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Seedling traits predict drought-induced mortality linked to diversity loss

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Trait-based approaches are increasingly used to predict ecological consequences of climate change, yet seldom have solid links been established between plant traits and observed climate-driven community changes. Most analyses have focused on aboveground adult plant traits, but in warming and drying climates, root traits may be critical, and seedlings may be the vulnerable stage. Relationships of seedling and root traits to more commonly measured traits and ecological outcomes are poorly known. In an annual grassland where winter drought-induced seedling mortality is driving a long-term decline in native diversity, using a field experiment during the exceptionally dry winter of 2017–2018, we found that seedling mortality was higher and growth of seedlings and adults were lower in unwatered than watered sites. Mortality of unwatered seedlings was higher in species with shorter seedling roots, and also in species with the correlated traits of small seeds, high seedling specific leaf area (SLA), and tall seedlings. Adult traits varied along an axis from short-stature, high SLA and foliar N, and early flowering to the opposite values, and were only weakly correlated with seedling traits and seedling mortality. No evidence was found for adaptive plasticity, such as longer roots or lower SLA in unwatered plants. Among these species, constitutive variation in seedling root length explained most of the variation in survival of a highly vulnerable life stage under winter drought. Selective loss of species with high adult SLA, observed in this community and others under drought stress, may be the byproduct of other correlated traits.

grasslands | functional traits | roots | specific leaf area | aridification

Plant functional traits are increasingly being shown to correlate with species-level variation in demographic rates (1, 2), life history strategies (3, 4), and the outcomes of species interactions (5, 6). One of the most ambitious goals in trait-based plant ecology is to predict the effects of environmental impacts on community composition and ecosystem function (7–9), ideally using relatively easily measured and, thus, widely available morphological, traits. For example, under the more frequent and intense droughts forecast in many parts of the world (10, 11), selective mortality may lead to future communities enriched in species with foliar (12), root (13), and life history (14) traits associated with drought resistance. In turn, such trait-based shifts may be found to exert predictable effects on soil biotas (15), rates of carbon and nutrient cycling (7), and other ecosystem-level outcomes.

Significant challenges that still confront the trait-based framework include the ambiguous functional interpretation of many morphological traits, the difficulty of measuring more directly relevant physiological traits, and the high correlations among some traits (9). While traits are usually measured on adult plants under unstressed conditions (16), and species-level mean values are used to predict ecological outcomes, such predictions may be undermined by within-species variation (17) arising from environmental, genetic, and developmental sources. Predictions are often derived from geographic correlations between mean trait values and climate, but such correlations are often weak (18) and inconsistent with experimental outcomes (19). Despite the proliferation of trait-demography and trait-environment correlations, few if any studies

have shown that interspecific trait variation predicts the outcomes of real climate-driven community changes, while at the same time taking key sources of intraspecific trait variation into account, and using experiments to reinforce observational evidence rather than to explore hypothetical scenarios.

California in the 21st century has undergone a dramatic "whiplash" from prolonged drought to extreme rainfall back to drought in a pattern of water cycle intensification that is predicted to increase in coming decades (20, 21). The documented long-term impact of this climate whiplash on a diverse annual grassland community (22, 23) formed the ecological background to the present study. From 2000 to 2017, species richness and diversity declined at 80 5-m² sites in a remote 2,600-ha biological reserve, with native annual forbs declining the most and no functional group increasing substantially. Native forbs having a trait that has been associated with drought intolerance [high specific leaf area (SLA), or leaf area/mass, measured at the adult stage] declined fastest as they "blinked out" more and "blinked in" less often over time than other species. These community changes were statistically attributable to a decline in winter (December–February) rainfall from 2000 to 2016, which was the only significant quarterly climatic trend during this period except for parallel declines in winter humidity and cloud cover. An extremely wet winter in 2016–2017 produced no recovery in grassland diversity and was followed by a return to severe winter drought in 2017–2018. A field experiment in 2015–2017 showed that winter drought induced high seedling mortality and that water addition only restored diversity when it was coupled with

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Significance

Tremendous effort is being devoted to developing large databases of plant traits, with the hope that these constitute a species-independent currency for predicting the nature and consequences of changes to plant communities driven by climate and other impacts. Yet in strikingly few cases has the efficacy of traits for understanding observed changes to the diversity of natural communities been tested. In an annual grassland undergoing drought-induced diversity loss, we found experimentally that critical outcomes were predicted only by seedling root length, while widely measured adult and aboveground traits were weakly correlated with outcomes. The need to pinpoint traits specific to the location and timing of a major environmental stress may pose a significant challenge to trait-based ecology.

seed addition. This combined evidence indicated that prolonged winter drought led to elevated seedling mortality, which depleted the seedbank and prevented a rebound of diversity in the wet year (23).

The present study used a field experiment to strengthen the mechanistic linkage between plant traits and drought-induced biodiversity loss. A central question was which of a suite of potentially interrelated aboveground and belowground traits was most strongly predictive of species loss in this system. Another question concerned the role of developmental variation. In this system as in many others, seedlings are likely to be the most drought-sensitive stage (13, 24), and their trait values may be quite different from those of adult plants. Finally, the role of plasticity was considered since some studies have found water stress to induce potentially adaptive changes, such as longer roots or lower SLA (25–27). Such trait plasticity may contribute to interspecific variation in stress tolerance (28), or it may parallel interspecific patterns and therefore not reduce the predictive power of species-level traits (29).

SLA was an obvious candidate trait based on earlier evidence linking high SLA to drought sensitivity (22, 23, 30, 31). However, drought-related interpretations of SLA are complicated by its unclear relationship to the physiology of water use (12, 32) and by its relationships to nutrient availability, shade tolerance, and herbivory resistance (33–35). Also, high SLA is correlated with other trait values that constitute the "fast growing" functional strategy, including small seeds, high foliar N, and height (18, 36–39), some of which could directly influence drought sensitivity. For example, smaller-seeded species are more prone to remain dormant in dry years while larger-seeded species can elongate their roots faster (40). Root traits, such as maximum depth, specific root length, elongation rate, diameter, and density, are likely to be critical for both drought tolerance and rapid growth (13, 41, 42), yet they are much less studied than aboveground traits, and there is little consensus as yet on their patterns of covariation (43) and correlation with other traits (44, 45).

The field experiment was conducted in the 2017–2018 growing season, when winter rainfall (December 1, 2017–March 1, 2018) was 133 mm, or 33% of its 30-y average. A rain event of 39 mm from November 8–11, 2017, stimulated strong germination in the grassland annuals over the ensuing 2 wk, but no rain fell from November 28, 2017 to January 2, 2018 and January 26 to February 25, 2018, and soil surfaces became hard and cracked during these monthlong dry spells. The experiment took place on 20 watered and 20 unwatered control sites in serpentine grassland that were used in the previous experimental study (23). Watering was applied weekly from December 1 to February 1 to maintain total rainfall at or slightly above its 30-y average. Because plants in the unwatered plots were subject to more extreme drought stress than those in the watered plots, they are referred to as the unwatered treatment rather than the control treatment. Seeds of 10 locally common native annual forb species were planted in the watered and unwatered sites in late October 2017 when the first rains were imminent and germinated in mid-to-late November 2017. Seedlings were marked and censused on December 1, 2017, and recensused on January 5 and February 1 and 27, 2018, to obtain three measures of cumulative mortality at monthly intervals. Seedling traits were measured in mid-January, and adult traits were measured in early April to late June when each species was at or near flowering (*SI Appendix*, Table S1).

Using the 10 experimental species as replicates, seedling mortality was analyzed in relation to trait variation. Given the prior evidence for wintertime seedling mortality as the key mechanism of community change, these analyses focused on seedling traits, but adult traits were measured so that their correlations to seedling traits and mortality could be examined. Constitutive values of traits (i.e., their species-level means in

watered plots) were the principal focus; however, based on earlier evidence for adaptive plasticity in SLA and root development (25–27), the proportional changes in these traits in drought-stressed plants relative to watered plants were also examined as potential predictors of seedling mortality across species.

Results

Watering Effects on Seedling Mortality. Cumulative seedling mortality was 27% in the unwatered treatment and 5% in the watered treatment by the end of December; 33% in the unwatered treatment and 10% in the watered treatment by the end of January; and 43% in the unwatered treatment and 12% in the watered treatment by the end of February (*SI Appendix*, Fig. S1). Watering significantly reduced cumulative seedling mortality in paired nonparametric tests (Wilcoxon signed-rank tests, $P < 0.008$ for December, $P < 0.005$ for January, $P < 0.005$ for February; significant after Bonferroni adjustment for three comparisons).

Variation in Seedling and Adult Constitutive Traits. The first two principal components of seedling traits explained 51.3% and 26.7% of the variance (Fig. 1 and *SI Appendix*, Table S2). Seedling trait PC1 reflected the variation from species that were small-seeded, short, and had high SLA and foliar %N as seedlings (low scores) to the opposite trait values (high scores). Seedling trait PC2 reflected variation from species with shorter roots—i.e., shallower rooting depths—in midwinter (low values) to those that developed longer roots (high scores).

The first two principal components of adult traits explained 69.5% and 16.7% of the variance (Fig. 1 and *SI Appendix*, Table S2). Adult trait PC1 reflected variation from species that as adults were on average tall and large (i.e., had high aboveground biomass) and had low SLA and foliar N (low scores) to the opposite trait values (high scores). Adult trait PC2 was moderately associated with adult biomass and foliar %N.

Adult trait PC1 strongly separated the three species that flowered in late spring and summer (*Clarkia*, *Calycadenia*, *Hemizonia*) from the seven species that flowered in April (Fig. 1). Considering individual traits, the three late-flowering species were distinctive for their much lower values of adult SLA, and also for the fact that their SLA declined from the seedling stage to the adult stage (*SI Appendix*, Table S1).

For the three traits that were measured at both the seedling and adult stages, correlations between the two life stages were very weak (height, $r = -0.26$; SLA, $r = 0.43$; foliar N, $r = 0.24$).

Trait Relationships to Seedling Mortality and Adult Growth. Seedling traits, represented as PC axes, affected species-level variation in seedling mortality in both treatments (MANOVAs; unwatered, Wilks' Lambda = 0.021, $P = 0.004$; watered, Wilks' Lambda = 0.023, $P = 0.005$; significant after Bonferroni adjustment for two comparisons). Bivariate analyses showed that seedling trait PC1, reflecting species with larger seeds, taller seedlings, and lower SLA and N, had marginally significant negative effects on seedling mortality in the unwatered treatment that increased in strength over the winter (*SI Appendix*, Fig. S2). Seedling trait PC2, reflecting longer roots and thus greater rooting depth, showed strong and increasing negative effects on mortality in the unwatered treatment, and weaker negative effects in the watered treatment (*SI Appendix*, Fig. S2). Among individual traits, in the unwatered treatment, root length showed a negative correlation with mortality that increased over time from moderate to strong; seed mass had a negative correlation that increased over time from weak to moderate; and seedling SLA had a positive correlation that diminished over time from moderate to very weak (Fig. 2).

Seedling mortality rates showed very weak correlations to constitutive adult traits, including both the trait principal components ($r = -0.04$–0.24) and individual traits ($r = -0.46$–0.32).
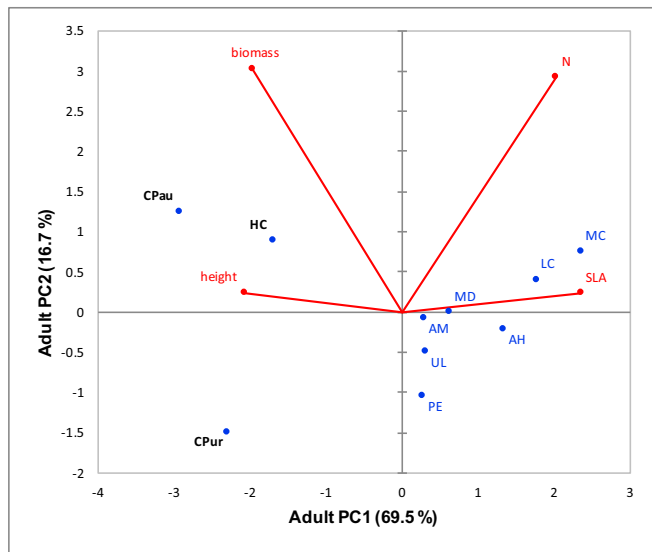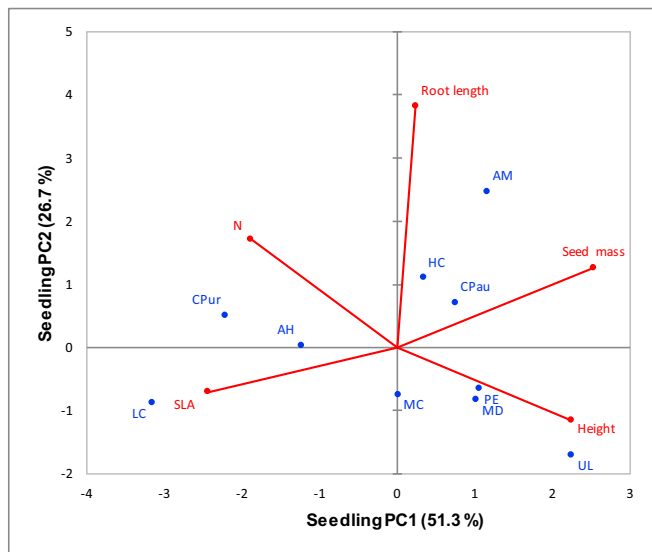
ECOLOGY

Fig. 1. Trait principal components. (*Upper*) Seedlings. (*Lower*) Adults (black = late-flowering species).

Drought sensitivity of growth, i.e., the proportional reduction of adult biomass in the unwatered relative to the watered treatment, showed very weak correlations to the seedling and adult trait principal components ($r = -0.4–0.01$) and to most individual traits ($r = 0.05–0.50$) except for seedling root length ($r = -0.60$).

Treatment Effects on Seedling and Adult Traits. Across species, seedling height averaged 10.1 ± 12.9% greater, absolute seedling root length averaged 6.8 ± 12.2% greater, and relative root length averaged 0.7 ± 5.3% lower in the watered than the unwatered treatment. Watering increased both seedling height and root length (general linear model; height: $P < 0.001$ for treatment, species, and interaction; root length, $P = 0.03$ for treatment, $P < 0.001$ for species, $P = 0.38$ for interaction), but did not consistently affect relative root length (general linear model; $P = 0.86$ for treatment, $P < 0.001$ for species, $P = 0.004$ for interaction). Watering significantly increased only seedling height after Bonferroni adjustment for three comparisons.

Across species, adult height averaged 25.6 ± 16.4% greater, adult biomass averaged 40.6 ± 32.8% greater, and adult SLA averaged 2.7 ± 10.2% greater in the watered than the unwatered treatment. Adult foliar %N (which was not replicated within species) averaged 4.1% higher in the unwatered treatment. Watering
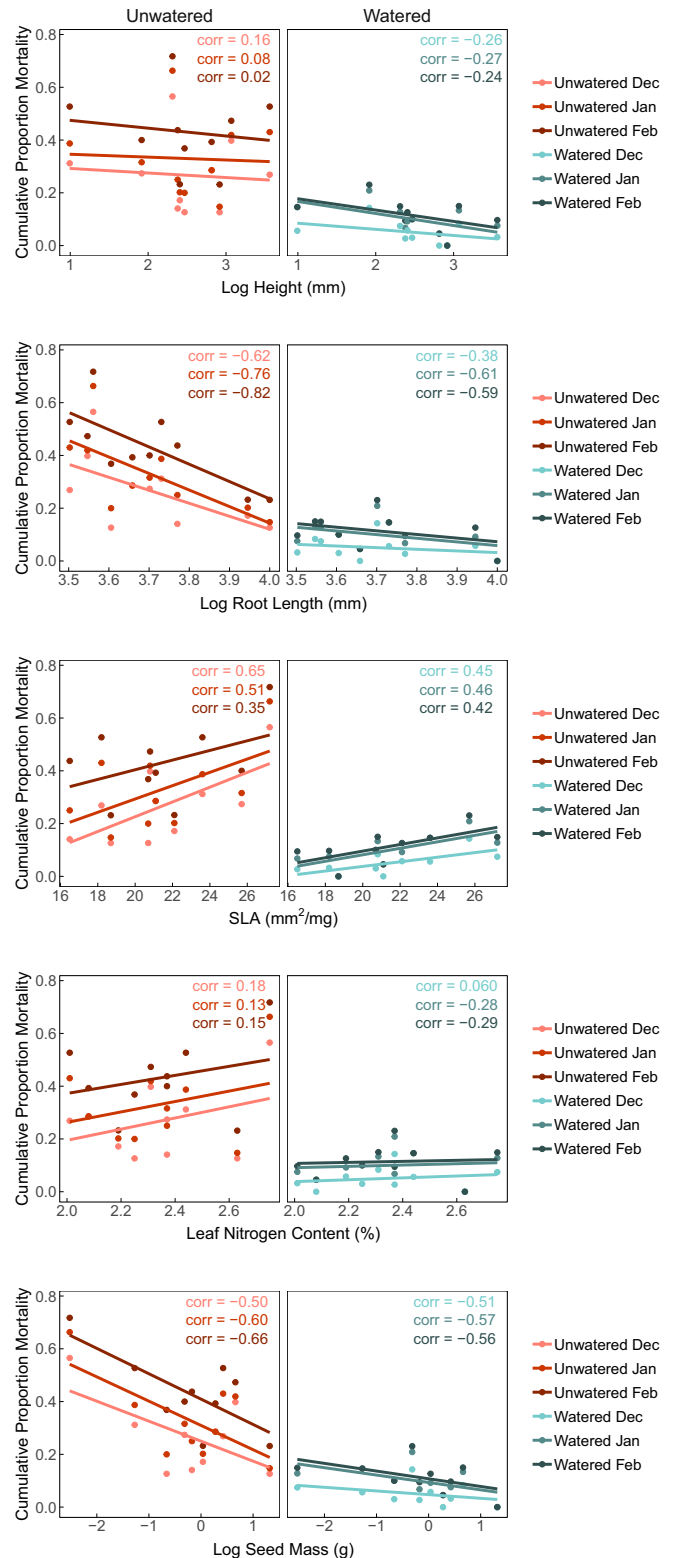

Fig. 2. Seedling mortality versus individual seedling traits.

significantly increased both adult height and biomass, which also varied among species (general linear model; height: $P < 0.001$ for treatment and species, $P = 0.009$ for interaction; biomass, $P < 0.001$ for treatment and species, $P = 0.25$ for interaction), but not adult SLA (general linear model; $P = 0.60$ for treatment, $P < 0.001$ for species, $P = 0.35$ for interaction). Watering still significantly increased adult height and biomass after Bonferroni adjustment for three comparisons.

Under the more conservative approach using paired nonparametric tests on species mean values, watering still significantly increased adult height and biomass but not seedling height (Wilcoxon signed-rank tests; adult height, $P = 0.005$; adult biomass, $P = 0.007$; seedling height, $P = 0.097$).

Seedling mortality rates showed very weak to weak negative correlations to plasticity in seedling root length ($r = -0.12$ to -0.31) and plasticity in adult SLA ($r = -0.10$ to -0.27 in the watered treatment, $r = -0.40$ to -0.55 in the unwatered treatment). Contrary to the hypothesis of adaptive plasticity, but consistent with the stress tolerance–growth tradeoff, these correlations indicated that seedlings survived better in species that changed less under water scarcity.

Discussion

Of all traits measured in this study, the best predictor of decreased drought-induced seedling mortality was greater root length, which measured the depth from which seedlings could extract water as surface soils dried in early winter. Greater seedling root length also showed a reasonably strong correlation to decreased drought sensitivity of adult biomass. Given that the unwatered seedlings in this study were severely stressed by drying soils, the central importance of extending their roots downward in their first 2 mo of growth is readily understandable. More surprising is that root length varied independently of other seedling traits (Fig. 1) as well as adult traits. Rapid early root elongation might have been expected to be associated either with larger seeds that provide more resources (40) or with high SLA and related traits that indicate the capacity for rapid early growth (37), but neither correlation was evident. It is possible that rapid seedling root growth is so critical to annual plant fitness that it can arise in association with either the drought-tolerant large-seeded syndrome or the fast-growing high-SLA syndrome. Such a conclusion would be broadly consistent with recent surveys finding that aboveground and belowground strategies cannot be reduced to a single continuum (44, 45). More broadly, herbaceous annuals are unlikely to show the growth-mortality tradeoff that is prominent in woody perennials (1, 3), since any mortality other than by senescence entails a complete loss of fitness. Growth-dormancy tradeoffs are more relevant for annuals (5, 6), yet neither the drought-tolerant nor the fast-growing and dormancy-prone syndrome is immune to the critical situation faced by small seedlings in an unusually dry winter.

A secondary contribution to decreased seedling mortality came from the cluster of correlated traits that included large seeds, low seedling SLA, and short seedling height. The effect of large seeds, which strengthened over time, may have reflected the ability of larger-seeded plants to allocate more energy to aspects of seedling growth not measured in this study, such as root biomass, total root length, or shoot biomass (35). Low seedling SLA may have related to survival directly through a relationship to higher water use efficiency and leaf turgor maintenance, or indirectly through a correlation with high seed mass or other stress-tolerant traits. In any case, the apparent effect of seedling SLA waned after the first month of drought, as mortality increased sharply for two species that had low seedling SLA but lacked long seedling roots (*Calycadenia pauciflora*, *Micropus californicus*).

Adult traits of these 10 species fell clearly into the classic continuum from high to low values of SLA and foliar %N that reflects the tradeoff between fast growth and stress tolerance (12, 31–34). However, these adult traits correlated weakly with

seedling mortality. The selective loss of native annual forbs with high mean adult SLA observed over the past 18 y (22, 23) may therefore have been the byproduct of the modest correlations between adult SLA on one hand and seedling root length ($r = -0.48$), seedling height ($r = 0.57$), seedling SLA ($r = 0.43$), and seed mass ($r = 0.14$) on the other. These correlations to seedling traits may have been strong enough to create a significant relationship of adult SLA to seedling mortality across many species and years, even if not in a short-term study of 10 species. In addition, the unexpectedly strong relationship of low adult SLA to late flowering phenology suggests an as yet unexplored avenue to explain the previously observed losses of high-SLA species under winter drought. Surviving individuals of summer-flowering species may be able to recover some of their growth and fecundity if there is spring rain, whereas spring-flowering species have little opportunity for such postwinter recovery.

Little evidence emerged for potentially adaptive plasticity in the traits used in this study. Seedling and adult height and adult biomass were lower in unwatered than watered plants, as expected under resource stress. However, water scarcity did not lead to greater absolute or relative root length, as might be expected if plants reallocated resources from water-losing aboveground parts to water-acquiring roots (46), nor did water scarcity induce lower SLA, which might be expected as a way to enhance water use efficiency (25, 28). Also, none of the indices of trait plasticity measured here suggested that plasticity reduced seedling mortality; if anything, lower mortality was associated with greater trait stability under stress. Our results appear consistent with studies finding constitutive trait values to explain most of the interspecific variation in drought responses (29), although adaptive trait plasticity has been found in other cases.

Our results suggest that in the quest for plant functional traits that accurately predict community responses to climate, it is important to pinpoint the relevant developmental stage, in this case seedlings, as well as the relevant part of the plant, in this case roots. Relatively little attention has been given to developmental stage as an important source of trait variation, despite the widespread attention to intraspecific trait variation in general (17) and the awareness that early life stages are often critically sensitive to environmental stress (13, 24). Although seeding and root traits may be highly valuable for predicting the fates of species and communities, however, they are not very well suited to the broader goal of using large databases of easily measured plant traits to predict ecosystem-scale and global-scale outcomes. If the importance of seedling roots as found here and elsewhere (e.g., ref. 24) has substantial generality to other cases of drought-driven community change, it casts additional light on why using traits to predict ecosystem effects continues to be the "Holy Grail" of trait-based ecology (9, 47).

Methods

Study System. The study took place at the University of California McLaughlin Reserve, a 2,776-ha research facility at 366- to 914-m elevation in the Inner North Coast Range (N 38°52′, W 122°26′), largely surrounded by >10^5 ha of public wildlands. The climate is Mediterranean with 30-y mean temperatures of 8 °C in January and 25 °C in July, and mean annual precipitation (=rainfall) of 620 mm mostly falling in November–March. Grasslands are dominated by exotic (Eurasian) annual grasses and native and exotic annual forbs that germinate in fall (September–November) shortly after the first substantial rains, are present as seedlings during winter (December–February), and flower in spring (March–April; most species) to summer (May–August; a few species). Grasslands on infertile soils derived from serpentine rock are richer in native forbs and less dominated by exotic annual grasses than the more widespread grasslands on fertile sedimentary-derived soils. Grassland community composition was studied from 2000 to 2017 at 80 5-m^2 observational sites and from 2015 to 2017 in a 100-site water manipulation experiment (22, 23).

Experimental Setup. The 40 experimental sites in this study were established in spring 2015 along two watering lines emanating from a rainfall catchment system. Each line supported 10 sprinklers 10–15 m apart that cast water over a 3-m radius (Mini Rotor Drip Emitters; Olson Irrigation). The 20 watered sites were centered on the sprinklers, and the 20 unwatered sites were interspersed with, and 4–8 m from, the nearest watered sites. Each site included a 1 × 1 m core observational plot (not used in the present study) and an adjacent 90 × 30 cm demography plot divided into 15 × 10 subplots for seed addition.

On October 28, 2017, when the season's first significant rainfall was imminent, seeds of each of 10 native annual forb species [*Achyrachanena mollis* (Ast.), *Agoseris heterophylla* (Ast.), *C. pauciflora* (Ast.), *Clarkia purpurea* (Onagr.), *Hemizonia congesta* (Ast.), *Lasthenia californica* (Ast.), *M. californicus* (Ast.), *Microseris douglassi* (Ast.), *Plantago erecta* (Plantag.), *Uropappus lindleyi* (Ast.)] were planted into one 15 × 10 cm subplot in each of the 40 sites. Seeds had been collected at the study location in spring 2017; species were selected based on availability, and amounts added to each plot were roughly equivalent to the output of one plant and to the amounts used in the previous study (20–200 seeds). After trimming dead biomass and the sparse perennials to roughly 1-cm height, seeds were planted using very light tillage and burial. Nearly all experimental seedlings emerged between the November 8–11 rain event and the December 1 first seedling census, which was also when the flush of naturally occurring seedlings occurred.

From December 1, 2017, to March 1, 2018, at the end of any week in which rainfall fell below its 30-y average for the week, sprinklers were operated for enough hours to bring natural plus supplemental rainfall up to the 30-y weekly average. Natural rainfall was reported by the Knoxville Creek weather station of the Western Regional Climate Center (https://wrcc.dri.edu/), near the center of our study landscape; supplemental rainfall was estimated by hours of watering times the measured application rate of 25 mm h^{-1}.

On December 1, 2017, up to five emergent seedlings per species and site were marked with toothpicks for a total of 1,704 marked seedlings. These marked seedlings were censused again on January 5, February 1, and February 27, 2018, to estimate cumulative seedling mortality for each species, treatment, and time period.

Seedling Trait Measurements. On January 6, following a rainstorm that left the ground soft, a total of 20 nonmarked seedlings per species and treatment were dug up from 10 watered and 10 unwatered sites (0–3 individuals per site). These 400 harvested seedlings were measured while fresh to obtain seedling height and maximum seedling root length (henceforth "root length"). Height was measured as the vertical extent of the fresh seedling in a natural position. Root length was measured as the extent of the gently straightened main root axis, and closely approximated maximum rooting depth; most root systems at this stage had a single main axis with only small branches. Relative root length was calculated as root length/(seedling height + root length).

Seedlings were washed, dried for 24 h at 80 °C, separated into roots and shoots, and weighed on an analytic balance (Mettler Toledo AB204); however, these weights approached the 0.1 mg lower readability limit of the balance and no significant results were found using them.

On January 20, 2018, enough leaves were collected from seedlings in the watered sites only to create one bulked sample of >10 mg of dry weight per species. These 10 samples were photographed while fresh, dried, and weighed. Total leaf area was computed from each photo using ImageJ software and divided by total dry weight to obtain a single measure of seedling SLA from watered individuals of each species. Seedling SLA could not be replicated because of the large number of leaves required for one measurement (>100 leaves to obtain 10 mg for each of the two smallest species).

Seed mass values for the 10 species were obtained from the Kew Seed Information Database (https://www.kew.org/). Seed mass was grouped with seedling traits because of its well-known positive effects on survival and growth of seedlings (40).

Adult Trait Measurements. Surviving plants were harvested aboveground in April–June 2018 when each species reached flowering (except *Hemizonia* and *Calycadenia*, which were harvested at the bolting stage just before their leaves became extremely resinous). Similarly to seedlings, up to 20 plants (when available) were collected for each species and treatment, using 0–3 plants from each of 10 sites of each treatment. These 342 plants were measured for adult height, dried, and weighed to obtain adult biomass, and some of each plant's leaves were used to obtain 342 measures of adult SLA.

Root traits were not possible to collect given the extensiveness of adult root systems and the rocky, clay-rich, dense, and hard (when dry) soils.

Samples of leaves collected during the above measurements were analyzed to obtain one measure of seedling foliar percent N per species, as well as one measure of adult foliar percent N per species and treatment (University of Georgia Soil, Plant, and Water Laboratory, Athens, GA).

Phenology (=observed month of peak flowering) was noted for each species and was grouped with the adult traits, although its skewed distribution made it unsuitable for quantitative analysis.

Trait Tabulation. For analyses of trait effects on seedling mortality, mean constitutive trait values were tabulated for each species (*SI Appendix*, Table S1). These were the species means from the watered treatment, except in the cases of traits lacking replication (foliar N, seed mass, phenology). Values from watered plants were used because it is standard to measure traits on "robust, well-grown" plants when studying the effects of interspecific trait variation on ecological outcomes (10); the word constitutive here refers to species mean trait values as expressed in these watered plants. In general, trait values from watered and unwatered individuals of the same species were well correlated with each other ($r > 0.8$), but values from watered individuals were stronger predictors of interspecific variation in seedling mortality.

For analyses of trait plasticity, which asked whether traits change in response to drought in either potentially adaptive ways (e.g., longer roots in drought-stressed plants) or nonadaptive ways (e.g., shorter roots in drought-stressed plants), we used individual plant values of seedling shoot length, root length, and relative root length (see below).

Analyses. Effects of drought on seedling mortality were examined in paired nonparametric tests (Wilcoxon signed-rank tests) with species ($n = 10$) as replicates, treatment (watered vs. unwatered) as the predictor, and cumulative proportional seedling mortality at the end of each winter month (December, January, February) as the dependent variables. Bonferroni adjustment was used for multiple comparisons, here and in subsequent analyses.

Variation among species in constitutive seedling traits was summarized using principal components analysis on species-level mean values. The central question of this study—how interspecific variation in seedling traits affected interspecific variation in seedling mortality—was tested by first using a multivariate analysis of variance with species ($n = 10$) as replicates, the first two seedling trait PCs as predictors, and cumulative mortality in each month as dependent variables. Next, the mortality for each month and treatment was analyzed in a bivariate general linear model with species as replicates and the first two seedling trait PCs as predictors. Finally, to aid in interpretation of the PC results, simple correlations of each seedling trait with each mortality rate were calculated.

Constitutive adult traits were likewise summarized using principal components analysis on species-level mean values, and their correlations with seedling traits and seedling mortality rates were calculated. Because adult traits cannot "cause" seedling mortality, they were not used as formal predictors. Correlations were also calculated between the species mean of each seedling or adult trait and the drought sensitivity of growth, which was measured for each species as (mean adult biomass in watered treatment − mean adult biomass in unwatered treatment)/(mean adult biomass in watered treatment).

To determine whether seedling traits changed in response to drought, general linear models were used with harvested seedlings ($n = 400$) as replicates; treatment, species, and species–treatment interactions as predictors; and individual values of seedling shoot length, root length, and relative root length (root length/[seedling height + root length]) as dependent variables. Treatment effects on adult traits were similarly tested in general linear models with harvested adult plants ($n = 342$) as replicates; treatment, species, and species–treatment interactions as predictors; and individual values of adult plant height, biomass, and SLA as dependent variables.

As a more conservative test of treatment effects on traits, we also used paired nonparametric tests (Wilcoxon signed-rank tests) with species ($n = 10$) as replicates, treatment (watered vs. unwatered) as the predictor, and species-specific and treatment-specific means of each trait as dependent variables.

To examine whether drought-induced trait plasticity was associated with lower mortality, correlations of seedling mortality to the plasticity of seedling root length and adult SLA were calculated, where plasticity of each trait was measured as (mean value in watered plants − mean value in unwatered plants)/(mean value in watered plants).

Correlations were described as very weak, weak, moderate, or strong if the absolute value of Pearson's r was respectively <0.50, 0.50–0.57, 0.58–0.70, or >0.70, corresponding to the critical values of α for $P > 0.1$, $0.1 > P > 0.05$, $0.05 > P > 0.01$, or $P < 0.01$ for simple correlations with $n = 10$. Formal statistical inferences were not attempted because of the large number of comparisons.

Variables were log- or square root-transformed as needed to meet normality assumptions. Analyses were conducted in Systat 13 (Systat Software

Inc.), except for the principal component analysis, which used XLSTAT (Addinsoft, New York).

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