growth (root and shoot elongation rates). Seedlings also tended to develop less efficient leaves (lower specific leaf area, or less light-acquiring surface per mass investment) and thinner roots and shoot diameters.

In contrast, allocation traits did not respond in a uniform way. Significant growth form by treatment interactions suggest that in 'dry' conditions, grasses increased biomass investment to roots (higher root mass ratio) without increasing relative investment in root length (root to shoot length ratio), while forbs did the opposite (Fig. 2). Other grass-forb differences in the *strength* of plastic responses support these findings: forbs reduced root diameter more and specific root length less under 'dry' conditions, suggesting a push to create thinner and more efficient roots (greater root surface per mass investment). Forbs also reduced shoot elongation rates, adjusted leaf construction (leaf dry matter content and specific leaf area), and slowed germination more than grasses under 'dry' conditions (Table S3).

H2: Seedling trait tradeoffs

Despite treatment effects on most individual seedling traits, water treatment explained little variation in multivariate trait space (RDA constrained $R^2 = 6.8\%$). PCA results based on separate 'wet' or 'dry' trait values were also qualitatively similar (Table S4). We therefore limit

our discussion to tradeoffs across averaged trait values. We captured a majority of the variation with four axes (83% of total variation with varimax-rotated PCA; Fig. 3A&B, Table S4). The first axis (31% of variation) captured variation in traits typically associated with a spectrum from resource conservative to acquisitive strategies. Species on the conservative end had larger seeds, tougher leaves (higher leaf dry matter content), and thicker roots and shoots, while small-seeded species on the acquisitive end had efficient leaves and roots (higher specific leaf area and specific root length). Root and shoot dry matter content were better represented on the second axis (22% of the variation), which captured a tradeoff between denser root/shoot tissues and deeper initial roots, clearly separating grasses and forbs (Fig. 3A).

The third axis (19% of variation) captured a tradeoff between slow and fast strategies, in which species requiring less time for germination also tended to grow more quickly (higher shoot and root elongation rates) (Fig 3B). The fourth axis (11% of variation) was also ecologically relevant, capturing variation in root mass ratio (which shared no significant correlations with other traits) (Fig. 3B, Table S5).

H3: Trait variation and survival

During the dry-down experiment, soil moisture decreased linearly from 18% to 9% VWC over the first four days, followed by a reduced rate of decrease, reaching a minimum of about 3.5% after 17 days (end of experiment; Fig. S2). There was no effect of water availability during germination on drought survival times across species (Fig. 2, Table S3). We therefore used trait and survival time values averaged across water treatments to assess relationships.

When four day-old seedlings were subjected to drought, species' survival times ranged from 7.5 days to 14.5 days. Survival time had a strong phylogenetic signal and was significantly longer in grasses than forbs (Fig. 1B, Fig. 2). The four continuous trait axes explained 51% of the variation in survival time, with stronger evidence of association with axes 2 and 4 (Table 2). The second axis reflected longer survival in species with higher root dry matter contents rather than high root to shoot length ratios (see also Fig. 4A), and could explain a possible mechanism for longer survival times in grasses. There was also evidence for longer survival with greater root biomass allocation (axis 4, $p = 0.06$; see also Fig. 4B). However, seed mass was the single trait with the strongest bivariate correlation with drought survival (Table S5, Fig. 4C). Although seed mass loaded most strongly on the first axis, other traits forming this axis collectively exhibited much weaker correlations with survival.

Discussion

Inherited traits and environmental conditions shape seedling trait variation.

To integrate seedling emergence into trait-based frameworks, we first characterized some potential drivers of phenotypic variation, finding that both evolutionary history and environmental conditions shaped trait expression (though this depended on the trait). Although we expected trait correspondence to phylogeny given fundamental structural and physiological differences between monocot and dicot seeds (Leck, Parker & Simpson 2008; Willis *et al.* 2014), the degree to which differences extended into continuous metrics of tissue dry matter content and resource allocation early on was surprising. Just as interesting are those traits without strong phylogenetic signals: seedling elongation rates and other aspects of tissue construction (root/shoot diameter, specific leaf areas and root lengths) were not patterned clearly across the phylogenetic tree, suggesting that these traits may have fewer evolutionary constraints.

Plastic responses reflected relatedness even more broadly than average traits – ten out of twelve species-level plasticity indices mapped onto phylogenetic trees or the grass-forb dichotomy (i.e. significant growth form by treatment interactions), compared to five average trait values. While some studies across closely-related species have found little correspondence between plasticity and phylogeny (e.g., Rutherford *et al.* 2017), Kembel and Cahill (2005) found a phylogenetic signal for root plasticity that separated monocots (less responsive) from dicots (more responsive). Our results across multiple plasticity metrics suggest that differences between these groups are complex and trait-dependent, but that coarse location on a phylogenetic tree can be a starting point to infer plastic responses. Although capturing phylogenetic signal within a small group of annuals is promising, we require a much wider diversity of species to fully characterize roles of phylogeny in the regeneration niche.

Young seedlings responded rapidly to water availability (see also Evans & Etherington 1991; Padilla, Miranda & Pugnaire 2007). Although we observed an overall shift towards reduced growth, resource-conservative strategies (tougher, less efficient tissues), and variable allocation responses, more work is needed to parse out mechanisms. While these responses could be beneficial adaptations under drought and part of species' larger ecological strategies (Grime & Mackey 2002), they could also result from physical constraints or slowed development (i.e. passive plasticity; Wright & McConnaughay 2002). Although plasticity induced by initial conditions did not strongly affect species' survival times at this stage, we must continue building

on efforts to understand when and how plasticity can be adaptive (or not; Freschet *et al.* 2018; Harrison & LaForgia 2019). By measuring *suites* of plastic responses for more species under a variety of conditions, we could begin integrating plasticity into our overall understanding of plant ecological strategies (e.g., exploring links to known trait tradeoffs Grime & Mackey 2002).

We did not explore some important sources of intraspecific trait variation in this study, including local adaptation and maternal effects (in part, because seedlots were collected from diverse growing environments. However, given strong evidence for the importance of maternal effects (Sultan, Barton & Wilczek 2009) and local adaptation (Leger, Atwater & James 2019) for seedling trait expression and performance, these sources of intraspecific variation must be more fully integrated into our understanding of trait expression and responses to drought.

Trait organization in new seedlings suggests broader ecological strategies.

In older plants, the suite of leaf, root, and shoot traits explored here typically fall along coordinated axes of trait variation interpreted as tradeoffs in ecological strategy (Garnier, Navas & Grigulis 2016). Adding to a vast literature on seedling function (Kitajima & Fenner 2000; Leck, Parker & Simpson 2008), our results quantitatively demonstrate that seedling strategies may fit within a similar trait-based framework within days of germination. Leaf (or cotyledon) and root (radicle) traits aligned to suggest separate tradeoffs related to resource acquisition, root development, growth, and biomass allocation. The first axis cemented a firm link between seedling tissue construction and seed mass (small seeded species typically have thinner, efficient tissues, i.e. greater resource-acquiring surface area per mass; Maranon & Grubb 1993; Kitajima & Fenner 2000). However, anticipated links between this dimension and growth (root/shoot elongation) or survival were not strong, suggesting other functional drivers of seedling performance in this context. Still, these tradeoffs could have functional implications for performance in relation to *other* recruitment challenges, including freezing, herbivory, or hard soils (e.g., thick shoots for emergence ability, dense leaves for herbivory, Alvarez-Clare & Kitajima 2007; Gardarin *et al.* 2016).

While the first axis suggests some degree of cohesiveness between tissue construction strategies above- and belowground, we found this pattern to be nuanced. Tissue toughness in roots (i.e. dry matter content) aligned with toughness in leaves across some (but not all) species and diverged from most other tissue construction traits. Instead, root dry matter content tied into a separate tradeoff between seedlings investing in tough roots or deep roots, which also separated

fibrous grasses (tough) and tap-rooting forbs (deep). This agrees with our general understanding that forbs and grasses differ predictably and early with respect to certain root traits (e.g., root topology) but not others (e.g., root diameter) (Tjoelker *et al.* 2005; Larson & Funk 2016).

Rapid growth rates are often associated with efficient root/leaf tissues (thought to be ‘resource-acquisitive’ traits) and smaller seed mass (Poorter, Remkes & Lambers 1990; Gibert *et al.* 2016), including in some Mediterranean annuals (at three weeks old; Maranon & Grubb 1993). In contrast, we found weak or opposite relationships between most traits and root/shoot elongation. Instead of biomass-based metrics, we measured length-based relative growth, which we associate with seedling ability to reach light or water resources. However, controls on growth rate have also been shown to shift through development (Gibert *et al.* 2016); a positive seed mass-growth relationship could suggest that relative growth potential mirrors absolute potential at early stages – more a result of internal resource provisions than leaf and root resource-capturing ability (see also Gardarin *et al.* 2016). Interestingly, the connection of rapid growth to rapid seed germination also hints that drought escape strategies may begin in the seed.

A critical next step is to understand how detected tradeoffs tie into drought response strategies at other stages. In seeds, dormancy is a key mechanism for drought avoidance enabling seeds to minimize the risk of drought exposure, yet studies rarely integrate dormancy with vegetative strategies. Drought tolerance also depends on aspects of leaf physiology and chemistry not explored here, e.g., different photosynthetic pathways (C3/C4), photosynthetic water use efficiencies, stomatal conductance, and water potentials (Pearcy & Ehleringer 1984; Comita & Engelbrecht 2014). It is unclear when, in development, these traits become meaningfully functional.

Trait variation explains seedling survival in the first weeks of life.

Whether and how trait variation impacts fitness is a fundamental question for trait-based inferences in plant populations and communities (Laughlin *et al.* 2018). Several studies have linked traits to seedling recruitment in field settings (e.g., Moles & Westoby 2004), but there is a need to mechanistically tie responses to stressors (e.g., drought, herbivory, or disease). We have shown that trait variation in the tiniest plants is substantial and structured, and that a few aspects of variation – seed and root investments – contribute to performance under drought. However, trait differences between growth forms also matter under water stress.

Across species, survival time increased with root dry matter content and biomass

allocation to roots (second and fourth axes), the former of which has been linked to lower respiration rates (Roumet *et al.* 2016) and greater tissue longevity (Eissenstat *et al.* 2000). These patterns initially suggest that allocating more to shallower but tougher roots (i.e. drought tolerance) could be a better strategy under rapid drought onset than the tradeoff of investing in deeper roots with fewer structural components (i.e. drought avoidance). However, variable root trait-survival relationships in other studies point to the possibility of different outcomes for different life stages, environmental contexts, or functional groups (Padilla & Pugnaire 2007; Butterfield & Briggs 2011). Harrison & LaForgia (2019) found that *deeper* roots increased drought survival among tap-rooting annual forbs in the field, where only one strategy type was being explored (tap-rooting), and resource reservoirs may have existed at depth. Similarly, while we found a positive effect of investing in *tough* roots across species, relationships differed within growth forms: grasses were uniformly tough-rooted (9 to 11 g·g⁻¹), while the relationship turned negative for forbs (Fig. 4A, forbs Pearson $r=-0.77$). This makes it difficult to conclude that investing in tougher roots is the key to higher drought tolerance, and suggests there may be other aspects of strategy that better explain survival patterns both across and within growth forms.

Seed mass, for example, was a more consistent driver of drought tolerance within and among functional groups (even though the broader first axis was not). This suggests a strong, direct benefit of a greater internal resource pool for survival under resource stress (e.g., Moles & Westoby 2004; Harrison & LaForgia 2019). Although this cements the importance of seed mass in seedling ecological strategies, the simultaneous importance of separate rooting tradeoffs demonstrates that understanding seedling ecological strategies is a multidimensional challenge (see also Alvarez-Clare & Kitajima 2007).

Moving forward, environmental context will also be a crucial consideration for trait-based frameworks to predict recruitment outcomes in the field. Seedling trait variation may have few consequences for recruitment patterns if stressors are too extreme (i.e. all die regardless of traits) or too benign (i.e. all survive regardless of traits). We documented survival times ranging from 7 - 14 days in rapidly drying soils—a 2-fold variation among annuals. Local climate data indicate that rain-free periods occurring somewhere within this window (7-14 days) are relatively common— 25% of all growing season events (Dec-Mar precipitation data, 1987-2017; California Irrigation Management Information System, Irvine, CA, USA). In this region, climate change is expected to result in greater evaporative demand and drier conditions (Cook *et al.* 2014), but the

Accepted Article

relative success of strategies to escape, avoid, or tolerate drought will likely depend on more dynamic intra-annual rainfall patterns (Donohue *et al.* 2010) and finer spatial variation in environmental properties affecting microsite quality. Designing recruitment field experiments at relevant spatiotemporal scales is thus a critical next step in the testing of trait-based frameworks.

Conclusion

Exploring trait variation in the first days of life revealed just how much plants can differ from the moment that root and shoot tissues emerge. Our findings suggest that plasticity should be explored as an important component of plant regeneration strategies, and that phylogenetic relatedness may serve as a useful proxy for multiple aspects of strategy. We also found a great deal of structure in seedling trait variation, including well-known tradeoffs related to tissue construction, growth, and allocation that provide support for early differentiation of plant ecological strategies. With observed links between seedling traits and drought survival, trait-based recruitment frameworks deserve further testing in the field, where environmental stressors are stronger and more dynamic.

References

- Alvarez-Clare, S. & Kitajima, K. (2007) Physical defence traits enhance seedling survival of neotropical tree species. *Functional Ecology*, 21, 1044-1054.
- Butterfield, B.J. & Briggs, J.M. (2011) Regeneration niche differentiates functional strategies of desert woody plant species. *Oecologia*, 165, 477-487.
- Comita, L.S. & Engelbrecht, B.M. (2014) Drought as a driver of tropical tree species regeneration dynamics and distribution patterns *Forests and Global Change* (eds D.A. Coomes, D.F.R.P. Burslem & W.D. Simonson), pp. 261-308. Cambridge University Press.
- Cook, B.I., Smerdon, J.E., Seager, R. & Coats, S. (2014) Global warming and 21st century drying. *Climate Dynamics*, 43, 2607-2627.
- Dobarro, I., Valladares, F. & Peco, B. (2010) Light quality and not quantity segregates germination of grazing increasers from decreasers in Mediterranean grasslands. *Acta Oecologica*, 36, 74-79.
- Donohue, K., de Casas, R.R., Burghardt, L., Kovach, K. & Willis, C.G. (2010) Germination, Postgermination Adaptation, and Species Ecological Ranges. *Annual Review of Ecology, Evolution, and Systematics*, Vol 41, 41, 293-319.
- Eissenstat, D.M., Wells, C.E., Yanai, R.D. & Whitbeck, J.L. (2000) Building roots in a changing environment: implications for root longevity. *New Phytologist*, 147, 33-42.
- Engelbrecht, B.M.J. & Kursar, T.A. (2003) Comparative drought-resistance of seedlings of 28 species of co-occurring tropical woody plants. *Oecologia*, 136, 383-393.
- Evans, C.E. & Etherington, J.R. (1991) The effect of soil-water potential on seedling growth of some British plants. *New Phytologist*, 118, 571-579.
- Freschet, G.T., Violle, C., Bourget, M.Y., Scherer-Lorenzen, M. & Fort, F. (2018) Allocation, morphology, physiology, architecture: the multiple facets of plant above- and below-ground responses to resource stress. *The New phytologist*.
- Funk, J.L., Larson, J.E., Ames, G.M., Butterfield, B.J., Cavender-Bares, J., Firn, J., Laughlin, D.C., Sutton-Grier, A.E., Williams, L. & Wright, J. (2017) Revisiting the Holy Grail: using plant functional traits to understand ecological processes. *Biological Reviews*, 92, 1156-1173.
- Gardarin, A., Coste, F., Wagner, M.H. & Durr, C. (2016) How do seed and seedling traits influence germination and emergence parameters in crop species? A comparative analysis. *Seed Science Research*, 26, 317-331.

- Garnier, E., Navas, M.-L. & Grigulis, K. (2016) Plant functional diversity: organism traits, community structure, and ecosystem properties. Oxford University Press, Oxford, U.K.
- Gibert, A., Gray, E.F., Westoby, M., Wright, I.J. & Falster, D.S. (2016) On the link between functional traits and growth rate: meta-analysis shows effects change with plant size, as predicted. *Journal of Ecology*, 104, 1488-1503.
- Griffin, D. & Anchukaitis, K.J. (2014) How unusual is the 2012-2014 California drought? *Geophysical Research Letters*, 41, 9017-9023.
- Grime, J.P. & Mackey, J.M.L. (2002) The role of plasticity in resource capture by plants. *Evolutionary Ecology*, 16, 299-307.
- Hardegree, S.P. & Emmerich, W.E. (1990) Effect of polyethylene-glycol exclusion on the water potential of solution-saturated filter-paper. *Plant Physiology*, 92, 462-466.
- Harrison, S. & LaForgia, M. (2019) Seedling traits predict drought-induced mortality linked to diversity loss. *Proceedings of the National Academy of Sciences of the United States of America*, 116, 5576-5581.
- Kembel, S.W. & Cahill, J.F. (2005) Plant phenotypic plasticity belowground: A phylogenetic perspective on root foraging trade-offs. *American Naturalist*, 166, 216-230.
- Kitajima, K. & Fenner, M. (2000) Ecology of seedling regeneration. *Seeds: The ecology of regeneration in plant communities* (ed. M. Fenner), pp. 331-360. CABI Publishing, Oxon, UK.
- Kitajima, K. & Myers, J.A. (2008) Seedling ecophysiology: strategies toward achievement of positive net carbon balance. *Seedling Ecology and Evolution* (eds M.A. Leck, V.T. Parker & R.L. Simpson), pp. 172-188. Cambridge University, Cambridge.
- Kursar, T.A., Engelbrecht, B.M.J., Burke, A., Tyree, M.T., El Omari, B. & Giraldo, J.P. (2009) Tolerance to low leaf water status of tropical tree seedlings is related to drought performance and distribution. *Functional Ecology*, 23, 93-102.
- Larson, J.E. & Funk, J.L. (2016) Seedling root responses to soil moisture and the identification of a belowground trait spectrum across three growth forms. *New Phytologist*, 210, 827-838.
- Larson, J.E., Sheley, R.L., Hardegree, S.P., Doescher, P.S. & James, J.J. (2015) Seed and seedling traits affecting critical life stage transitions and recruitment outcomes in dryland grasses. *Journal of Applied Ecology*, 52, 199-209.

- Laughlin, D.C., Strahan, R.T., Adler, P.B. & Moore, M.M. (2018) Survival rates indicate that correlations between community-weighted mean traits and environments can be unreliable estimates of the adaptive value of traits. *Ecology Letters*, 21, 411-421.
- Lebrija-Trejos, E., Reich, P.B., Hernandez, A. & Wright, S.J. (2016) Species with greater seed mass are more tolerant of conspecific neighbours: a key driver of early survival and future abundances in a tropical forest. *Ecology Letters*, 19, 1071-1080.
- Leck, M.A., Parker, V.T. & Simpson, R.L. (2008) *Seedling ecology and evolution*, 1st edn. Cambridge University Press.
- Leger, E.A., Atwater, D.Z. & James, J.J. (2019) Seed and seedling traits have strong impacts on establishment of a perennial bunchgrass in invaded semi-arid systems. *Journal of Applied Ecology*, 56, 1343-1354.
- Leishman, M.R. & Westoby, M. (1994) The role of seed size in seedling establishment in dry soil-conditions - Experimental evidence from semiarid species. *Journal of Ecology*, 82, 249-258.
- Maranon, T. & Grubb, P.J. (1993) Physiological basis and ecological significance of the seed size and relative growth rate relationship in Mediterranean annuals. *Functional Ecology*, 7, 591-599.
- Moles, A.T. & Westoby, M. (2004) Seedling survival and seed size: a synthesis of the literature. *Journal of Ecology*, 92, 372-383.
- Muscarella, R., Uriarte, M., Forero-Montana, J., Comita, L.S., Swenson, N.G., Thompson, J., Nytech, C.J., Jonckheere, I. & Zimmerman, J.K. (2013) Life-history trade-offs during the seed-to-seedling transition in a subtropical wet forest community. *Journal of Ecology*, 101, 171-182.
- Padilla, F.M., Miranda, J.D. & Pugnaire, F.I. (2007) Early root growth plasticity in seedlings of three Mediterranean woody species. *Plant and Soil*, 296, 103-113.
- Padilla, F.M. & Pugnaire, F.I. (2007) Rooting depth and soil moisture control Mediterranean woody seedling survival during drought. *Functional Ecology*, 21, 489-495.
- Pearcy, R.W. & Ehleringer, J. (1984) Comparative ecophysiology of C3 and C4 plants. *Plant Cell and Environment*, 7, 1-13.
- Poorter, H., Remkes, C. & Lambers, H. (1990) Carbon and nitrogen economy of 24 wild species differing in relative growth rate. *Plant Physiology*, 94, 621-627.
- R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

- Reader, R.J., Jalili, A., Grime, J.P., Spencer, R.E. & Matthews, N. (1993) A comparative study of plasticity in seedling rooting depth in drying soil. *Journal of Ecology*, 81, 543-550.
- Rebollo, S., Perez-Camacho, L., Garcia-de Juan, M.T., Benayas, J.M.R. & Gomez-Sal, A. (2001) Recruitment in a Mediterranean annual plant community: seed bank, emergence, litter, and intra- and inter-specific interactions. *Oikos*, 95, 485-495.
- Reich, P.B., Buschena, C., Tjoelker, M.G., Wrage, K., Knops, J., Tilman, D. & Machado, J.L. (2003) Variation in growth rate and ecophysiology among 34 grassland and savanna species under contrasting N supply: a test of functional group differences. *New Phytologist*, 157, 617-631.
- Roumet, C., Birouste, M., Picon-Cochard, C., Ghestem, M., Osman, N., Vrignon-Brenas, S., Cao, K.F. & Stokes, A. (2016) Root structure-function relationships in 74 species: evidence of a root economics spectrum related to carbon economy. *New Phytologist*, 210, 815-826.
- Rutherford, S., Bonser, S.P., Wilson, P.G. & Rossetto, M. (2017) Seedling response to environmental variability: The relationship between phenotypic plasticity and evolutionary history in closely related *Eucalyptus* species. *American Journal of Botany*, 104, 840-857.
- Saatkamp, A., Cochrane, A., Commander, L., Guja, L.K., Jimenez-Alfaro, B., Larson, J., Nicotra, A., Poschlod, P., Silveira, F.A.O., Cross, A.T., Dalziell, E.L., Dickie, J., Erickson, T.E., Fidelis, A., Fuchs, A., Golos, P.J., Hope, M., Lewandrowski, W., Merritt, D.J., Miller, B., Miller, R.G., Offord, C.A., Ooi, M.K.J., Satyanti, A., Sommerville, K.D., Tangney, R., Tomlinson, S., Turner, S. & Walck, J.L. (2019) A research agenda for seed-trait functional ecology. *New Phytologist*, 221, 1764-1775.
- Stamatakis, A. (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22, 2688-2690.
- Stampfli, A. & Zeiter, M. (2008) Mechanisms of structural change derived from patterns of seedling emergence and mortality in a semi-natural meadow. *Journal of Vegetation Science*, 19, 563-576.
- Sultan, S.E., Barton, K. & Wilczek, A.M. (2009) Contrasting patterns of transgenerational plasticity in ecologically distinct congeners. *Ecology*, 90, 1831-1839.
- Tjoelker, M.G., Craine, J.M., Wedin, D., Reich, P.B. & Tilman, D. (2005) Linking leaf and root trait syndromes among 39 grassland and savannah species. *New Phytologist*, 167, 493-508.
- Uselman, S.M., Snyder, K.A., Leger, E.A. & Duke, S.E. (2015) Emergence and early survival of early versus late seral species in Great Basin restoration in two different soil types. *Applied Vegetation Science*, 18, 624-636.

Valladares, F., Sanchez-Gomez, D. & Zavala, M.A. (2006) Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. *Journal of Ecology*, 94, 1103-1116.

Willis, C.G., Baskin, C.C., Baskin, J.M., Auld, J.R., Venable, D.L., Cavender-Bares, J., Donohue, K., de Casas, R.R. & Grp, N.E.G.W. (2014) The evolution of seed dormancy: environmental cues, evolutionary hubs, and diversification of the seed plants. *New Phytologist*, 203, 300-309.

Wright, S.D. & Mcconnaughay, K.D.M. (2002) Interpreting phenotypic plasticity: the importance of ontogeny. *Plant Species Biology*, 17, 119-131.

Table 1 Blomberg's K values indicating the extent of phylogenetic signal for each trait (upper table) and plasticity index (lower table). Corresponding P-values indicate the significance of each signal (randomization test). Traits and plasticity indices with significant differences between grass and forb groups are indicated in the last column (P-values from two-way ANOVAs, Table S3).

Traits	K	P	ANOVA P (Growth form)
<i>Seed traits</i>			
Seed mass	0.370	0.084	--
Time to germination	0.109	0.954	0.607
<i>Tissue construction</i>			
Shoot diameter	0.279	0.221	0.626
Root diameter	0.300	0.143	0.968
Specific root length	0.354	0.103	0.189
Specific leaf area	0.282	0.227	0.181
Shoot dry matter content	0.498	0.007	0.011
Root dry matter content	1.190	0.000	<0.001
Leaf dry matter content	0.457	0.025	0.277
<i>Biomass allocation</i>			
Root to shoot length ratio	1.019	0.000	<0.001
Root mass ratio	0.693	0.001	0.064
<i>Growth</i>			
Root elongation rate	0.212	0.459	0.682
Shoot elongation rate	0.243	0.341	0.391
<i>Fitness metric</i>			
Survival time	0.896	0.000	<0.001
<i>Plastic Responses</i>			
Plastic Responses	K	P	ANOVA P (Growth form x Treatment)
<i>Seed trait</i>			
Time to germination	0.276	0.214	<0.001
<i>Tissue Construction</i>			
Shoot diameter	0.193	0.551	0.008
Root diameter	0.238	0.349	0.011

Specific root length	0.390	0.067	0.002
Specific leaf area	0.555	0.003	0.003
Shoot dry matter content	0.600	0.005	<0.001
Root dry matter content	0.202	0.498	0.87
Leaf dry matter content	0.480	0.009	<0.001
<i>Biomass allocation</i>			
Root to shoot length ratio	0.522	0.008	<0.001
Root mass ratio	0.602	0.013	<0.001
<i>Growth</i>			
Root elongation rate	0.203	0.513	0.686
Shoot elongation rate	0.349	0.122	0.031
<i>Fitness metric</i>			
Survival time	0.202	0.531	0.131

Table 2 Seedling survival time under drought as a function of species' positions along four PCA trait axes (see Fig. 3). Bolded attributes were associated with longer survival times.

PCA Axis	Axis Interpretation	t	p
<i>(Intercept)</i>		28.796	1.04E-11
RC1	Small & thin tissues ↔ Large & thick tissues	1.379	0.19516
RC2	Deep Roots ↔ Tough Roots	3.438	0.00555
RC3	Slow ↔ Fast	1.404	0.1878
RC4	Mass put into shoots ↔ Mass put into roots	2.053	0.06465
		Adjusted R-squared: 0.5147	

Figure captions

Fig. 1 A) Final phylogenetic tree for study species, and the distribution of species' traits (B-F; gray bars) and plasticity indices (G-K; black bars) across the tree. For traits, bars show species mean values with standard errors (pooled across treatments). For plasticity indices, bars show PI_v values (see main text). Below each panel, p-values indicate whether there was a significant phylogenetic signal and/or difference between grasses and forbs (see Table 1). All other traits and plasticity indices are in Fig. S1.

Fig. 2 Trait variation as a function of growth form (forb, "F", circles; grass, "G", triangles) and water treatment (wet, "W", black; dry, "D", gray). Traits were scaled to a mean of 0 and standard deviation of 1. Points are group means and bars are ± 1 std. error. Significant factors are indicated to the right of each trait (T, treatment; G, growth form; T*G, their interaction (see Table S3 for model results).

Fig. 3 A-B A) First and second axes, and B) third and fourth axes from PCA including 13 traits ('RC' for rotated principal components). Points are species (forbs, closed circles; grasses, open triangles) in trait space. Only traits which load strongly on one of the displayed axes ($|r| > 0.5$) are labelled. See Fig.2 for trait abbreviations.

Fig. 4 Relationships between individual traits and survival (all bivariate correlations in Table S5). A) Root dry matter content ($r=0.60$), which loaded on PCA Axis 2; B) Root mass ratio ($r=0.54$), which loaded on PCA Axis 4; C) Seed mass ($r=0.73$) which loaded on PCA Axis 1.

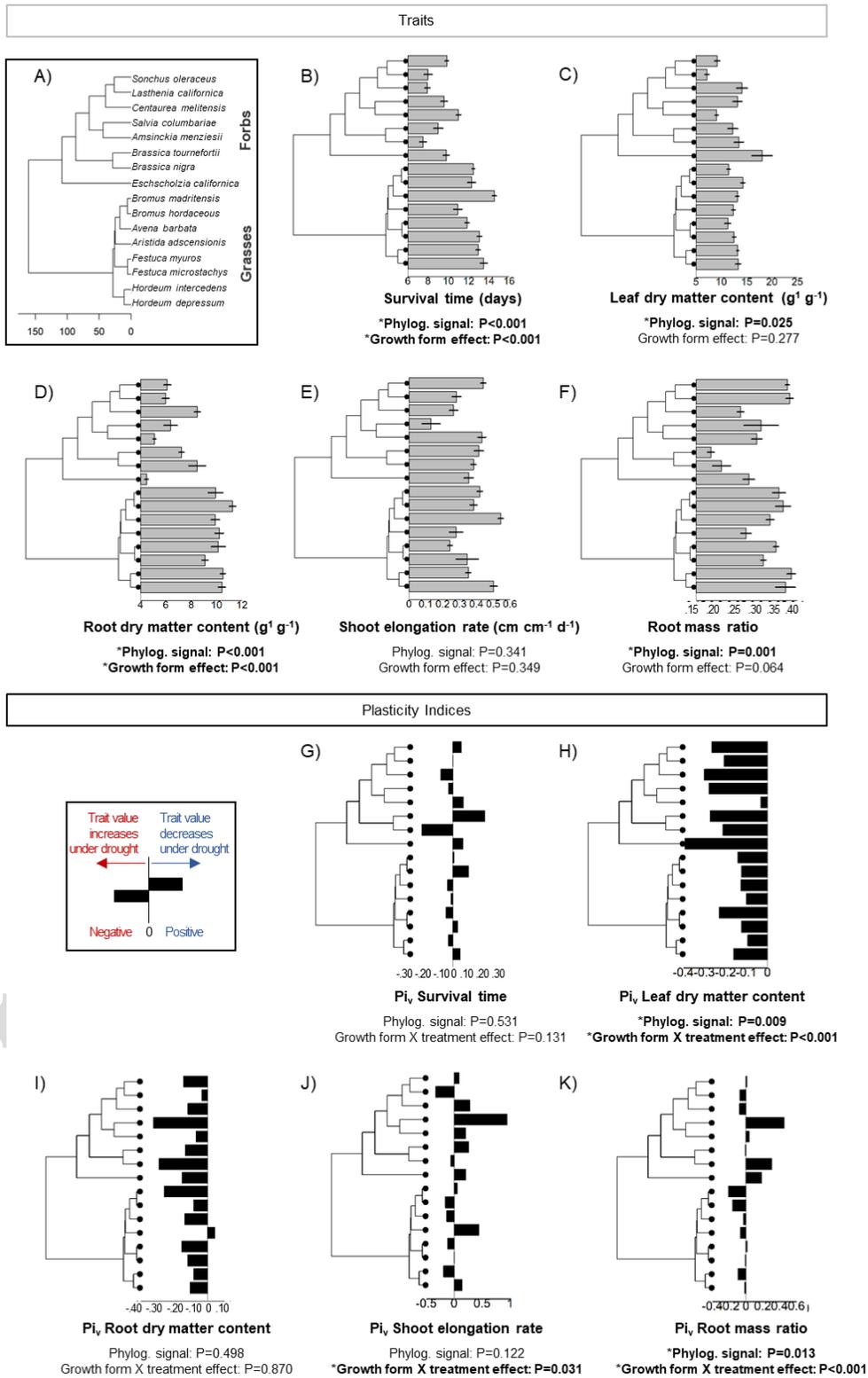


Fig. 1

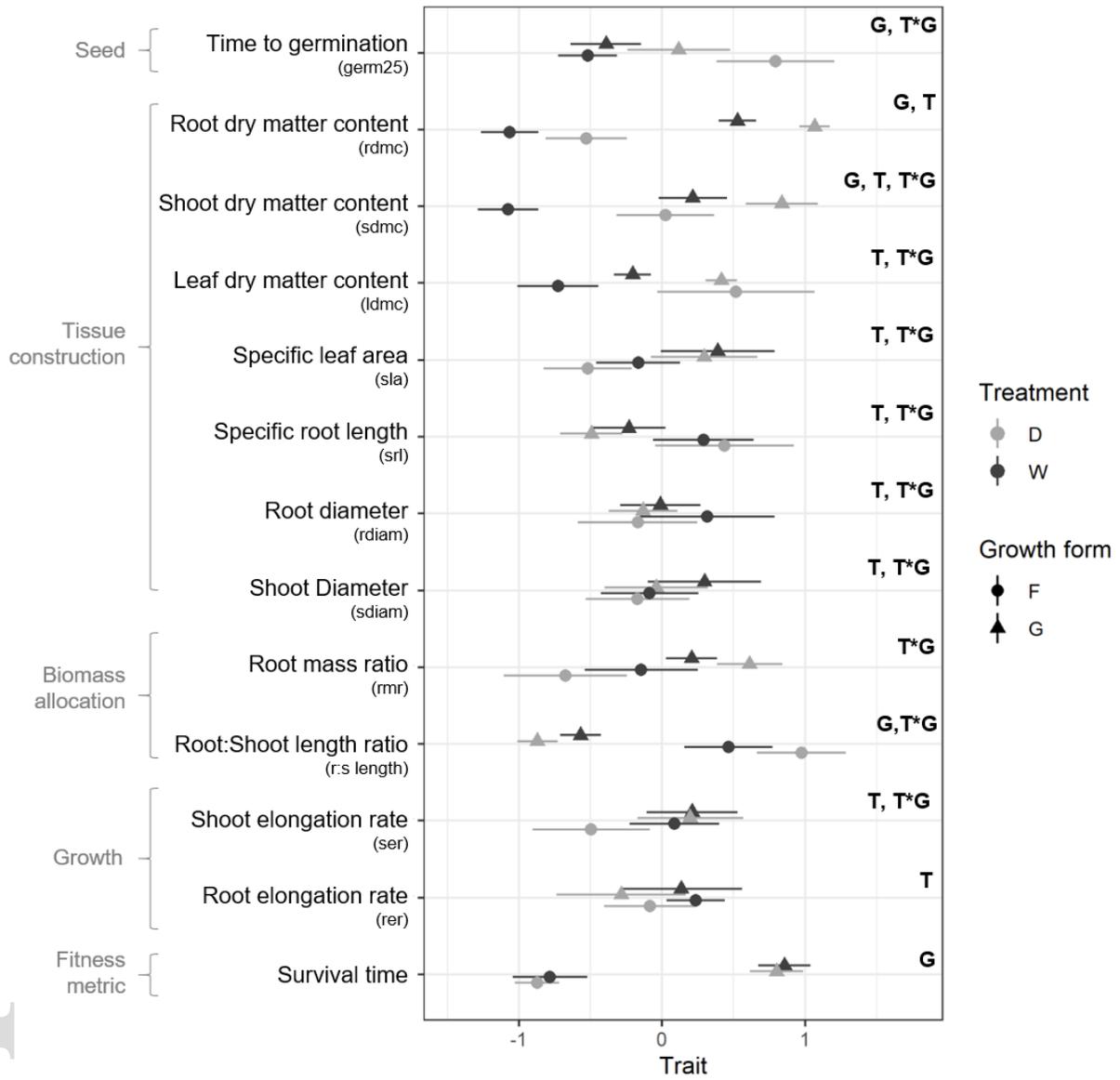


Fig. 2

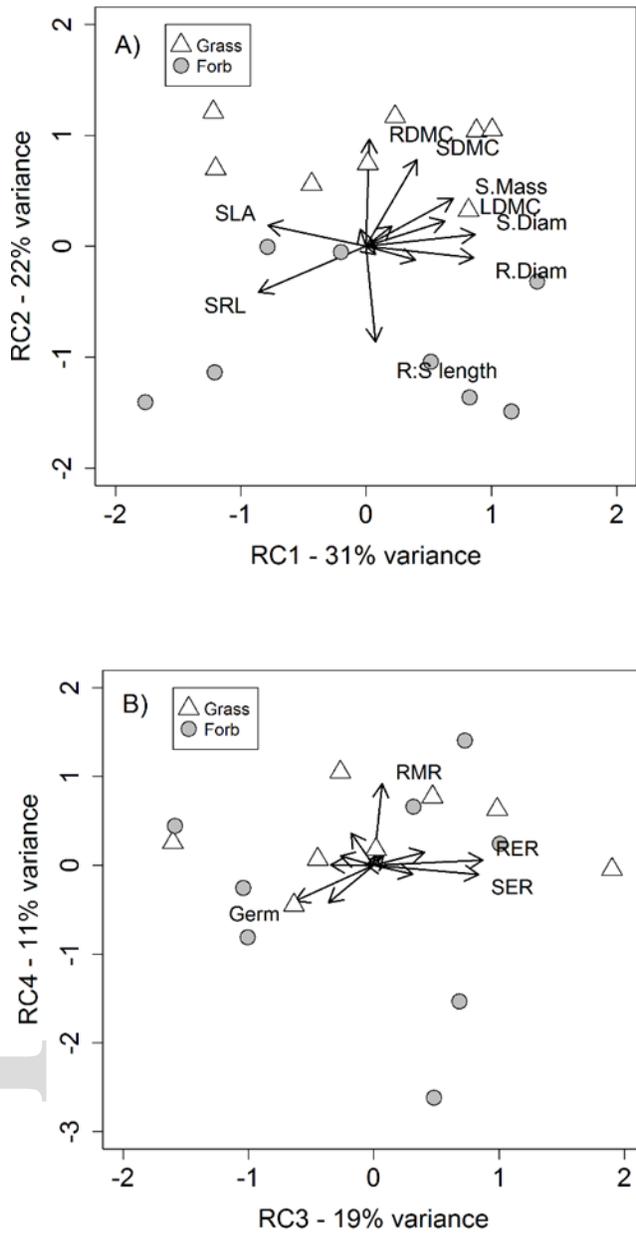


Fig. 3 A-B

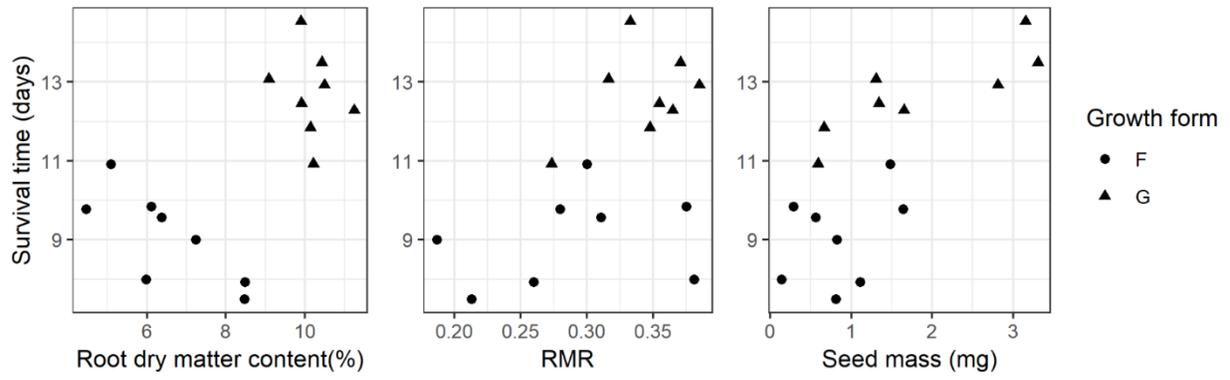


Fig. 4

Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Annual species used in the study.

Table S2 Species average trait values and standard errors.

Table S3 Mixed model results: survival time and traits as a function growth form (grass (G) or forb (F)), treatment (wet (W) or dry (D)), and their interaction.

Table S4 Trait correlations with PCA axes for a) species average trait values b) species ‘wet’ trait values, c) species ‘dry’ trait values.

Table S5 Pearson correlation coefficients (r) between species mean trait values, including mean survival time under drought (days).

Fig. S1 Barplots showing A) average species traits and B) plasticity indices distributed across the final phylogenetic tree, along with Blomberg’s K statistics and associated p-values (randomization test).

Fig. S2 Soil volumetric water content (VWC, %) across the dry-down experiment.

Appendix S1 Methods and results from phylogenetic generalized least squares models exploring the explanatory power of growth form to account for phylogeny in models.