Title

Global change factors interact with functional traits to influence seed banks and recruitment in grassland communities

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Global change factors interact with functional traits to influence seed banks and recruitment in grassland communities

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Abstract

Grasslands are biodiverse communities that offer many ecosystem services such as erosion prevention, carbon sequestration, and water filtration. Global change factors, such as increases in temperature, unpredictable precipitation patterns, and nitrogen deposition, impact grassland communities which can lead to species loss, dominance of non-native annual grasses, or habitat conversion. While many grassland and dryland species are adapted to low precipitation and high temperatures, they may not tolerate further shifts in environmental conditions. Morpho-physiological traits characterizing trade-offs in growth strategies are useful in predicting how species, populations, and communities will respond to shifting global change factors. While traits of adult plants are well studied, trait-performance relationships for early regeneration stages (seeds, seedlings) are less understood, and these stages may be particularly vulnerable to shifts in environmental conditions. Here, I examine how the traits and species identity of adult graminoids influence seedling establishment of nearby plants (Chapter 1), how seed traits interact with global change factors to shift seed bank composition (Chapter 2), and how differences in populations and maternal environment conditions drive intraspecific variation in seedling traits, seedling drought resilience, and first-year reproduction (Chapter 3). The main finding from Chapter 1 was that forb establishment was more likely next to graminoids with low leaf dry matter content (LDMC) and similar flowering phenology. For Chapter 2, I found that non-native seeds were larger than natives, and that N-fixing forbs had strong seed barrier traits. Additionally, community weighted seed traits shifted with resource availability, where higher nutrient availability increased seed size and higher water availability reduced seed coat thickness. For Chapter 3, in a survey of three populations of a perennial grass, I found that plants from the most arid population had root traits associated with higher resource acquisition, which were correlated with higher reproduction but not survival. Further, the maternal water environment impacted first year reproduction, e.g. transgenerational plasticity, where the direction of the reproductive response depended on the population. Collectively, my results have advanced our understanding of how traits respond to environmental variation and how they may be used to predict species, population, and community response to global change factors. My results also have important implications for management of grassland systems. As graminoids with low leaf dry matter content appear to be good nurse plants for forbs, my work suggests that incorporating these species, or increasing leaf trait diversity, in seeding mixes may enhance forb cover in dryland restoration efforts. Finally, my finding that seed provenance and maternal water availability affect first-year reproductive output reinforces the value of using locally adapted seed sources and suggests that using different watering regimes for the cultivation of seed to use in restoration plantings may enhance restoration outcomes.

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Chapter 1

Some graminoids are better neighbors - phenological synchrony and leaf traits may impact seedling establishment

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ABSTRACT

Species interactions affect plant establishment success, however, how traits of neighboring species influence species interactions is less understood and may depend on environmental context. A better understanding of which traits associated with dominant species facilitate establishment of new recruits, which is a critical transition in population dynamics, may be relevant to predicting or preserving community richness. Neighbors may alleviate effects of environmental stress on recruits as well as increase local environmental heterogeneity. In this study, we examined how dominant graminoid species and their functional traits related to the abundance and richness of forb recruits and whether this varied based on phenological timing in grassland communities. Abundance and richness depended on the species identity of a neighboring graminoid and phenological timing, such that spring-active forbs were more commonly associated with early flowering graminoids and summer-active forbs with later flowering graminoids. Local richness was higher near graminoid species with higher aboveground resource acquisitive traits (low leaf dry matter content - LDMC) and more conservative belowground resource acquisitive traits (low specific root length). Finally, abundance of establishing individuals depended on forb phenology, where only spring-active forbs were more abundant near graminoids with low LDMC. Together, our results suggest that phenology and traits are important considerations when predicting the impact of species interactions on community composition. Understanding how species interactions affect establishment will help inform the management of grassland systems confronted with climate change.

INTRODUCTION

Plant-plant interactions vary greatly between species and influence community composition and succession. Although neighbors can compete for resources, leading to reduced performance, interactions between neighbors can be neutral or even beneficial (Bruno et al. 2003, Brooker et al. 2008, Bronstein 2009, Maestre et al. 2009). Facilitation, defined as positive interactions between organisms, is a widespread phenomenon (Brooker et al. 2008, Maestre et al. 2009). Facilitation may benefit both partners, such as when multiple species flower concurrently to attract more pollinators (Bronstein 2009). However, facilitation may come at a cost to the facilitator, such as increased competition (Callaway 2007, Michalet et al. 2006). These interactions, though small scale, can influence ecosystem dynamics by altering species distributions over time and space (Richardson et al. 2013, Usinowicz and Levine 2018). The goal of this study is to determine the extent to which different graminoid species act as facilitators, and how the capacity for facilitation can be predicted based on their morphological characteristics and phenology. By identifying which species facilitate the establishment of other plants, especially in challenging arid environments, we can better predict community response to environmental changes and enhance management and restoration strategies for these ecosystems (Butterfield 2009, Butterfield et al. 2017a, Copeland et al. 2018).

As newly germinated individuals have limited resources and defenses, establishment is a highly vulnerable transition during which plant-plant interactions may have a large impact on survival (Larson and Funk 2016a, Gallien and Zurelli 2018). Adult plants may enhance survival of young seedlings (i.e., nurse plant effects, Soliveres et al. 2011, Howard et al. 2012, Cavieres et al. 2014) by improving the local abiotic conditions (e.g., reducing solar radiation, Pueyo et al. 2016), or adding heterogeneity to the environment, such as creating areas with low or moderate light availability (Koutzoukis et al. 2023). For example, *Carnegiea gigantea* (saguaro cacti) are nursed by *Cercidium microphyllum* (palo verde) trees, which provide protection from heat (Shreve 1931), frost, and predation (Steenberg and Lowe 1977). In contrast, a sedge species, *Carex nudata*, increased local richness by creating areas of full shade, which increased the heterogeneity of the habitat and prevented a single forb species from competitively excluding others (Levine 1999). Positive effects from nurse plants may increase survival during vulnerable life stages, such as for seedlings, especially under harsh conditions (Arroyo et al. 2015, Ziffer-berger et al. 2014).

Nurse plants affect their local environment through morphological, physiological, and phenological traits and, consequently, may increase the survival of neighbors (Temperton et al. 2004, Soliveres et al. 2011). Plants modify their physical environment by changing light quantity and quality, influencing wind flow through surface roughness, and regulating the flux of water, carbon, and heat through canopy conductance (Callaway 2007, Howard et al. 2012, Pueyo et al. 2016, Richardson et al. 2013). Phenology of green-up and flowering affect the timing and duration of evapotranspiration and light reflectance, as green plants absorb more energy (Ryu et al. 2008). Further, plants with more resource acquisitive traits, such as high specific leaf area, leaf nitrogen (N), and root nitrogen (N), tend to have higher evapotranspiration and photosynthetic rates (Wright et al. 2004, Reich 2014), leading to higher relative humidity and more moderate temperatures (Richardson et al. 2013). Plant height may also impact the local environment through affecting wind speed and direction, as well as shade, where taller plants reduce wind flow and sunlight, slowing the rate of water loss from surrounding plants (Richardson et al. 2013). Additionally, higher specific root length and smaller root diameter may provide increased potential for inoculation by beneficial mycorrhizae to individuals establishing nearby (Comas et al. 2014, Bergmann et al. 2020, Rutten and Allen 2023). Finally, higher leaf P and root N may speed up the processes of litter decomposition, which can alter the heterogeneity of the habitat and increase nutrient availability (Howard et al. 2012). Thus, the morphological, physiological, and phenological traits of dominant plants not only shape their environment but can also impact community composition by enhancing the survival of neighboring plants.

Plant growth strategies influence plant-plant interactions, and species with particular traits may be more or less likely to benefit from a nurse plant (Levine 1999, Brooker et al. 2008). Forb species may employ various growth strategies to achieve fitness in dry environments such as drought avoidance, where rapid resource acquisition allows plants to reach maturity before periods of drought; or drought tolerance, where plants tolerate hot and dry periods through resource conservation (Volaire et al. 2018, Kooyers 2015). Plants with greater drought tolerance and similar phenology to the nurse plant may benefit most from this shelter, and microhabitat changes in environmental conditions, while forbs that avoid drought through fast growth during wet periods, and forbs with very different phenological timing may be less affected. However, trait similarity, such as phenology, between neighboring plants could also increase competition and reduce fitness (Ackerly and Cornwall 2007, Gastauer et al. 2017). Resource efficient C4 grasses are often photosynthetically active later into the summer, maintaining growth during drier and hotter conditions (Epstein et al. 1997, Edwards and Still 2008). Thus, competitive effects between late flowering C4 species and summer active forbs may result in predominantly negative interaction for forbs, while facilitative effects of earlier flowering graminoids on summer-active forbs may shift interactions from negative to positive.

In grassland systems, the survival of forb seedlings may vary based on their specific phenology, specific graminoid species present, and abiotic conditions. This study examines whether there are patterns consistent with facilitation and whether they vary among graminoids with distinct functional traits in a diverse grassland system. In this paper, we ask (1) do different graminoid species vary in the presence, richness, and abundance of neighboring forbs, and does climate or plant size affect the presence of neighbors? Next, we explore if forb phenology changes the relationship with nurse plants and ask, (2) do summer active forbs establish near graminoids more frequently than spring active forbs, and does graminoid phenology affect this? Finally, we explore further differences in traits potentially relating to nurse ability within the graminoid community, and ask (3) which traits inform differences in forb richness and abundance and does this change depend on forb phenology? We hypothesize that forb neighbors are more likely to persist as neighbors of larger individual graminoids, and we expect greater effects of different graminoid neighborhoods on forb communities when conditions are harsher (dry and hot). We hypothesize that summer active plants, which must endure stressful environmental conditions, may benefit more from a nurse plant than spring active forbs. Further, graminoids with later flowering phenology may have limited ability to facilitate summer active forbs as they overlap in reproductive timing and may be competing for resources. We expect that traits relating to habitat amelioration or differentiation through greater canopy conductance (leaf resource acquisitive traits, phenology, photosynthetic pathway), reduced wind (taller plants), litter buildup, or traits that promote mycorrhizal relationships (high specific root length) will correlate with neighborhood richness and abundance, but do not expect this to differ for spring or summer active forb communities.

METHODS

Study site and data collection

This research takes place in semi-arid grasslands of the Colorado Plateau, encompassing a range of elevations (2,070 - 2,500 m), soil textures, and plant communities found in the region (Moore et al. 2022). Plant communities are dominated by a mixture of perennial C3 and C4 grasses and sedges (Table 1.1) with a rich diversity of annual and perennial forbs and some shrubs. The study sites in Northern Arizona experience a bi-modal precipitation pattern with winter precipitation and late summer monsoons with dry periods in early summer and fall and an average growing season of 103 days between the last spring freeze in late May and the first fall freeze in early October (Moore et al. 2022). The mean annual precipitation and temperature range from 500-750mm and 6.2-9 degrees Celsius, respectively (Munson et al. 2019, Figure S1.1). All 60 plots are 1-m2 and located within 40 km of Flagstaff in northern AZ across 12 sites. In these plots, all plants were mapped using a chart-quadrat method, where basal area was traced for all grass, sedge, and ground spreading forbs, and shrubs and forbs were mapped as points (Lauenroth and Adler 2008, Laughlin et al. 2018, Moore et al. 2022). Maps were hand drawn in the field and digitized using ArcGIS software (Figure S1.2). We followed the methods of Lauenroth and Adler 2008 and Laughlin and Moore 2018 to track perennial individuals across years. This research uses data from 2002 – 2021 during which time the plots were censused annually (Moore et al. 2022).

In order to assess differences in nurse plant potential among graminoids, we used maps of the plant community to determine which forbs were within the neighborhood of each individual graminoid for the most abundant graminoid species (Table 1.1). Neighborhood zones and distances to nearby points and polygons were calculated using the functions buffer, near, and distance in ArcGIS (ESRI 2019). We defined the local neighborhood as 10 cm from the edge of the basal area of a graminoid polygon in every direction. A 10 cm neighborhood area was used because it was large enough to allow tracking of graminoid polygons (Lauenroth and Adler 2008) and small enough to reduce neighborhood overlap between graminoids while detecting species interactions (Gridzak et al. 2024). All graminoid polygons within 10 cm of the edge of the plot were excluded from the analysis, as were all graminoids with less than 1 cm2 basal area. Each forb seedling was assigned to the neighborhood of the closest graminoid, where seedlings included all annuals as well as perennials that had not been present in the previous year. Within each neighborhood, forb abundance was determined as the number of forbs, and forb richness was the number of different forb species. Other graminoids present in the neighborhoods were not included in our analysis.

Statistical analysis

To evaluate whether graminoid species varied in their likelihood of having forbs in their neighborhood (Q1), we used a binomial generalized linear mixed model with a logit link function. The response variable for this model was whether a graminoid individual had at least one forb seedling nearby (1) or did not (0). We included graminoid size, graminoid species, as well as the annual temperature and precipitation for the site of each observation as fixed factors in the model. We used climate data downloaded from PRISM for each site for each year of the study (http://www.prism.oregonstate.edu). Site annual precipitation and temperature values were log transformed for all analyses. In order to account for potential site differences, plot nested within the site was initially included as a random intercept, however plot was removed due to issues of model convergence (lme4, Bates et al. 2015). We then evaluated if the richness and abundance of forbs varied based on graminoid species identity. In order to account for the nonlinear species-area relationships in species richness and abundance (Scheiner 2003, Arrhenius 1921) we used the residuals for each individual from a power model where the log richness or abundance was predicted by individual neighborhood area as the response variable (Table S1.1). We used a non-parametric analysis of variance on these residuals (ANOVA; Kruskal Wallis rank sum test) to test for differences among species and calculated the significance of pairwise comparisons using a pairwise Wilcoxon rank sum test (R Core Team 2023). Using the mean rank sum from the Kruskal Wallis test, we ordered graminoid species from lowest to highest in terms of the forb abundance and richness in their neighborhoods. Following extraction of spatial data from ArcGIS, all data manipulations, calculations, and analyses were executed in R version 4.3.1 "Beagle Scouts" (R Core Team 2023).

In order to determine if the probability of having a seedling neighbor shifted depending on the phenology of the forb species, and how similar this was to the phenology of the graminoid, we used a binomial generalized linear mixed model including the forb activity period instead of species identity. We compared flowering phenology of species as a proxy for green up timing as flowering phenology data is widely available. Green-up timing as well as factors relating to photosynthetic activity such as plant size and degree day thresholds influence flowering phenology (Wang et al. 2014). Activity period was determined using the trait dataset from Laughlin et al. (2010) compiled from regional flora databases and data was supplemented using peak flowering time from GBIF observations for species missing data (R dismo package: Hijmans 2023). Cool season species had a peak flowering date before the end of June (Julian date range of 121 to 259, median of 213). As above, the presence (1) or absence (0) of any forb neighbor was the response variable, here modeled against the type of forb (spring or summer active), the flowering phenology of the graminoid species, the interaction between these two factors, as well as graminoid basal area, and the precipitation and temperature for each site and year. Further, we included the site as a random effect.

Finally, to determine if traits explained variation in nurse potential, we used published data from the study system collected on randomly selected adult plants at each site in which the species occurred (Laughlin et al. 2010). We included 8 traits that we hypothesized may affect nurse plant potential: plant height, peak flowering date, leaf N, leaf P, specific leaf area (SLA), root N, and specific root length (SRL). We did a Principal Component Analysis (PCA) to determine the main axes of trait variation among graminoids in the system. Next, we determined if graminoid trait values impacted neighborhood richness and abundance using a rank order test. We used Pearson's correlation coefficient to determine if the ranked order of graminoids for each trait correlated to the richness and abundance rank orders as calculated from the Kruskal Wallis test (R Core Team 2023). We looked at PCA axes to determine trait groupings and then considered individual traits due to the presence of correlations between trait values. Furthermore, in order to determine if the relationship between traits and nurse ability shifted depending on the phenology of the forb species, we calculated mean rank order values of graminoid richness and abundance separately for spring and summer active forbs. Leaf dry matter content (LDMC) was not included in the PCA analysis as this trait was not available for one graminoid species (POAPRA); this species was removed from the analysis in order to determine rank order values for LDMC.

RESULTS

As hypothesized for Q1, we found that graminoid species differed in potential nurse ability. While most graminoids were neutral, the graminoid species that frequently had seedling neighbors also had high neighborhood abundance and species richness. Specifically, MUHTRI and POACOM had neighbors more frequently than other graminoid species, while ELYTRA, SPOINT, and POAPRA displayed a lower probability of having neighbors (Figure 1.1). While POAPRA and BROCIL were less likely to have neighbors, if any neighbors were present, they had neighborhoods with relatively high richness (Figure 1.2, Figure S1.3). Presence of seedling neighbors decreased under increasing annual temperatures but was not affected by precipitation. Further, graminoid species varied in size (Figure S1.4) and larger graminoids were more likely to have neighbors (Table S1.2). We found significant differences between graminoid species for both abundance and richness (Kruskal-Wallis test, chi-square = 713.5 and 896.5, $p<0.001$, Table S1.3). Richness and abundance rankings were similar for most graminoid species, particularly those with the highest (MUHTRI, POACOM) and lowest (SPOINT, BOUGRA) rankings (Figure 1.2). MUHWRI and FESARI had lower richness rankings but had higher abundance or neighbor presence, while BROCIL and MUHRIG had higher richness but lower abundance (Table 1.2).

For our next question (Q2), we considered the phenology of the forb seedlings and found that seedlings adjacent to graminoids were slightly more likely to be summer active forbs. This supported our prediction that summer active forbs may benefit more from a nurse plant than spring active forbs. Further, when comparing spring and summer active forbs with the flowering phenology of graminoid species, we found that forbs were more likely to be present in the neighborhood of a graminoid with similar flowering phenology (Figure 1.3). Similar to findings from the presence/absence model with graminoid species identity (Q1), the presence of forb seedlings decreased with increased temperature and increased with larger graminoid size (Table S1.4).

Finally, we identified the main axes of trait variation among our graminoid species and determined if trait axes affected local abundance and richness. We found that root resource acquisitive traits, height, and flowering phenology loaded strongly on PC1 (Figure 1.4, Table S1.5), separating tall species with high SRL, high root N, and later flowering from shorter species with less resource acquisitive root traits and earlier flowering (PC1, 35.3% of variance explained, Figure 1.4). The second PC axis differentiated C3 and C4 plants, where, as predicted, C3 plants had higher SLA and leaf N (PC2, 24.2% of variance explained, Figure 1.4). Leaf P did not contribute to the main leaf trait resource acquisition axis separating C3 and C4 species, but loaded as its own axis (PC3, 16.8% of variance explained, Figure S1.5). Once the trait axes were identified, we then tested if trait axes and individual traits related to residual neighborhood abundance and richness (Q3). We found no significant correlation between the rank order of PC values and neighborhood richness or abundance when considering all forb species (Table 1.2). However, when we conducted analyses separately for cool season, spring active forb seedlings and warm season, PC2 was positively correlated with neighborhood abundance for spring species. Next, we explored relationships between individual traits and neighborhood richness and abundance of all forb seedlings due to trait correlations (Figure S1.6). We found negative correlations between local richness and LDMC ($p < 0.01$), and a negative correlation between neighborhood abundance and leaf P content (Table 1.2). In analyses considering spring and summer forbs separately, the finding that LDMC trait values were negatively correlated with neighborhood richness remained true for both groups, further SRL was negatively correlated with richness for spring and summer forbs. However, leaf P was only negatively correlated with summer forb abundance, while LDMC was negatively correlated to spring forb abundance. However, if BROCIL, which has a small sample size, was removed from the rank order correlation test, LDMC was not correlated with abundance for spring forbs (p=0.17). In summary, we found that for both spring and summer seedlings low LDMC and low SRL values were correlated with higher neighborhood species richness. However, neighborhood abundance varied with phenology, such that high PC2 and low LDMC values aligned with increased abundance for spring active forbs, while low leaf P values aligned with increased abundance for summer active forbs.

DISCUSSION

Arid environments pose significant challenges for seedlings (Larson and Funk 2016b, Butterfield 2009) and facilitation from adult plants, such as graminoids, may be essential for forb establishment (Soliveres et al. 2011, Howard et al. 2012, Cavieres et al. 2014). Variation in the neighborhood composition of graminoids would suggest different strengths of forb - graminoid interactions. We found that some graminoid species are likely to be better neighbors and that graminoids are better facilitators to forbs with similar phenology. Further, we found that graminoids with resource acquisitive leaf traits had higher neighborhood richness, while neighborhood abundance shifted based on forb phenology, with LDMC increasing spring forb abundance and leaf P reducing summer forb abundance.

Seedling establishment is influenced by graminoid identity and temperature

While shrubs and trees are commonly identified as nurse plants with known mechanisms of facilitation in stressful habitats (Howard et al. 2012, JankJu 2013, Pueyo et al 2016, Farzan and Ehtegadu 2018, Gholami et al. 2018, Gomez-Aparicio et al. 2004), few studies have demonstrated facilitative effects of graminoid plants (Shaw 2018, Reyl et al. 1996). We tested the probability of having seedling neighbors for 12 different species across time and space and found clear distinctions between species. Specifically, MUHTRI and POACOM individuals were more likely to have neighbors (50% more likely to have neighbor seedlings than average) and, when seedlings were present, their neighborhoods had high species richness and abundance. In contrast, few SPOINT plants had seedling neighbors. Other studies of nurse plants similarly found species-specific patterns but focused on one or a few species (Levine 1999, Kikvidze and Nakhutsrishvili 1998). Our findings suggest that the dominant graminoid species in a stressful habitat may greatly influence the ability of seedlings to establish. The demographic impact of increased seedling survival when some graminoids are dominant will impact community composition over time which has important implications for habitat restoration (Butterfield 2009, Copeland et al. 2018, Navarro-Cano et al. 2021) and understanding how species-interactions influence ecosystem functioning.

Negative and positive interactions occur between many species concurrently and shift with time, climate, and resource availability (McAuffle 1984, Maestre et al. 2009, Brooker et al. 2008). In arid systems, such as those studied here, climate should strongly influence these dynamics, as facilitation and competition are highly dependent on the abiotic environment (Arroyo et al. 2015, Ziffer-berger et al. 2014). In our system, a harsh dry period in the early summer is followed by extensive, warm monsoon rains, and another period of very low precipitation before the winter rain season begins (Gremer et al. 2018). Surprisingly, our findings emphasize the role of temperature instead of precipitation, as higher temperatures reduced the probability that a graminoid had a seedling neighbor, and there was no effect of precipitation.

This pattern was not due to fewer total seedlings present under higher temperatures, resulting in two possibilities. First, this could be due to an increase in total graminoid presence with higher temperatures, in which case, seedlings will be neighbors to a smaller proportion of graminoid individuals. Over half of our species spread vegetatively and a large graminoid recorded in an ambient year may dieback in a year with higher temperatures, thus creating two or more distinct patches of aboveground biomass recorded as separate graminoid individuals. Alternatively, this pattern could arise from an increase in spatial aggregation where few graminoids have neighbors under higher temperatures, however these graminoids have more neighbors. The second possibility may occur if competition increases for seedlings under higher temperatures, reducing seedling survival; however, in the neighborhood of the few graminoid species that show evidence of facilitation, seedlings have higher survival. Temperature determines a species' climatic niche in the Colorado plateau, and this may increasingly impact communities as climate change raises temperatures (Butterfield and Munson 2016).

Synchrony of graminoid and forb flowering influences seedling presence

We found that graminoids are more likely to have seedling neighbors that have similar phenology, i.e., later flowering grasses are more likely to be associated with summer active forbs, while earlier flowering grasses are more likely to be associated with spring active forbs. This contrasts with our hypothesis that an increase in phenological overlap would reduce presence of neighbors given that negative interactions are more acute with increasing trait similarity (Ackerly and Cornwall 2007, Gastauer et al. 2017), which may be more prevalent in extreme climates (Gallien et al. 2018), such as the climate studied here. Phenological matching between forbs and graminoids may instead suggest that facilitative effects shift with seasonal timing. In our system, early-season graminoids may be most active in the spring following snowmelt and have higher leaf area and photosynthetic activity than late-season graminoids, which may benefit neighboring forbs during the dry early summer. Larger early-season graminoids may also benefit forbs through hydraulic lift (Maestre et al. 2001). Smaller, late-season graminoids would not provide these benefits to early-season forbs resulting in no facilitative effect. However, by early fall, when early-season grasses and forbs have reduced photosynthetic activity, summer-active seedlings may benefit from the greater shelter provided by late-flowering grasses. As data collection occurred in August, it is possible that some early forb species were not captured in our survey and are underrepresented in our dataset. While this would not affect our finding of preferential phenological synchrony for summer-active forbs, the spring-active pattern could change from a negative slope to neutral, where spring-active forbs are also equally likely to occur near late-flowering graminoids.

Neighborhood richness increases near graminoids with resource acquisitive leaf traits

Positive effects of nurse plants are often species-specific (Levine 1999, Kikvidze and Nakhutsrishvili 1998) and typically involve habitat amelioration, although an increase in habitat heterogeneity may also facilitate species (Bulllari et al. 2016). Habitat amelioration creates a less stressful physical environment for the seedling by dampening light, wind, heat, and/or increasing moisture content (Richardson et al. 2013). We found that resource acquisitive traits of leaves, but not roots, of graminoid neighbors positively correlated with neighborhood richness. The plants with low LDMC may enhance neighborhood richness by increasing relative humidity and moderating temperatures (Richardson et al. 2013), allowing new species to establish. Further, plant density, height, and other resource acquisitive traits are associated with dominant species, which can greatly impact habitat structure and heterogeneity (Bulllari et al. 2016). Dominant species may also alter community dynamics through suppressing a shared competitor. Levine (1999) showed that variation in cover of a graminoid sedge (*Carex nudata*) changed light availability such that denser sedges indirectly facilitated species through the suppression of a shared competitor *Mimulus guttatus,* via reducing the physical dominance of Mimulus and increasing habitat heterogeneity. While we are unable to determine the mechanisms driving patterns in our system, we know that light availability and soil moisture change within the canopies of some of our species (Elwood, unpublished data). This microsite variation suggests increased habitat heterogeneity near some graminoids. Our results suggest that resource acquisitive plants, through leaf traits or large stature, may facilitate species through increasing habitat amelioration and heterogeneity.

Root traits relating to plant-soil interactions rather than resource acquisition were more strongly coupled to neighborhood richness. Low SRL, which was correlated with higher richness for both spring and summer forbs, may indicate a greater collaboration between the plant and soil-microbes (Bergmann et al. 2020). Mycorrhizal associations of neighboring plants may facilitate other plants (Othman et al. 2004), and graminoids with thicker roots may provide a greater potential for establishing individuals to benefit from positive associations (Rutten and Allen 2023). This opportunity may benefit various species including both spring and summer active forbs. While we are unable to know the exact mechanisms for increased neighborhood richness, our finding that increased richness is associated with lower SRL, and highly resource-acquisitive leaf traits suggests that these traits may modify the physical environment in a way that benefits many species. Additionally or alternatively, these traits may maintain competitive pressures that prevent any single species from becoming dominant.

Neighborhood abundance differs with graminoid leaf traits and seedling phenology

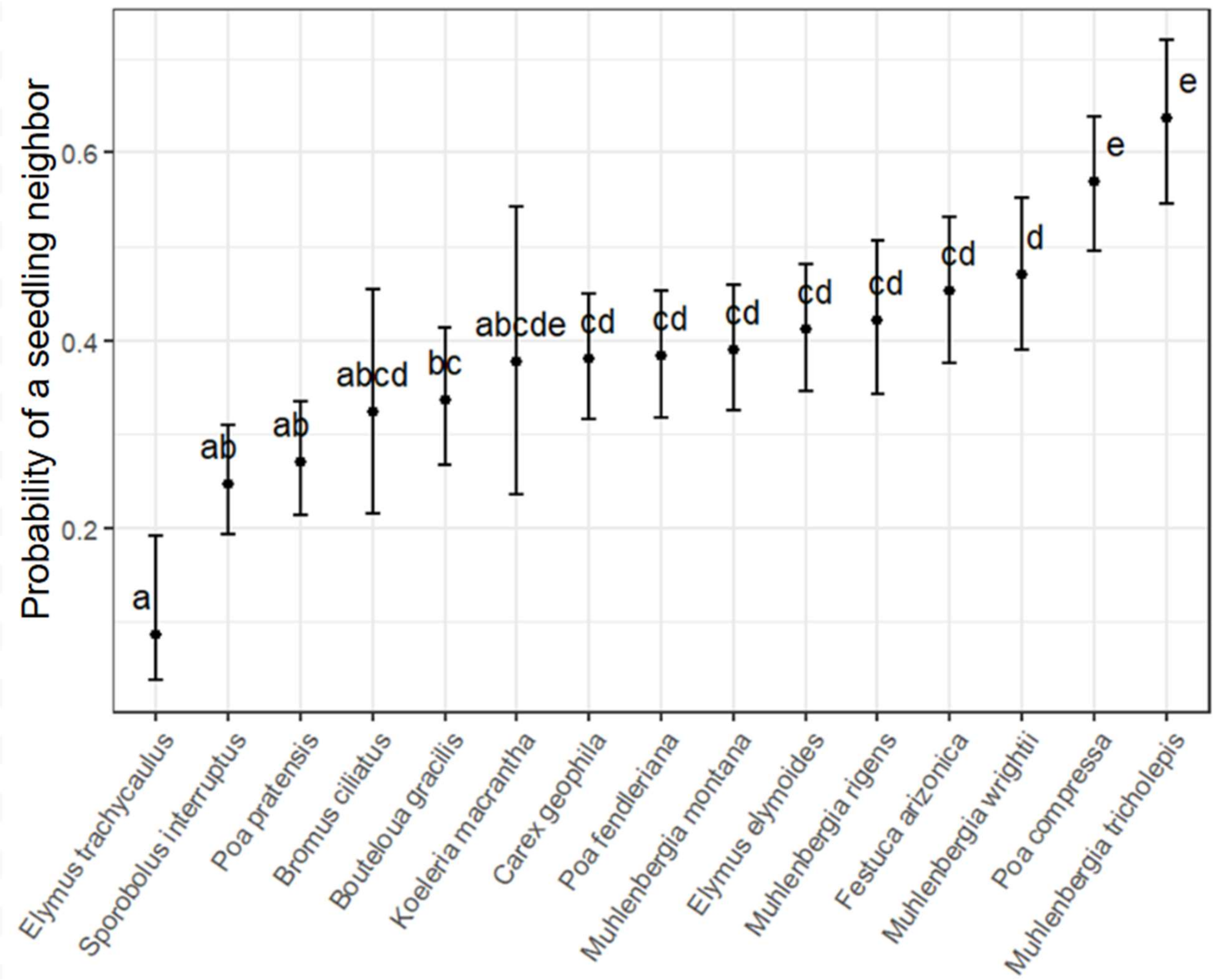
While neighborhood richness of spring and summer active forbs was similarly influenced by graminoid traits, graminoid traits influenced the abundance of spring and summer active forbs differently. The abundance of summer active forbs increased near graminoids with low leaf P while the abundance of spring active forbs increased near graminoids with high aboveground resource acquisitive traits (LDMC, PC2). Leaf P, essential for a variety of plant functions, may differentially affect graminoid water use efficiency and resource-acquisition (Cooksley et al. 2023, Lambers et al. 2008), which may increase resource competition between neighboring plants. High leaf P in graminoids like SPOINT and MUHWRI, which are poor neighbors, may correlate with nutrient acquisition traits, enhancing their competitive impact on neighbors. This idea aligns with Cooksley et al. (2003), who observed that woody species with high root acid phosphatase activity, indicative of phosphorus acquisition, tended to outcompete neighbors in water-limited environments. While some woody nurse plants increase local nutrient availability (Mihoč et al. 2016, Koutzouki et al. 2023), which might offset an increase in competition, there is little evidence that graminoids increase local nutrients (Reyl et al. 1996). It is not clear why leaf P has a greater negative effect on summer active forbs, although we speculate that competition for this nutrient may be particularly high late in the season as it is essential for flowering (Li et al. 2019). Graminoids with traits related to high aboveground resource acquisition may improve the local habitat through creating shelter and increasing local moisture, however, the benefit to forbs may decrease later in the season due to the arrival of monsoons. The impact of resource acquisitive leaf traits on the abundance of spring active but not summer active forbs may be a result of this early season shelter. Furthermore, resource acquisitive leaf traits may increase habitat heterogeneity, enhancing richness of all forb species.

Implications

We found higher neighborhood richness near some graminoid species with resource acquisitive leaf traits, while neighborhood abundance varied depending on forb phenology. We suspect that patterns in neighborhood abundance are primarily due to habitat amelioration while increased richness may be driven by greater niche partitioning changing competitive interactions amongst species. Our study supports the idea that graminoids may serve as nurse plants to forbs; however, these facilitative outcomes remain difficult to predict based on graminoid traits alone. Few studies have considered how phenology and functional traits influence facilitation. Experimental manipulations of facilitating and facilitated plants with different phenology would determine whether and how traits affect relationships between phenological synchrony and facilitation. The answer to this question will have important implications for management in these stressful systems, as phenological timing and planting order will influence restoration success (Navarro-Cano et al. 2021, Browning 2019). Further, as phenological data has become far easier to collect due to technological advances in remote sensing, this creates an opportunity to fine tune timing of management actions (Browning 2019). While the importance of species interactions is currently recognized in species distribution modeling (Norberg et al. 2019) and restoration approaches, adding phenological synchrony may improve management outcomes as species respond differently to novel stressors induced by our changing climate (Nakazawa and Doi 2012).

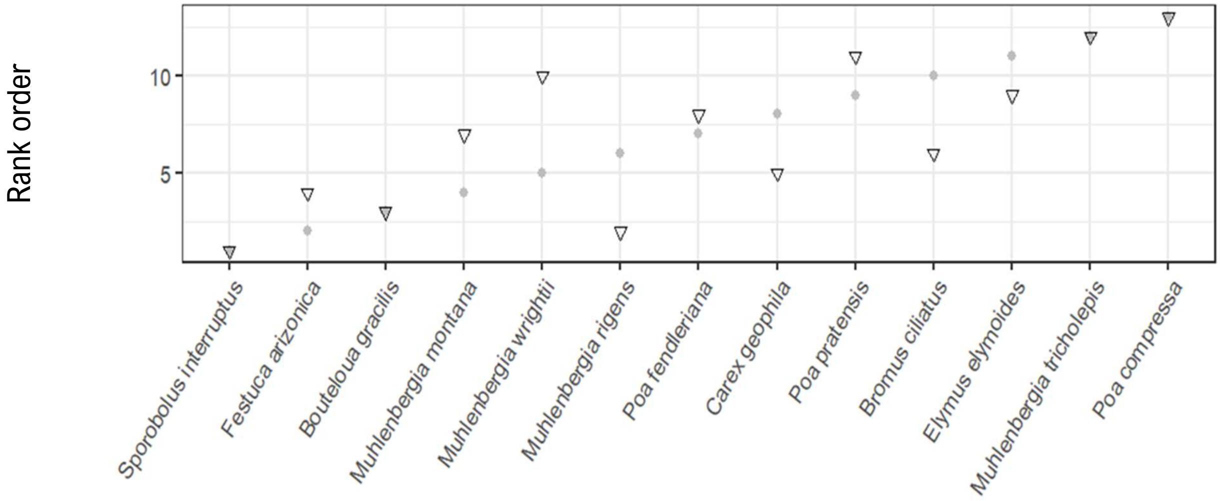
FIGURES AND TABLES

Figure 1.1: Probability that a graminoid neighbors an establishing forb



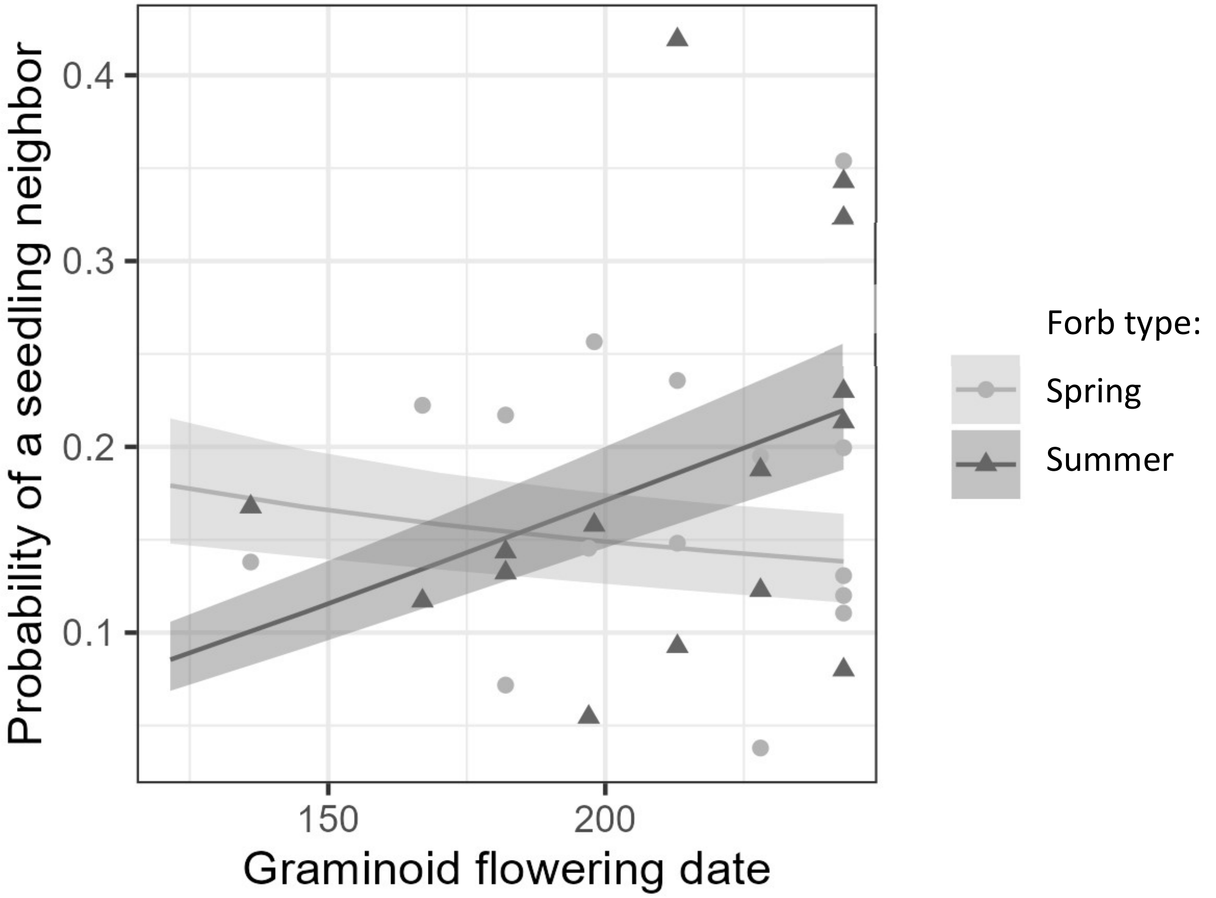
Legend: Points indicate the estimated marginal mean as determined by the model in Table S1.2, back transformed into a probability, that a graminoid species will have any seedling neighbors with SE bars from the model. Species are in ascending order with different letters denoting a significant difference in a post-hoc pairwise comparison between species.

Figure 1.2: Rank order of graminoid neighborhood richness and abundance



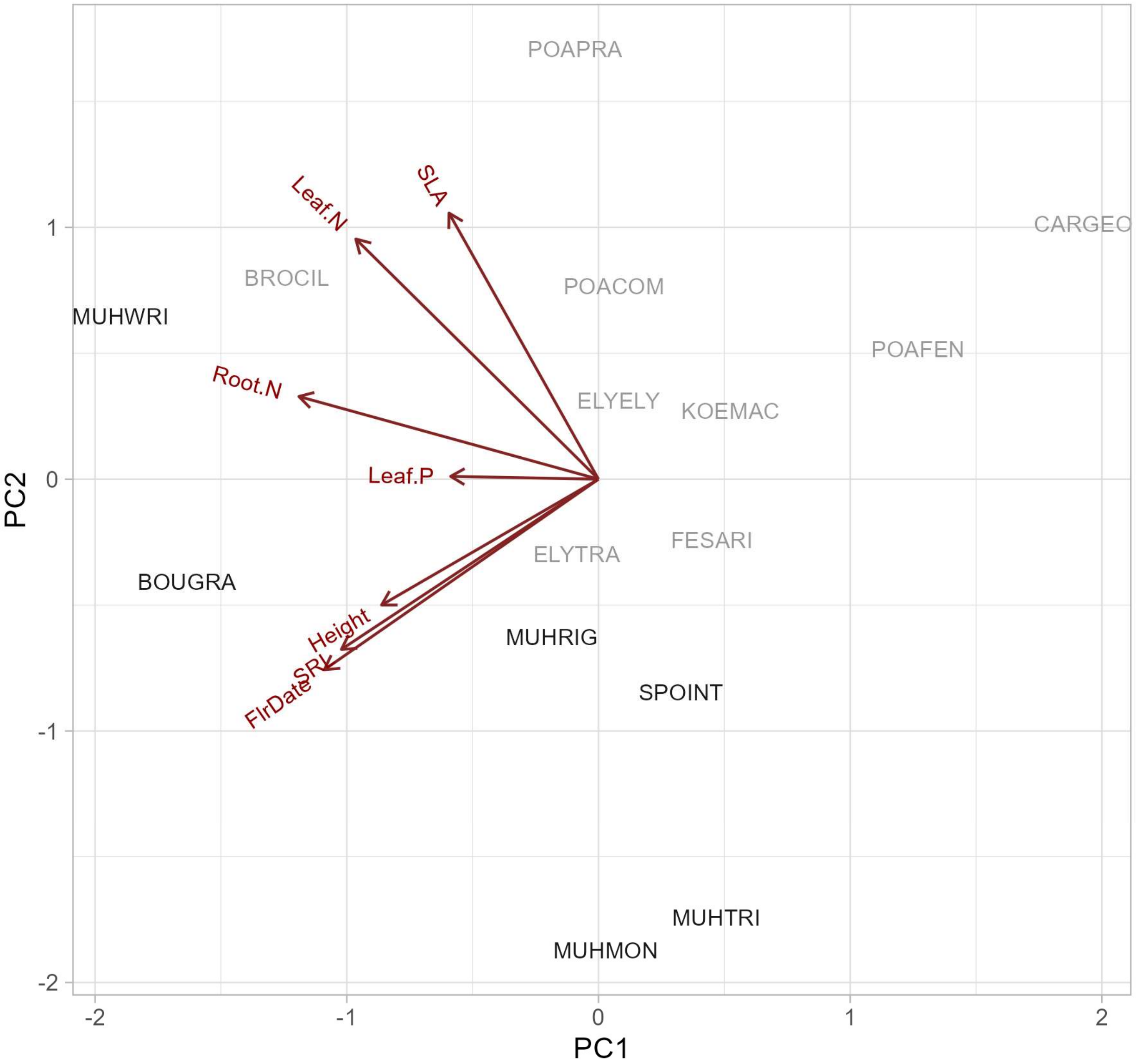
Legend: Richness and abundance rankings of species, where low ranks have the neighborhoods with the least richness and abundance, and high ranks have the greatest richness and abundance. Graminoid species are ordered from left to right along the x-axis by increasing neighborhood richness rank order (circles). Abundance rank order shown as triangles for each species. Rank order was determined from the mean rank sum of species calculated for the Kruskal Wallis test.

Figure 1.3: Phenological timing impacts the probability that graminoids neighbor a seedling



Legend: The change in probability that a graminoid species will have any seedling neighbors that are spring (light) or summer (dark) active given the graminoid flowering date. Lines are back transformed from the binomial estimated marginal slopes from Table S1.4. Points represent the observed proportion of individuals for each graminoid species with a spring (circles) or summer (triangles) seedling neighbor(s). Julian flowering date increases from left to right (x-axis).

Figure 1.4: Principal component analysis of graminoid traits



Legend: First and second Principal Component (PC) axes for the dominant graminoid species included in the study (Table 1.1). Arrows indicate direction (positive or negative) and strength (length of arrow) of trait loadings for each axis (Table S1.5). Traits included in the analysis are: Specific root length (SRL), Root nitrogen content (Root N), Specific leaf area (SLA), Leaf phosphorus content (Leaf P), Leaf nitrogen concentration (Leaf N), Height, and Flowering date (FlrDate). Species abbreviations (Table 1.1) are positioned to indicate trait loadings along the PC axes for each species. Species abbreviations are colored according to photosynthetic pathway, as either C3 (gray) or C4 (black).

Table 1.1

Abbreviation	Graminoid species	Photo-synthetic pathway	Peak flowering date	Total observations	Richness rank order	Abundance rank order
BOUGRA	*Bouteloua gracilis*	C4	243	1692	3	3
BROCIL	*Bromus ciliatus*	C3	243	75	10	6
CARGEO	*Carex geophila*	C3	136	1449	8	5
ELYELY	*Elymus elymoides*	C3	182	2432	11	9
ELYTRA	*Elymus trachycaulus*	C3	197	55	NA	NA
FESARI	*Festuca arizonica*	C3	198	456	2	4
KOEMAC	*Koeleria macrantha*	C3	213	54	NA	NA
MUHMON	*Muhlenbergia montana*	C4	228	1524	4	7
MUHRIG	*Muhlenbergia rigens*	C4	243	431	6	2
MUHTRI	*Muhlenbergia tricholepis*	C4	243	260	12	12
MUHWRI	*Muhlenbergia wrightii*	C4	243	528	5	10
POACOM	*Poa compressa*	C3	213	1243	13	13
POAFEN	*Poa fendleriana*	C3	167	2460	7	8
POAPRA	*Poa pratensis*	C3	182	1407	9	11
SPOINT	*Sporobolus interruptus*	C4	228	2004	1	1

Legend: Graminoid species included in the study, along with their photosynthetic pathway, peak flowering (median Julian date of flowering season), and richness and abundance rank orders. Species abbreviations are used throughout the manuscript. Rank order was determined from the mean rank sum of species calculated for the Kruskal Wallis test. KOEMAC and ELYTRA were excluded from the abundance and richness analysis due to low sample sizes, however, were included in the binomial presence of neighbor model (Figure 1.1).

Table 1.2

	All seedlings		Spring active forbs (Cool)		Summer active forbs (Warm)	
Trait axis or trait	P-value, Correlation Coefficient					
	Richness	Abundance	Richness	Abundance	Richness	Abundance
PC1	p = 0.65, r = 0.14	p = 0.90, r = 0.04	p = 0.51, r = 0.20	p = 0.84, r = -0.06	p = 0.43, r = 0.24	p = 0.71, r = 0.11
PC2	p = 0.16, r = 0.42	p = 0.27, r = 0.33	p = 0.06, r = 0.54	**p = 0.027, r = 0.61**	p = 0.09, r = 0.48	p = 0.64, r = 0.14
PC3	p = 0.78, r = 0.08	p = 0.30, r = 0.31	p = 0.69, r = -0.12	p = 0.97, r = 0.01	p = 0.84, r = -0.06	p = 0.10, r = 0.47
Leaf P	p = 0.07, r = -0.52	**p = 0.03, r = -0.60**	p = 0.20, r = -0.38	p = 0.52, r = -0.20	p = 0.086, r = - 0.49	**p = 0.008, r = - 0.70**
Leaf N	p = 0.83, r = 0.07	p = 0.84, r = 0.06	p = 0.72, r = 1.1	p = 0.16, r = 0.41	p = 0.92, r = - 0.03	p = 0.59, r = - 0.16
SRL	p = 0.09, r = -0.48	p = 0.61, r = -0.15	**p = 0.046, r = -0.56**	p = 0.43, r = -0.24	**p = 0.025, r = -0.62**	p = 0.67, r = -0.13
Flowering Date (graminoid)	p = 0.66, r = -0.13	p = 0.64, r = -0.14	p = 0.43, r = -0.24	p = 0.81, r = -0.07	p = 0.22, r = -0.37	p = 0.36, r = -0.27
SLA	p = 0.47, r = 0.22	p = 0.36, r = 0.27	p = 0.43, r = 0.24	p = 0.12, r = 0.45	p = 0.31, r = 0.31	p = 0.64, r = 0.14
Root N	p = 0.39, r = 0.26	p = 0.25, r = 0.35	p = 0.78, r = 0.08	p = 0.17, r = 0.41	p = 0.83, r = 0.06	p = 0.53, r = 0.19
Height	p = 0.39, r = 0.26	p = 0.62, r = 0.15	p = 0.53, r = 0.19	p = 0.62, r = 0.15	p = 0.80, r = 0.08	p = 0.91, r = - 0.03
LDMC	**p = 0.009, r = -0.71**	p = 0.12, r = -0.47	**p < 0.001, r = - 0.83**	**p < 0.001, r = -0.84**	**p = 0.007, r = - 0.73**	p = 0.75, r = - 0.11

Legend: Pearson's Correlation coefficients (r) and p-value for the rank ordered richness or abundance with the rank ordered PC and trait values for each graminoid. The direction and strength of the correlation are indicated by the r value where positive or negative values indicate a positive or negative correlation of this trait with increased neighborhood richness and abundance rankings, where greater absolute value indicates a stronger correlation. Analyses were run separately including either summer active forbs, spring active forbs, and with both spring and summer active forbs (all). Significant correlations (p-value < 0.05) are bolded.

Chapter 2

The role of nutrient and water on seed bank trait composition in a serpentine grassland system

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ABSTRACT

The composition and diversity of vegetative and seed bank communities are shifting in response to changes in the abiotic and biotic environment. During periods of stressful conditions, dormant seeds in the seed bank may act as a buffer against species loss. Germination and persistence processes are mediated through seed traits which affect the detection of light and water cues, the risk of seed predation, and the length of time seeds remain viable. However, seed traits have been largely understudied except for seed mass, particularly in grasslands where plants spend much of their lives in the seed bank. Past studies in serpentine grasslands show that the colimitation of nutrients and water maintains diverse native vegetation and that resource additions alter the composition of both the vegetative and seed bank communities. This study investigates if morphological (length, mass, shape), chemical (starch, C content, C:N ratio), and barrier (coat permeability, coat thickness) seed traits vary among functional types and between native and non-native species. Further, we ask if seed traits are correlated with changes in seed persistence in the seedbank under water and nutrient additions. We found that barrier traits differed between N-fixing forbs and other groups, while few trait differences separated non N-fixing forbs from grasses. Non-native species had longer seeds and thinner seed coats relative to native species. We found that seed banks in habitats with more productive soils had longer seeds that were heavier, and more spherical in contrast to light rod-shaped seeds. In the less productive habitat, nutrient addition increased the relative proportion of longer seeds in the seed bank while other morphological traits remained unchanged. Barrier traits responded to increasing water availability in the less productive habitat, where seeds with thinner, less permeable seed coats became more prevalent. Patterns of community weighted changes towards longer seeds with thinner seed coats driven by additional resource additions are consistent with increased seed

inputs of species with fast growth strategies, such as non-native grasses, into the seed bank. Such community level shifts in seed traits were observed only in the less productive habitat, which suggests that initial resource availability of the community will determine if global change factors impact seed bank composition. Thus, conservation efforts should be focused on sites with the lowest habitat resource availability that are predicted to experience global change factors.

INTRODUCTION

Global change factors, such as alterations in precipitation and nitrogen deposition, are causing shifts in plant community composition and diversity. Seed banks can be important sources of diversity as species can persist in the seed bank through unfavorable conditions, buffering populations and communities from interannual environmental variation (Kalisz and McPeek 1993, Fenner and Thompson 2005). Furthermore, seed banks can promote coexistence by storing species in the seed bank (DeMalach et al. 2021, Chesson et al. 2004). The density and diversity of the seed bank hinges on multiple abiotic and biotic factors governing seed persistence in, and seed input into, the soil (Figure 2.1). Given the important role of seed banks in community dynamics, understanding which traits mediate seed bank responses to environmental factors, such as water and nutrient availability, will further our ability to predict community responses to global change factors (Baskin and Baskin 1978, Thompson et al. 1993, Baskin and Baskin 2006, Saatkamp et al. 2014, Saatkamp et al. 2019, Agneray et al. 2022, An et al. 2022). However, research to date has focused on morpho-physiological traits of seedlings and adults and much less is known about seed traits, which mediate processes such as seed persistence, germination, and entry into the seed bank (Diez et al. 2016, Westoby and Wright 2006, Kleyer et al. 2008, Kraft et al. 2015, but see Thompson et al. 1993, Saatkamp et al. 2019).

Morphological, chemical, and barrier (coat thickness and permeability) traits affect seed bank dynamics through their effects on dispersal, establishment, persistence, and germination phenology (Saatkamp et al. 2019). Morphological traits (e.g., length, mass, shape) affect persistence. For instance, large seeds are attractive to birds, rodents, and other larger predators (Fenner 1995, Hulme 1998, Diaz 1994). However, elongated seeds with lighter mass are less easily buried in soil and less likely to be eaten (Thompson et al. 1993) and have higher potential for longer distance dispersal (Nathan et al. 2011, Wilson 1993, Bullock and Clark 2000, Nathan 2006). Thus, there are trade-offs in morphological traits relating to spatial dispersal versus persistence in the seed bank (Venable 1986, Ehrlen and Groenendael 1998, Tigano and Friesen 2016). Traits favoring dispersal might include lower mass and an elongated shape with structures aiding in dispersal, while traits promoting seed bank persistence might include a heavier, shorter, and rounder shape (Bekker et al. 1998, Thompson et al. 1993, Wilson 1993, Nathan et al. 2011). Seed chemistry, such as nutrient composition, affects seed predation rates and early seedling survival (Saatkamp et al. 2019, MacNeill et al. 2017). Seeds characterized by a chemical composition that is appealing to predators, featuring high absolute amounts of carbon and nitrogen, a low carbon-to-nitrogen ratio, and high starch (MacNeill et al. 2017, Ramirez and Traveset 2010), may have lower persistence. However, a low carbon-to-nitrogen ratio may better provision the seed for a successful establishment. Further, seed coat traits that determine the barrier between the seed and external environment, such as seed coat thickness and seed coat permeability, impact predation and germination rates (Traveset et al. 2008). Species with thicker seed coats have higher persistence as they are less likely to lose viability over time (Gardarin et al. 2010, Davis 2007), take longer to germinate, and provide more protection from predation by microbial pathogens and fungi (Hulme 1998, Dalling et al. 2011). Similarly, an impermeable seed may be more persistent as it is less susceptible to predation (Saatkamp et al. 2014), but high permeability may enable rapid germination and establishment (Nooden et al. 1985, Evenari et al. 1966).

Traits and associated ecological strategies, which mediate responses of species to global changes, may help explain shifts in species abundance in the seed bank between native and non-native species among functional types (LaForgia et al. 2018, Loydi and Collins 2021). For example, under conditions of increased nutrient and water availability, non-natives can increase in abundance (Eskelinen and Harrison 2015b, Grimes 1974, Gross et al. 2005, Huenneke et al. 1990, Flores-Moreno et al. 2016), driven by competitive traits like high growth rates, high resource-use efficiency, and long-distance dispersal abilities (Daehler 2003, Lau and Funk 2023). This may extend to seed traits, as non-native species might exhibit seed characteristics linked to fast germination and low persistence (high seed mass, low C:N ratio, low seed coat thickness and high permeability), while natives may have higher persistence and slower germination (thick seed coats, high C:N chemical content). For example, a seed with a long rod-like shape and a thin seed coat, characteristic of many grasses, may have higher spatial dispersal, and remain close to the soil surface where they may quickly imbibe water leading to rapid germination (Benvenuti 2007). However, these traits could also result in heightened susceptibility to seed predation and lower persistence in seed banks. As N-fixers depend largely on seed pod ballistic mechanisms or animals for dispersal, N-fixing forbs differ chemically and morphologically from non-N-fixers (Baskin 2003, Wilcots et al. 2019). N-fixers may possess high chemical stores, and large, heavy seeds that are less likely to develop wind dispersal structures (McKey 1994, Baskin 2003, Wilcots et al. 2019). Through investigating seed traits we enhance our understanding of what mechanisms may be driving differences in seed bank dynamics between native and non-native species and functional types.

Low productivity habitats, such as serpentine grasslands, can support high native diversity and endemism of species with diverse sets of traits (Brushmann and Hobohm 2013) and may be more sensitive to global change factors (Knapp et al. 2015). Studies in low productivity grasslands have demonstrated that soil resource availability drives differences in seed bank dynamics between natives and non-natives, and across plant functional types (Eskelinen et al. 2021, LaForgia et al. 2018, Larson and Suding 2022). Further, low productivity soils exhibit greater changes to community trait values in response to global change factors (Eskelinen and Harrison 2015b). We expect that soils with lower productivity will have higher abundances of seeds with slow germination and high persistence traits (Spasojevic and Suding 2012, Hooper et al. 2012). However, with resource additions, traits associated with fast germination and low persistence may become less common in the seed bank community, as species with these traits are consumed or germinate. However, if fast-germinating species are successful in establishing, then these traits may increase in abundance as offspring are added to the seed bank.

Global change factors may greatly impact seed bank diversity, mediated by trait values, soil habitat, and changes to the composition of the emergent community (Eskelinen et al. 2021). In this paper, we explore whether seed traits vary along axes that represent morphological, barrier, and chemical traits which mediate critical regeneration processes (Saatkamp et al. 2019). We then ask if seed traits differ between native and non-native species, and among functional types (grass, forb, N-fixer). Finally, we ask if seed traits of the community shift with the nutrient and water addition, and if so, how habitat productivity (i.e., soil fertility) affects these shifts. We predict trade-offs between traits associated with germination speed and ability to persist in the seed bank and that natives will display traits associated with slow germination and high persistence. Further, we predict that grasses will exhibit traits favoring dispersal such as longer, lighter, oblong seeds with dispersal appendages in contrast with forbs that will have traits focused on high persistence. As the functions of persistence and dispersal differ between N-fixers and non-N-fixers (Wilcots et al. 2019), we expect N-fixing forbs will prioritize persistence with well-developed barrier traits (Baskin et al. 2000, Baskin 2003) as well as nutritious chemical traits (Wilcots et al. 2019). We expect that seedbanks in low productivity habitats (i.e., low soil fertility) will have traits favoring slow germination and high persistence. Further, we expect that seedbanks from the low productivity habitat will experience stronger shifts in trait composition in response to resource additions. In response to nutrient and water resource additions we expect that seeds with high germination and/or low persistence traits will be removed from the seed bank, unless resource additions are predominantly affecting seed bank composition through seed production, in which case traits associated with high germination will become more common.

METHODS

Study site and experimental set-up

Our study builds off of work done at the McLaughlin reserve in a native serpentine grassland system in the Inner North Coast Range of California (Eskelinen and Harrison 2014, 2015, Eskelinen et al. 2021). This site is characterized by a Mediterranean climate with winter precipitation and dry hot summers (Eskelinen et al. 2021), with a mean annual temperature of 15.1 degrees C and precipitation of 761.9 mm (1990- 2020) (Western Regional Climate Center 2021). While initial treatment years had normal precipitation (2010- 2012), there was a drought for some treatment years (2013-2015, average precipitation of 434.0 mm). Precipitation was 870.5, 1043.0, 532.4, and 1079.5 for the seed collection years (2016 - 2019). In this study, we considered “harsh serpentine” grassland habitats as a low productivity soil (Eskelinen and Harrison 2014). Harsh serpentine is characterized by coarse rocky soils, high native diversity, and very low biomass. In contrast, “lush serpentine” soils were higher productivity soils characterized by fine-textured, alluvial soils, intermediate biomass between the harsh serpentine and the non-serpentine, and a mixture of natives and non-native species (Eskelinen and Harrison 2015a). In addition to being characterized by low nutrient availability, serpentine soils have high concentrations of heavy metals and low water holding capacity (Damschen et al. 2012). Further, in this habitat, soil fertility varies greatly with soil depth and texture (Damschen et al. 2012), affecting plant community composition and resistance of the community to invasion (Daehler 2003, Eskelinen and Harrison 2015b).

A full factorial manipulative experiment was conducted from 2010- 2014, when 90 harsh and lush serpentine experimental plots were selected and randomly assigned to a water addition, nutrient addition, control, or both treatments. There are 10-12 replicates per treatment combination for each soil-type. Nutrient addition followed the Nutrient Network protocol and corresponded to upper estimates of nitrogen deposition in CA (slow-release granular NPK 10-10-10 with micronutrients, with 3.3 g /m2 of each element in November, February and March each year) (Borer et al. 2014, Eskelinen and Harrison 2015b, Fenn et al. 2003). Water addition plots increased average annual rainfall by approximately 18%. This was achieved through the addition of 2.5cm of rainwater applied overnight from a height of 50cm. Watering occurred weekly to extend the spring water availability for an additional 8 weeks; water treatment started when winter precipitation stopped for the season (1+ weeks without rain) in March or April. Watering treatment was designed to extend the rainy season following previous work in California grasslands, based on previous climate projections (Suttle et al. 2007, Eskelinen and Harrison 2015b).

We grew out the persistent seed bank in order to capture responses to the plant community composition to global change factors (Eskelinen et al. 2021). We sampled the persistent seed bank by collecting soil samples following spring germination but prior to seed set (April 4th - 9th, 2017). To do so, we collected soil from a 25 by 25 cm square from within the treatment plots to a depth of 5 cm. Soil was then stored under similar conditions to the field, experiencing a hot dry summer, and transferred to a greenhouse in the fall to mimic field germination conditions. Soil was carefully sifted, and all large rocks and organic debris were removed from the sample (> 1 cm3). Soils were then spread over a mix of potting soil and sand in 1080 flat trays in a fully randomized design and watered daily to maintain moist soils. All stems that emerged were counted and removed from the trays. The experiment continued for 2 years during which time there was periodic manual disturbance of soils and a dry down at the end of the spring period to mimic field conditions. Unknown seedlings were transplanted and grown until ID could be verified. Following the grow-out, total abundance for each species was determined for each plot (Eskelinen et al. 2021).

Seed collection and trait measurements

Seeds were collected at McLaughlin reserve near the experimental site on serpentine soil using protocols from the LEDA Trait database (Kleyer et al. 2008). Collection took place during the spring and summer of 2016, 2017, 2018, and 2019. Seeds were stored in envelopes by the maternal family in a dry room-temperature cabinet or in a -18 degrees Celsius freezer after seeds were oven dried at 63 degrees Celsius for a minimum of 72 hours. We measured morphological, chemical, and barrier traits (Table 2.1; Saatkamp et al. 2019). Traits were selected in accordance with commonly and efficiently measured morphological traits such as seed mass, seed shape, and seed length (Thompson et al. 1993). We further characterized investment in morphological dispersal structures through a binary categorization of seeds with or without an ephemeral appendage (wing, pappus). Additional morphological data was collected using VideometerLab multispectral acquisition system (Videometer A/S, Denmark), which uses 19 wavelength imaging to determine characteristics of seeds (reflectance, texture, seed dimensions, deviation from ovoid shape) (Table 2.1; Boelt et al. 2018). To measure chemical traits, carbon, nitrogen, and carbon-to-nitrogen (C:N) ratio samples were bulked from 5 maternal families and ground up and placed in tin capsules for combustion sampling (PDZ Europa ANCA-GSL elemental analyzer with PDZ Europa 20-20 IRMS, Sercon Ltd., Cheshire, UK) at UC Davis Stable Isotope laboratory.

Finally, the starch-iodine test was used to determine the presence or absence of starch content (starch), via observing if a color change occurred on imbibed, dissected, and starch treated seeds under magnification (Table 2.1, S14; Jones and Earle 1966, Jeong et al. 2010). Further, barrier traits that may respond to changes in nutrient and water availability (seed coat permeability, seed coat thickness). Seed coat permeability (SCP) was measured as the percent change in mass of seeds exposed to water for 24 hrs (+/-2hrs) (Traveset et al. 2008). Seed coat thickness (SCT) was measured using 80x magnification of dissected seeds under a microscope. We measured SCT on seeds dissected in a horizontal direction (Table 2.1). All traits were measured on seeds bulked from 10 maternal families in approximately equal proportions whenever possible (smallest # families = 3).

Statistical analyses

We used Principal Component Analysis (PCA) on standardized and centered data to differentiate axes of trait variation using R package vegan (Oksanen et al. 2022), ANOVA to compare PC values between functional types and species origin groups and determined correlations between trait values using package vegan (Oksanen et al. 2022) We checked trait distributions and transformed traits: SCT was square root transformed, seed compactness was transformed through taking the natural exponent, and all other traits were log transformed (mass, length, texture, shape, SCP; Table 2.1). Texture data was missing for seven species, here the median texture value was substituted (Dray and Josse 2015). Results from analyses excluding species with missing values were similar. We ran post-hoc testing on ANOVA results using Tukey pairwise comparison. Highly correlated traits were removed from the analysis (Figure S2.1, Table S2.1).

To determine shifts in traits at the community scale, we ran mixed effect models using package nlme (Pinheiro 2023). We ran models for each habitat type (lush, harsh) for PC1 through PC4. In our model the response variable was the community weighted mean (CWM) of the PC for the plot, calculated as the PC value for each species multiplied by the species abundance and divided by the absolute abundance of the plot, summed across all species in the plot. Models included fixed effects for nutrients, water, and their interaction. Further, to account for community heterogeneity within the field site and experimental design, we included watering lines from the field experiment as a random effect. There were 5 watering lines serving the 90 plots. On average trait data covered 82% (se= 2.3%) of individuals within a plot of the overall community. Differences between species origin and treatment groups were identified via post hoc tests using package emmeans (Lenth et al. 2023) and model assumptions were checked using package DHARMa (Hartig 2022). All data processing and analyses were done using R version 4.3.1 "Beagle Scouts"(R Core Team 2023).

RESULTS

Primary axes of variation were for morphological traits on PC1 and 2, followed by barrier traits orthogonally positioned on PC 3 and 4 (Figure 2.2). Chemical traits were associated with both morphological and barrier trait axes (Figure 2.2). Seed size (length) loaded most strongly on PC1, and longer seeds were positively associated with greater mass, more oblong shape, less compact shape, higher texture, and lower likelihood of having an ephemeral dispersal structure. In contrast, shorter seeds tended to have a somewhat rounder, compact shape, with lower texture and fewer dispersal structures (PC1, 27.3% of variance explained). We also found a trade-off between seed shape and mass, such that more oblong seeds were lighter while rounded seeds were heavier and had less carbon (PC2,16.1% of variance explained, Figure 2.2, Table S2.2). While barrier traits (SCT and SCP) represented separate axes from morphological traits, they were not combined into a single axis but clearly occupied separate axes with or without chemical traits. SCT was associated with chemical traits where thicker seed coats and carbon content trade-off with higher starch content and a higher C:N ratio (PC3, 13.1% of variance explained), while SCP dominated the 4th PC axis (PC4, 9.9% of variance explained). Further, chemical traits were incorporated in the morphological axes as larger seeds displayed higher C:N ratios and less carbon content than smaller seeds (PC1), and heavier, rounder seeds had less carbon content (PC2; Figure 2.2, Table S2.2).

Non-native species exhibited significantly different traits compared to natives, and forbs showed significantly different traits from N-fixing forbs (Figure 2.2, Table 2.2). As expected, non-natives were larger in seed size (PC1) and with thinner seed coats and greater starch content (PC3), than natives. However, non-natives and natives did not differ in morphological proportion nor SCP. Forbs and grasses did not differ in barrier traits (PC3, PC4); however, grasses were larger (low PC1 values) representing many traits including long length, rod-like shape, presence of an ephemeral dispersal structure, rougher texture, heavier mass, and lower carbon content (Figure 2.2, Table 2.2, Table S2.3). N-fixing forbs and non-N-fixing forbs had similar overall size (PC1), SCT, and starch values (PC4). However, N-fixing forbs were less permeable (PC4) and had different morphological proportioning with a higher mass and rounder shape compared to non-N-fixing forbs (PC2). Finally, we found that N-fixing forbs were smaller (PC1), rounder and heavier (PC2), and had thicker seed coats and less starch (PC3) than grasses (Figure 2.2, Table 2.2). For individual species PC values see Table S2.4.

Low productivity (harsh serpentine) communities tended to have smaller, lighter, rod-like, and thicker coated seeds than the high productivity (lush serpentine) communities (Figure 2.3, Table 2.3). Further the productivity of the community had a significant impact on the trait responses to treatments in all models (Table 2.3). For harsh serpentine soils exposed to nutrient addition, the community shifted towards longer seeds, and seeds with a thinner seed coat and/or higher starch content (PC1, PC3). Exposure to water also shifted the community towards seeds with a thinner seed coat in addition to less permeable seed coats (PC3, PC4). The shape and mass, i.e. morphological proportioning, axis (PC2) showed different responses to water and nutrients based on soil type and when combined. In harsh soils, water and nutrients shifted the community towards species with lighter, less spherical seeds (Figure 2.3, Table 2.3). In lush serpentine soil, nutrient addition reduced SCT and increased starch (PC3), while water reduced the community seed size (PC1; Figure 2.3, Table 2.3).

DISCUSSION

In this study we asked whether seed traits vary along axes associated with morphological, chemical, and barrier traits, how they vary across functional types, and if these relationships shift under global change factors. We found four main axes of variation describing traits that align with germination and establishment (PC1), persistence (PC2, PC3) or both functions (PC4). Further, we found that while forbs and grasses differed in seed size (length; PC1), they did not differ in barrier or chemical traits. However, non-N-fixers differed broadly from N-fixers. We observed more changes in seedbank trait composition, and a greater magnitude of change, in the low productivity habitat with resource additions. Responses of traits (PC1, PC3) support the idea that resource addition moves the community toward fast germination and low persistence. SCP was more affected by moisture levels, whereas SCT responded more to nutrient additions. This suggests that highly permeable seeds may be removed from the community in response to resource addition as hypothesized. Thus, our results demonstrate that seed traits within the seed bank are indeed responsive to global change factors and highlight important interactions between seed traits and environmental factors across functional types and habitats.

Seed trait trade-offs

Trait trade-offs exist within morphological traits and among barrier and chemical traits which relate to functions associated with regeneration processes. As expected, we found a trade-off between seed shape and mass, ranging from light, rodlike seeds to heavy, round seeds. This may correspond to distinct strategies characterized by slow germination and a greater seed burial rate (rounder, heavier seeds) versus fast germination and greater long distance dispersal ability (lighter, rod-shaped seeds) (Ramirez and Traveset 2010, Gong et al. 2015, Bekker et al. 1998, but see Benvenuti 2007). High SCT and low starch content, which were aligned in our study, may increase persistence via a reduction in predation as predators prefer higher starch content and thinner seed coats due to their reduced fiber content (MacNeill et al. 2017, Gong et al. 2015, Janzen 1971). While a literature review found that additional chemical defenses, such as phenolic compounds may be higher in seeds with thinner seed coats, there is no correlation between desirable seed chemistry to predators (protein and oil content) and SCT (Gardarin et al. 2010). Surprisingly, however, we found that SCT and SCP aligned onto orthogonal axes, suggesting that these traits may not be coordinated in environments with limited water availability. This suggests that SCT may not relate directly to dormancy strategies in dry environments and illustrates the complex role of seed coat mechanisms in dormancy breaking behavior (Saatkamp et al. 2014, Baskin 2003), however, it remains to be tested if SCT and SCP represent separate barrier axes and processes in wetter climates. More work on a greater range of chemical traits and barrier traits as well as further investigation of how these traits relate to one another would be useful. Further, quantification of intraspecific variation in these traits remains largely unknown (Saatkamp et al. 2019) and would allow more direct links to the processes of persistence and germination.

Seed traits vary across species

Seed traits varied across native and non-native species and functional groups, although not all of our predictions were supported. We predicted that grass species, which seldom maintain long-term persistent seed banks (Fenner and Thompson 2005), would have a different set of chemical and barrier traits than forb species, which can persist in the soil for many years (Thompson 1987, Loydi and Collins 2021, Saatkamp et al. 2014). However, we found no substantial differences between barrier traits of forbs and grasses despite substantial divergences between seed dormancy strategies, and the acknowledged role of barrier traits in influencing dormancy, seed germination (Nooden et al. 1985, Baskin et al. 2000), and predation (Gardarin et al. 2010). Indeed, seed banking of grasses may be more common than previously thought (Dairel and Fidelis 2020, Xavier et al. 2021), or barrier traits may not be reliable indicators of dormancy. Permeable seed coats were common, although highly variable, within forb and grass functional types and species origins. As SCP determines a seed's response to water availability, high SCP may be useful in drylands where ambient seasons are short (Nawaz et al. 2013, Dalil 2014).

Further, we found N-fixing forbs have strong barriers to the environment with less permeable coats than forbs and thicker coats than grasses. However, past studies suggest that N-fixing forbs may shift allocation of resources to adjust barrier traits in response to environmental conditions, resource availability (Nooden et al. 1985), and as seeds mature (Marbach and Mayer 1974). Thus N-fixing barriers and predation rates may be highly context dependent although, as high N generally increases allocation to seed defense (Grubb 1998), N-fixers are likely well defended chemically if not physically (Wilcots et al. 2019). While there was no difference in SCP based on species origin, we did find that native species, which are potentially well adapted to the system, possess thicker seed coats with less starch content (PC3) than non-native species, supporting our prediction that non-natives may have less defense against predation in contrast with natives.

We found that seeds of natives, forbs, and N-fixers were shorter in length than grasses and non-natives, and these traits were consistent with our prediction that non-natives prioritize faster germination and establishment compared with native species. This differs from some research contrasting native and non-native species which show no differences across multiple habitats in carbon capture strategies (Leishman et al. 2010) or leaf economic spectrum traits (Tecco et al. 2010) and research contrasting carbon assimilation and leaf economic strategies within low-fertility serpentine systems worldwide (Funk et al. 2016). In contrast to expectations (Moravcová et al. 2010), when accounting for overall seed size, natives were similar to non-natives, and grasses similar to forbs with respect to seed mass and shape (PC2). While we find that non-natives and grasses are larger in overall seed size, which may indicate a greater competitive advantage in early establishment, grass and forb species; being lighter, more oblong, and often having appendages, may be better suited for wind dispersal than N-fixing forbs (Moravcová et al. 2010, Sperry et al. 2021). The heavier, rounder seeds of N-fixing forbs, relative to grasses and forbs, may assist with seed bank persistence through higher burial rates. In conjunction with strong barrier traits, this may allow N-fixing forbs to undergo variable dormancy periods and may reflect bet-hedging strategies. N-fixing seeds have been found to have largely clumped dispersal patterns (Wilcots et al. 2019), and high clumping may reduce offspring survival due to intra-specific competition; however, dispersing germination over both time and space may contribute to population resilience (Chen et al. 2020) and community composition (Plue and Cousins 2017).

Effects of resource addition on seed traits depends on soil fertility

In low productivity soil, we found fewer seeds and seeds that were shorter, lighter and more rod-like with thicker seed coats. This may be due to differences in nutrient and water availability as well as other factors. Harsh serpentine has a higher pH (Gravuer and Eskelinen 2017) and higher fungi presence (Basto et al. 2015b, Du et al. 2023), which may increase predation rates reducing seed persistence. As increased water and soil fertility attracts additional arthropods and fungi predators (Schafer and Kotanen 2003, Wagner and Mitschunas 2008), seeds with traits increasing burial (heavier, more spherical) may be more protected from predation than seeds near the soil surface. Another potential reason for the differences between habitats in mass and shape may be that morphological proportioning is primarily associated with dispersal or establishment success and indirectly with persistence in the seed bank. Past studies support this through findings that seed mass is correlated with establishment success (Moles and Westoby 2006). Further, seed shape and seed mass are related to seed dispersal potential, whereby lighter, more rod-like seeds disperse further, and we expect a trade-off with lower seed bank persistence (Peart 2016, Thompson 1987, Wilson 1993).

In our study, water addition had stronger effects on seed bank trait composition for barrier and chemical axes and these effects were restricted to harsh soils. We hypothesized that we would see shifts in the community towards thicker, less permeable seed coats in seed banks exposed to water addition, as thinner, permeable coats may germinate at higher rates as well as be targeted by predators. While we found lower SCP with water addition, SCT unexpectedly showed the opposite pattern. Further, we found that seed length (size) increased. These findings mirror those from LaForgia et al. (2018) who found that drought increased the relative abundance of native forbs in the seed bank (smaller seeds) and reduced non-native grass abundance (larger seeds). This pattern may be due to overall growth strategies that correlate with small seeded species using a drought escape strategy (Kooyers 2015, LaForgia et al. 2018). Due to the nature of the experiment whereby water extended the growing season, this may have provided additional time for reproduction to occur (Suttle et al. 2007, Eskelinen and Harrison 2015b). Further, with added water, the competitive advantage conferred to seeds with high permeability and rapid water response as part of a drought escape strategy may be reduced (Dalil 2014, Nawaz et al. 2013).

Similar to the effects of water addition, there was a greater effect of nutrient addition on harsh soil communities. Nutrients increased seed length and reduced community SCT on harsh soils but only slightly reduced SCT on lush soils. This mirrors patterns observed in the emergent community, where nutrient addition on low fertility soils shifts the community in favor of species with more acquisitive strategies, such as taller plants with higher SLA (Eskelinen and Harrison 2014, 2015b). This shift may occur if N fixers, which we found to have high SCT, are preferentially removed from the seed bank, although this would be surprising as thicker seed coats should offer greater protection. Alternatively, this community shift to species with more acquisitive traits (longer seeds, thinner coats) may be due to large increases in non-native abundance, as was found in the aboveground community (Eskelinen et al. 2014, 2015b). It may be that N fixers are competitive under nutrient poor conditions, when they have access to more N than non-natives, but are unable to compete with non-natives with nutrient additions to the community. Regardless of seed predation rates, if N fixers are unable to compete with non-natives, they will have a much smaller proportion of seed inputs and the SCT of the community will shift towards the thinner seed coats of non-natives. Thus, while we are unable to explicitly determine the mechanism behind the community shift in seed traits with our data set, we suspect that decreased SCT with additional nutrient availability is primarily due to differences in seed inputs, and that seed inputs are driving changes in the seed bank community composition.

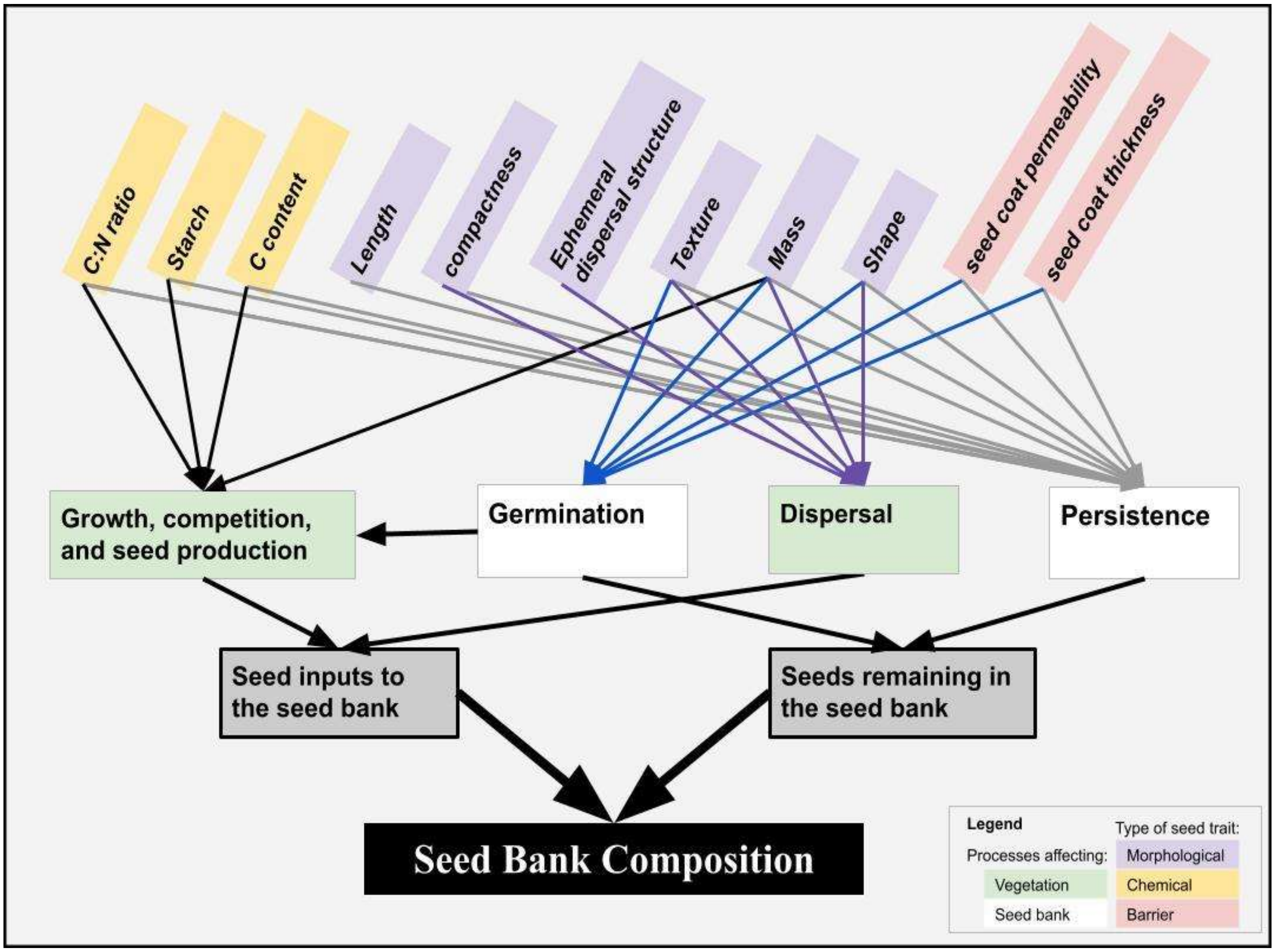
Conclusion

Our findings identify key axes of seed trait variation that change at the community level when exposed to global change factors. Morphological trait axes, while accounting for the largest amount of variation in the data, had limited responses to resource additions, and only in resource limited, low productivity habitat. As seeds with a thick seed coat were not aligned with low SCP as we expected, but rather aligned with chemical traits, barrier traits may be relevant to different processes. Higher SCT, a trait that native species have, may indicate higher defensive traits while SCP may relate primarily to seed germination strategies and operate independently from other traits. Our findings from comparing functional types suggest that while grasses and forbs have similar trait values, N-fixing forbs are distinctive in barrier traits and are more spherical and heavier than non-N fixers. The intra-functional type variation within barrier traits further signifies the value of considering individual species and connecting relevant traits to seed bank persistence. Further, additional exploration of chemical traits and how they relate to SCT would be useful. In contrast with other trait axes, SCP does not differ between habitats or with nutrient addition, yet SCP decreased with water addition supporting our initial hypothesis that seeds with high SCP may be removed from the community under water addition.

Our results demonstrate the relevance of habitat resource availability in determining community seed trait responses to changing nutrient and water availability. Changing resource inputs will differentially affect communities in stressful habitats and on low productivity soil types. Changing nutrient availability may result in more varied and substantial changes in low productivity community composition than in areas of greater productivity. Further, as community responses were stronger in harsh serpentine habitat than on soils with greater resource availability, this suggests that conservation efforts should be focused on sites with low resource availability, and where shifts in precipitation and nutrient availability are expected. As seed bank abundance and diversity influence the long-term resilience of communities to global changes, preserving the seed bank and understanding how barrier seed traits interact with global changes is critical to the conservation of grassland communities.

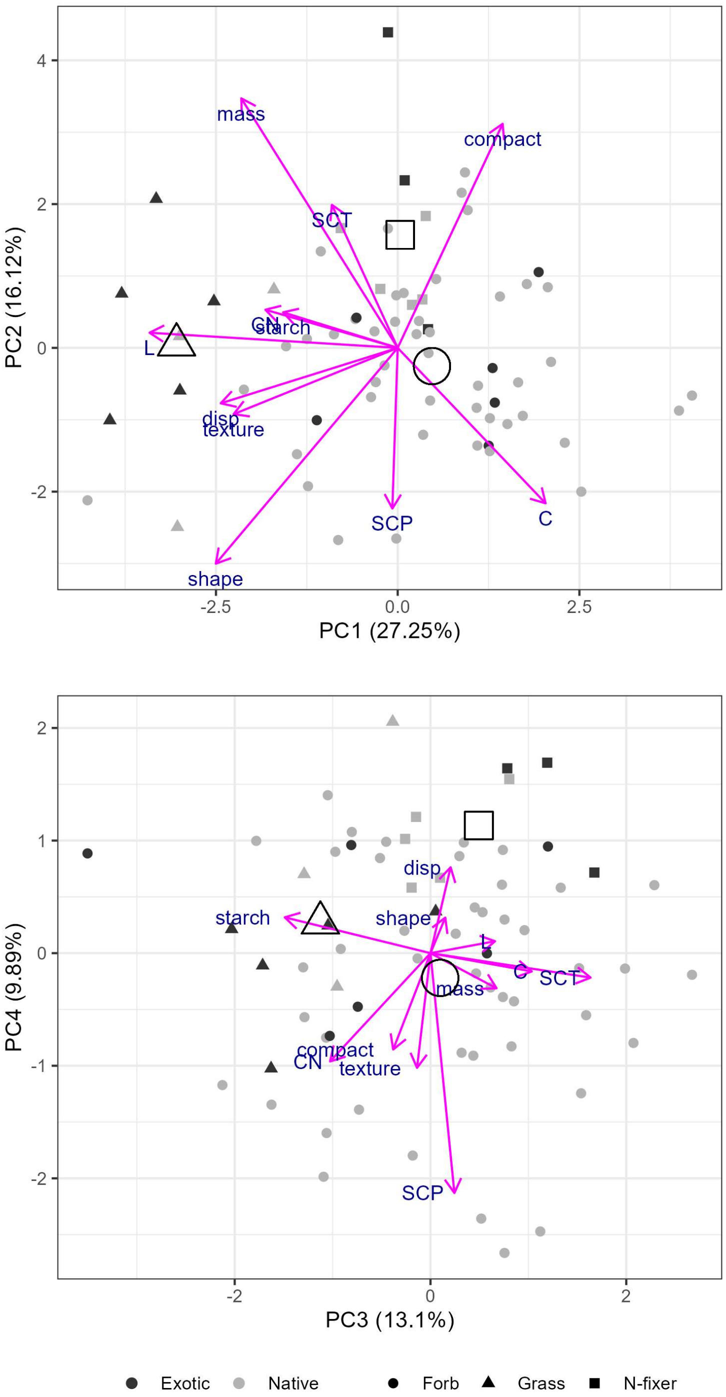
FIGURES AND TABLES

Figure 2.1: Seed bank composition conceptual diagram



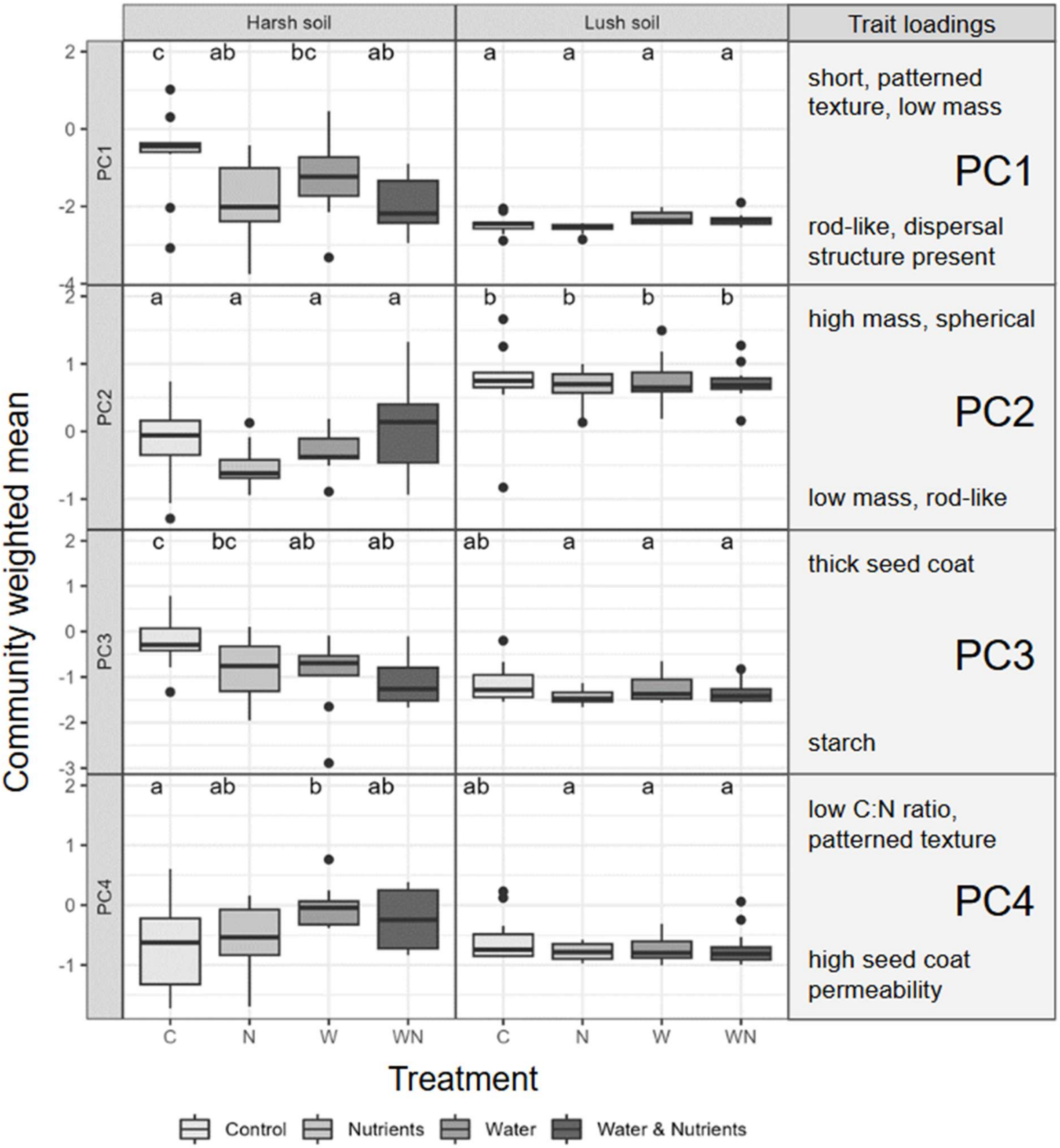
Legend: This diagram illustrates processes affecting vegetative plants with green boxes and seeds with white boxes. Seed traits in yellow are chemical traits, purple are morphological traits, and pink are seed coat barrier traits. Arrows from seed traits to processes indicate known mechanisms where a particular seed trait influences a process. Arrows do not indicate the strength or frequency of this relationship and additional relationships between seed traits and processes not shown are likely. Most literature shows how resource addition affects processes; here we explore how traits mediate the effects of resource addition on vegetative and seed bank processes which ultimately determine seed bank composition. Resource additions of water and nutrients will affect vegetative growth, competition, and seed production (Keller et al. 2023, Leishman et al. 2000) as well as germination (Bekker et al. 1998, Davis 2007, Basto et al. 2015a, Carta et al. 2022, Saatkamp et al. 2014) and persistence in the seed bank (via reduced seed viability: Dalling et al. 2011, Pakeman et al. 2012, via increased predation: Davis 2007, Schafer and Kotanen 2003, Wagner and Mitschunas 2008). Additionally, vegetative growth reduces germination by limiting light availability (Jankowska and Daws 2007) and litter accumulation (Jessen et al. 2023, Van Mourik et al. 2005).

Figure 2.2: Principal component ordination



Legend**:** Principal components analysis where points represent mean trait values for each species; shapes illustrate whether the species is a forb (circles), grass (triangle), or N-fixing forbs ("N-fixer"; squares) and native and non-native species are gray and black respectively. Arrows represent vectors indicating strength and direction of trait loadings. The large white shapes outlined in black indicate the group mean for each functional type.

Figure 2.3: CWM response to treatments



Legend: Community weighted mean (CWM) of PC values for different treatments and soil types. Color indicates treatment: lightest gray (control), light mid-gray (nutrient addition), dark-mid gray (water addition), dark (combined water and nutrient addition). The left panel is the harsh serpentine, less productive soil and the middle panel is the lush serpentine, more productive soil. Different letters indicate significant differences in estimated CWM across all treatments and soil types. Trait loadings for each PC are indicated in the right panel. See full models in Table 2.3.

Table 2.1

Trait	Trait category	Units and formulas	Methods
Mass	Morphological	grams	Cornelissen et al. 2003
Length of longest axis (size)	Morphological	mm	Thompson et al. 1993
3D Shape (shape)	Morphological	non-dimensional: 0 (spherical) to 0.3 (rod-like) variance of seed length, width, and depth, after transforming values so that length is unity	Thompson et al. 1993
2D Seed compaction (compact)	Morphological	non-dimensional: variance from an ellipse calculated as (4*area) / (pi*length*width).	VideometerLab Manual
Ephemeral dispersal structure (disp)	Morphological	binary: Presence of ephemeral dispersal structure (1), no dispersal structure or persistent structure (0)	Modified from LEDA dispersal structure categorizations: Kleyer et al. 2008
Seed texture (texture)	Morphological	ratio of vertical to horizontal grain energy where higher values indicate a smooth surface and lower values indicate a rough or patterned surface calculated as (horizontal covariance) / (vertical covariance)*(1/mean)	Videometer Lab Manual
Starch content (starch)	Chemical	binary: no starch content (0) starch content (1)	Jones 1966, Jeong et al. 2010
Carbon: nitrogen ratio (C:N ratio)	Chemical	ratio of carbon (g) / nitrogen (g)	UC Davis Stable Isotope Laboratory
Carbon content (C)	Chemical	percent carbon (g)	UC Davis Stable Isotope Laboratory
Seed coat thickness (SCT)	Barrier	mm	
Seed coat permeability (SCP)	Barrier	change in mass after 24 hours calculated as: (wet mass (g) - dry mass(g)) / (dry mass (g)	Traveset et al. 2008

Legend: Seed traits measured with trait type, definition of measurement, and methods citation. VideometerLab multispectral acquisition system software (Videometer A/S, Denmark) was used to measure seed length, shape, compaction, and texture.

Table 2.2

ANOVA table	**Estimate**	**Estimate**		**F value**	**p-value**
PC * species origin	**Non-native**	**Native**			
PC1	-0.86	0.23		7.37	**p=0.009**
PC2	0.49	-0.13		2.93	p=0.10
PC3	-0.50	0.13		3.45	p=0.07
PC4	0.38	-0.10		2.77	p=0.10
PC * Functional type	**Grasses**	**Forbs**	**N-fixing forbs**		
PC1	-3.04 b	0.47 a	0.04 a	20.24	**<0.001**
PC2	0.04 a	-0.25 a	1.57 b	6.84	**p=0.002**
PC3	-1.12 a	0.01 ab	0.49 b	3.66	**p=0.03**
PC4	0.27 ab	-0.22 a	1.13 b	6.07	**p=0.004**

Legend: Pairwise differences of PC values by species origin and functional type; estimates are the group means. The p-value indicates if there is a significant difference between any of the groups and significant (p-value < 0.05) contrasts are bolded. Letters shared by groups indicate that those group means are not significantly different from each other in a pairwise Tukey comparisons across groups.

Table 2.3

Response variable: CWM	Fixed parameters	p-value of model (loglikelihood ratio comparison)	Estimate value (standard error)	R2 Marginal / R2 Conditional
PC1	Intercept (Harsh control)	NA	-0.634 (0.231)	0.49 / 0.57
	Lush soil	NA	-1.975 (0.272)	
	Water	NA	-0.609 (0.278)	
	Nutrients	NA	-1.254 (0.280)	
	Water: Nutrients	p=0.236	0.621 (0.398)	
	Water: Soil	p=0.069	0.781 (0.389)	
	Nutrients : Soil	p=0.069	1.201 (0.385)	
	Water: Nutrients : Soil	p=0.267	-0.584 (0.550)	
PC2	Intercept (Harsh control)	NA	-0.112 (0.151)	0.51 / 0.62
	Lush soil	NA	0.828 (0.167)	
	Water	NA	-0.110 (0.171)	
	Nutrients	NA	-0.350 (0.171)	
	Water: Nutrients	p=0.066	0.636 (0.244)	
	Water: Soil	p=0.340	0.158 (0.238)	
	Nutrients : Soil	p=0.340	0.289 (0.236)	
	Water: Nutrients : Soil	p=0.053	-0.630 (0.337)	
PC3	Intercept (Harsh control)	NA	-0.207 (0.188)	0.38 / 0.59
	Lush soil	NA	-1.065 (0.184)	
	Water	NA	-0.714 (0.187)	
	Nutrients	NA	-0.515 (0.188)	
	Water: Nutrients	p=0.122	0.384 (0.267)	
	Water: Soil	p=0.004	0.621 (0.261)	
	Nutrients : Soil	p=0.004	0.294 (0.257)	
	Water: Nutrients : Soil	p=0.554	-0.208 (0.369)	
PC4	Intercept (Harsh control)	NA	-0.650 (0.132)	0.22 / 0.22
	Lush soil	NA	0.068 (0.187)	
	Water	NA	0.623 (0.196)	
	Nutrients	NA	0.060 (0.196)	
	Water: Nutrients	p=0.901	-0.283 (0.280)	
	Water: Soil	p=0.005	-0.777 (0.273)	
	Nutrients : Soil	p=0.005	-0.254 (0.270)	
	Water: Nutrients : Soil	p=0.181	0.496 (0.386)	

Legend: Community weighted mean (CWM) of the top four PC axes modeled using the parameters of soil type, treatment, the interaction between soil type and treatment, the interaction of nutrient and water treatments and the full three-way interaction of water, nutrients, and soil type. All models include a random effect of the watering line assigned to the plot as these plots may be spatially correlated.

Chapter 3

Trait-performance relationships vary across populations in response to drought

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ABSTRACT

As precipitation patterns change, populations with particular traits or life history strategies may be better able to cope with changes and persist. Shifting water availability for plants may trigger transgenerational plastic effects, i.e., resulting in different trait expression in offspring. Transgenerational effects may impact early seedling traits and, consequently, future plant performance. We asked whether seedling traits varied across three populations along a precipitation gradient, and in response to three maternal environment treatments (drought, control, well-watered). Further, we examined whether seedling traits impacted seedling survival under drought and first year reproductive output. We found that the low elevation population, which was the driest, had the most resource acquisitive root traits, such as high specific root length and small root diameter. Surprisingly, maternal environment treatments had few impacts on seedling traits, but we observed greater resource acquisitive root traits for seedlings from maternal plants in water addition treatments in contrast with offspring from droughted plants. Survival was similar across populations and maternal treatments, yet longer survival aligned with higher seedling root mass fraction and earlier emergence. The low elevation population generally had a higher probability of flowering and higher seed production. However, effects of maternal environment treatment differed across the gradient, with drought maternal treatment tending to increase the probability of flowering at the low elevation site but decreasing the probability of flowering at the high elevation site. Across sites, the probability of flowering increased with greater resource acquisitive root traits. Our findings suggest that water availability for the maternal plant and population may be relevant for determining first-year flowering, and that traits associated with resource acquisitive roots or fast growth may facilitate reproduction. A

better understanding of maternal environment on traits and performance will inform management and provide insight into processes affecting population persistence.

INTRODUCTION

As droughts become longer and more frequent (Dutkowski and Potts 2012), plants must tolerate stressful conditions in order to survive and reproduce. Faced with limited resources, plant performance will vary across and within populations (Baughman et al. 2019, Butterfield et al. 2017b) due to differences in phenotypic plasticity (Sultan and Bazzaz 1993), local adaptation (Blumenthal et al. 2021), and genetic diversity (Donohue and Schmidt 1999, Baskin and Baskin 2004). By identifying functional traits affecting survival, growth, or fecundity (Violle et al. 2007), and understanding their variation and impact on performance under limited water availability, scientists can predict which populations are most likely to persist in a changing climate (Kimball et al. 2016, Angert et al. 2011, Demarche et al. 2020, Laughlin et al. 2020). However, trait expression depends on the environment, and changes in water availability can alter trait expression in individuals (Sultan and Bazzaz 1993) as well as in future generations (i.e., their offspring), which cannot be easily inferred without study (Zirbel and Brudvig 2020). Studies considering the effects of water availability on offspring trait expression, and if these traits link to plant performance, remain rare (Drenovsky et al. 2016).

Traits associated with plant growth strategies hold promise for predicting individual and population responses to environmental changes (Westoby et al. 2002, Garbowski et al. 2020, Harrison and LaForgia 2019, Zirbel and Brudvig 2020, Roybal and Butterfield 2019). Various frameworks, such as the plant economic spectrum, differentiate fast-growing, resource-acquisitive species (with high specific leaf area [SLA], high nitrogen [N] content) from slower growing species that have longer-lived tissues (Reich et al. 2014, Wright et al. 2004, Diaz et al. 2016). Recent efforts have integrated root trait dimensions into these economic frameworks (Carmona et al. 2021, Weigelt et al. 2021). Trade-offs between growth rate (high root nitrogen content) and structural endurance of roots (high root tissue density, RTD) create a belowground resource conservation gradient which aligns with the leaf economic spectrum (Carmona et al. 2021). Furthermore, a fungal collaboration gradient differentiates plants that acquire resources themselves through acquisitive tissues (e.g., high specific root length, SRL) from plants that outsource resource uptake to mycorrhizal symbionts (e.g., larger root diameter; Bergmann et al. 2020). Resource acquisitive traits may be essential for plants with drought escape strategies that include fast growth and senescence to avoid dry conditions, while stress tolerance traits are needed for withstanding the negative effects of drought (Blanco-Sánchez et al. 2022, Kooyers 2015). For instance, a large root diameter may be particularly useful in drylands as thick roots reduce the risk of hydraulic failure, store non-structural carbohydrates which allow further osmoregulation (Chaves and Oliveira 2004), and enhance tissue lifespan (Weemstra et al. 2016, Lozano et al. 2020). The significance of root traits is demonstrated by studies showing populations in drier climates have thicker roots (Zhou et al. 2019), and that thicker roots may develop when plants are exposed to drought (Lozano et al. 2020, Zhou et al. 2018, although see Comas et al. 2013). By using trait variation to identify plant strategies and measuring how these strategies relate to performance, we will further our understanding of long-term population viability and how shifting environmental conditions will differentially impact species (Butterfield et al. 2017a, Leger et al. 2019, Leger et al. 2020).

The narrow window from germination to establishment is a perilous time for a plant during which the ability to survive harsh conditions depends on seedling trait strategies

(Harrison and LaForgia 2019, Atwater et al. 2015, Grubb 1977). There is evidence that trait-environment relationships may change as species age (Funk et al. 2021, Tautenhahn et al. 2019); and more resource acquisitive traits may provide a greater benefit to younger individuals compared to adult plants (Funk et al. 2021). However, differences in seed and seedling traits and how these relate to performance remain understudied compared to adult traits (Larson and Funk 2016a, Garbowski et al. 2021, Saatkamp et al. 2019). These studies have demonstrated that seedlings can enhance establishment and drought response through a variety of mechanisms. For example, acquisitive root traits (high SRL, high root tip number, high root length) may enhance water uptake and increase establishment rates while traits that conserve resources (small stature, higher relative root mass ratio, low SRL) may enable seedlings to endure drought stress (Leger et al. 2020, Larson et al. 2020, Atwater et al. 2015).

Traits may vary greatly among and within populations (Roybal and Butterfield 2019, Tautenhahn et al. 2019), understanding how this variation arises, and which trait values are beneficial under drought conditions is necessary to predict population performance. This intraspecific variation can arise from plasticity to the environment, genetic differences, transgenerational (maternal) effects, or combinations of these factors. Transgenerational effects are plastic responses of offspring due to the environmental conditions experienced by the preceding generation, often from the maternal plant (i.e., maternal effects). We know that maternal effects vary between populations and may be an important aspect of local adaptation (Hereford and Moriuchi 2005). For instance, a seedling's ability to germinate, establish, and compete varies with seed traits (mass, resource stores, seed coat thickness). These traits develop depending on how the maternal plant responds to the environment (Roach and Wulff 1987, Donohue and Schmidt 1999, Philippi 1993, Hereford and Moriuchi 2005, Tielborger and Valleriani 2005).

Through their potential influence on functional trait expression, maternal effects can have strong effects on performance in response to environmental conditions. Plants growing under low water availability may produce poorly provisioned seeds in contrast to well-watered individuals, however, there is some evidence of adaptive plasticity whereby maternal plants in dry conditions may produce more offspring, or offspring that have more competitive or resource acquisitive traits (Drenovsky et al. 2016, Zolfaghari et al. 2022, Vasques et al. 2013). For instance, offspring from maternal plants that experienced drought had higher seed production along with more resource acquisitive traits than offspring from well-watered plants in some grass species (Nguyen et al. 2016). Higher seed production increases the likelihood that at least one offspring will find a favorable microsite for germination (Mojzes et al. 2021). In contrast, another study found that offspring from droughted *Quercus brantii* trees had traits associated with high drought tolerance including high root to shoot ratio (greater seedling water uptake) and reduced xylem diameter (prevents cavitation; Zolfaghari et al. 2022). If trait expression is driven by resource availability in the maternal environment, seedlings from mothers from more benign environments may have high performance only when water is available, whereas seedlings from mothers in dry environments may be less dependent on water availability to maintain performance (Vasques et al. 2013). However, high maternal resource availability can increase offspring performance through factors such as higher seed mass increasing establishment (Atwater et al. 2015) regardless of seedling environment. Despite much recent work in this area exploring how seedling traits are linked to performance under dry conditions, it remains unclear if interactions between seedling traits and maternal environment result in differential performance.

In this study, we investigate *Elymus elymoides*, a perennial bunchgrass species, to address the following questions: 1) How do traits and trait trade-offs vary between populations and maternal environments? 2) Does water availability experienced by the maternal plant influence seedling survival under drought and first-year reproductive performance? and 3) Which traits predict performance? Here we consider three performance metrics: seedling survival under drought, probability of flowering in the first year, and seed set of those individuals that flower. We expect seedlings will exhibit trade-offs among resource acquisitive and conservative traits across both leaf (SLA, leaf N) and root traits (RTD, root N, SRL, root diameter). We expect population and maternal environment treatment to influence seedling traits, with seedlings from the low elevation population and dry maternal treatment possessing more resource acquisitive traits to quickly reach an age of maturity before water becomes scarce. Further, we hypothesize that resource acquisitive traits will increase reproduction while plants from populations and maternal treatments with greater stress tolerance traits will survive longer under drought.

METHODS

Study species and seed collection:

We explored intraspecific variation of *Elymus elymoides* (Poaceae), a short-lived perennial bunchgrass with a wide distribution in arid and semi-arid rangelands across the western United States (Leger et al. 2019). *Elymus elymoides* exhibits high intraspecific trait variation across populations (Parsons et al. 2011, Leger et al. 2019, Clary 1975), where traits correlate with environmental conditions, such as precipitation. Individuals receiving less precipitation have lower biomass, lower seed mass, and smaller leaf size than individuals from wetter populations (Blumenthal et al. 2021).

This study leverages a 5-year field manipulation spanning three grassland sites across an elevational gradient with one population from each site. Experimental plots along an elevational gradient of grassland communities were established in 2016 near Flagstaff, Arizona in the southwestern United States (Munson et al. 2019, 2022). Sites included a mixed conifer meadow (elevation 2591 m; MAP = 658 mm, MAT= 6.9), a ponderosa pine meadow (elevation 2179 m; MAP = 579 mm, MAT= 7.3), and a juniper savanna (elevation 1930; MAP = 486 mm, MAT= 9.9; Munson et al. 2019, Munson et al. 2022). This region experiences a bimodal precipitation pattern with winter rains, a dry foresummer, followed by late summer monsoons and a dry fall (Moore et al. 2022). At each of the three sites, four 2 x 3-meter plots were established each for control, drought (- 53 ± 3% summer precipitation), and water addition treatments (i.e., well-watered, + 32 ± 3% summer precipitation; Munson et al. 2022). The drought treatment was achieved through rain-out shelters consisting of a metal frame supporting a roof with bands of ultraviolet transmitting acrylic (Solacryl SUVT; 11 × 200 cm, bent into a V shape at 60°) placed at 20° angles that covered a total of half of the plot and faced toward the prevailing wind direction (southwest) to minimize precipitation blown into the plot. Water from the rain-out shelters was collected in barrels and redistributed to the water addition plots after rainfall events greater than 10mm, using PVC piping and sprinklers at the four corners of the water addition plots. Plots were surrounded by metal flashing buried 30cm beneath the soil surface to hydrologically isolate plots. Shelters and sprinklers were in place at all sites from the beginning of May through the end of September (Munson et al. 2022) starting in 2016. In August 2020, after five summers of experimental treatments, we collected a ripe inflorescence from all flowering plants within the plot; sample sizes ranged from 0 up to 5 extant plants per plot (median of 2, mean 2.4). We collected seed from flowering individuals outside of plots at the lowest elevation site to supplement the control treatment due to an insufficient number of plants with undispersed seed in control plots. Seed was stored dry at approximately 20°C.

Seedling grow out and trait measurements:

In order to assess maternal environment effects on seedling traits and link them to seedling performance, we grew seedlings from each population and each of the three maternal treatments (drought, control, well-watered) in a controlled environment. Seeds were moved to a refrigerator with no light and approximately 60% humidity at 4ºC for two weeks prior to planting in order to mimic temperatures of fall germination conditions (M. Moore, *pers. comm.*), though past work suggests that *Elymus elymoides* is relatively unaffected by pre-germination temperatures (Meyer et al. 2000). We randomly assigned seeds to one of three different growing groups: trait measurement group, dry-down survival group (hereafter "dry-down group"), and well-watered reproductive performance group (hereafter "reproductive performance group"). We planted n=30 seeds per maternal environment treatment per site, i.e., population, for the trait group and n=30-40 seeds per maternal environment treatment per population for the dry-down and reproductive performance groups; n = 930 seeds were planted in total, although not all seeds germinated. We recorded the mass of each seed prior to planting seeds in a fully randomized design in a greenhouse at the Orchard Park facilities at UC Davis in May 2021. Seeds were planted into individual cone-tainers (Stewe and Sons; 164 mL capacity, 1.5 x 8.25 in). All seeds were sowed in a soil mix of local commercial topsoil (60%: equal parts soil, sawdust), sand (20%), and greenhouse agricultural mix (20%: equal parts redwood sawdust, peat moss, pumice rock, stone) to mimic field soil while also facilitating root cleaning of seedlings (Leger et al.

2020). Mean daily high and low temperatures were 27.6 and 15.5 ºC (May 9 – May 29) for the trait measurement plants. Temperatures increased to 34.9 and 18.4 ºC, with approximately 14.3 hours of light per day and midday light levels around 800 PAR (May 9 – August 20) for the performance plants. All trays were moved every 2 days to account for variation in conditions in the greenhouse through mid-July, after which trays were rotated weekly. We scored seedling emergence daily for 30 days after sowing. For all seedlings, we recorded the day of emergence, as well as height at day 5. We hand watered all seedlings with 30mL of water each day, enough to dampen but not fully saturate the soil, from the time of sowing until 10 days after emergence for all seedlings.

While individual trait data of seed mass, days to emergence, and height were taken for every individual including reproductive performance, dry-down, and trait groups; destructive trait measurements were measured only on trait group plants. These plants were harvested at 10 days, and separated into root, shoot, and leaf tissue, with cotyledons and true leaves also separated upon collection. Some samples were refrigerated (4ºC) for up to 7 days when immediate processing was not possible, as tissue preserves well for this period (J. Funk, *pers. comm.*). Roots were carefully removed from containers and cleaned until no soil remained, using methods from Leger et al. 2019. Roots were scanned using an Epson perfection scanner at 800 dpi and analyzed using WinRHIZO software (WinRHIZO 2021 32-bit). We massed the total fresh leaf mass and cotyledon separately and measured fresh leaf area on the cotyledon using a LiCOR 3100C leaf scanner. After drying in an oven at 60ºC until fully dry (minimum of 3 days), root, shoot, and dry leaf biomass were measured for each individual. Total seedling biomass was calculated as the sum of the above and belowground biomass.

Seedling performance:

Seedlings in the reproductive performance group were randomly interspersed with dry-down seedlings and experienced identical conditions except that they continued to receive 30 mL water daily throughout the experiment. Seedlings in the dry-down group were watered to soil saturation 10 days after emergence (date varied), and then did not receive water for the rest of the experiment (Larson et al. 2020). Seedlings in the reproductive performance and dry-down groups were monitored 2-3 times a week and visually scored for signs of wilting. Categories included: healthy (fully green, upright), slightly wilted (up to 25% non-green aboveground or leaves fold, roll, or angle down), moderately wilted (leaves partially lacking color and elasticity), strongly wilted (leaves with less than 25% color or maintaining some elasticity) or dead (leaves fully dead, no green and no elasticity; modified from Engelbrecht et al. 2003). Reproductive performance seedlings were monitored until July 7 (60 days after planting). During this period these plants remained healthy: one plant from the reproductive performance group died at day 31 from unknown causes. Dry-down seedlings were monitored until August 20 (103 days after planting), when all seedlings in the dry-down group had died (n=243). After seedlings were categorized as dead, they were watered to saturation for the following three days to test for mortality (Larson et al. 2020). Any seedlings that revived, showed new green leaf growth, were reclassified as strongly wilted instead of dead and were subsequently removed from survival analyses.

All reproductive performance plants (167) were transplanted into larger pots with additional soil 79-81 days after planting (July 27 – 29) in order to allow plants to mature towards reproduction. Sample sizes varied greatly due to differences in germination proportions and were the smallest for the lowest elevation (9 – drought; 13 – control; 6 – well watered maternal treatments), with 20-27 individuals per maternal treatment for the mid and high elevation populations. These plants received 66 mL of greenhouse fertilizer mix from irrigation (150 ppm N, 50 ppm P, 200 ppm K, 175 ppm Ca, 55 ppm Mg, 120 ppm S, 2.5 ppm Fe, 0.5 ppm B, 0.5 ppm Mn, 0.02 ppm Cu, 0.01 ppm Mo, 0.05 ppm Zn) for 9 days and 33 mL per day for another 14 days (August 2 – 24). From August 25th through March, reproductive performance plants received 33mL of water per day. Plants began flowering mid-September (~4 months after planting) and were monitored weekly until the end of December (~8 months after planting) when the final inflorescences had matured. Each ripe inflorescence was measured for length and stored in an individual envelope. We sampled a subset of inflorescences for seed production through counting the number of viable seeds as well as total viable seed mass. In March 2021 (~11 months after planting), we collected and measured dry aboveground biomass for all reproductive performance plants and belowground biomass for a subset of those individuals (n=64).

Statistical analyses:

Trait correlations and difference between populations and treatments:

We assessed the data from the trait measurement group to determine if there were coordinated trait strategies and whether traits varied by population and maternal treatment. We first analyzed trait correlations for all traits measured using Pearson's correlation with adjustment for pairwise correlation coefficients (ggcorrplot package: Kassambara 2023). Trait data was screened for collinearity, and traits were narrowed to remove highly correlated traits (r > 0.5). Seedling traits that were measured and assessed for correlations but not included in further analysis included root N, root tissue density (RTD), root tip number, root mass, height at day 5, total biomass, leaf N, and average leaf area (Table S3.1).

Following this, we used a Principal component analysis (PCA) to determine which traits and trait trade-offs best differentiated individuals from different populations and maternal environments. Traits included in the analysis were seed mass, days to emergence, root mass fraction (RMF), specific leaf area (SLA), specific root length (SRL), root length, root diameter, and root tips per root length (Table 3.1). Missing values for dry leaf mass were imputed for five individuals from the predicted value of a linear model relating dry leaf mass to wet leaf mass for the lowest elevation population (Supplement S3.0). We used ANOVAs to compare PC values between populations, maternal environments, and their interaction for the top 4 PC axes. When significant differences between trait groups existed, we conducted pairwise comparisons using Tukey multiple comparison of means. We further compared traits between populations and maternal environment treatments using a PERMANOVA test (vegan package: Oksanen et al. 2022).

Population and maternal treatment differences in performance:

We assessed if performance varied by population and maternal treatment, using survival under a dry-down (dry-down group), and measures of reproductive performance including probability of flowering and expected seed production as performance metrics for the reproductive performance group. Expected seed production was estimated from a subset of seeds (counted from 33 individuals) using a linear model with population, maternal treatment, the interaction of population and maternal treatment, and inflorescence length as predictors. Using these predictors, we fitted a model estimating seed set and used this model to predict the estimated seed set for all flowering individuals (r2 = 0.61, Supplement S3.0).

To evaluate differences between populations and maternal environment treatments in seedling performance, we measured seedling survival under drought (days alive without water), probability of flowering, and seed set if an individual flowered. Each of these response variables was modeled against population and maternal treatment as well as the interaction between these factors. We used a linear (gaussian) model to determine survival time, i.e., days since last watered, during a dry-down; a binomial logistic model for probability of flowering with a logit link (glmmTMB package: Brooks et al. 2017) where each seedling either flowered (1) or didn't (0); and a negative binomial model for seed set (MASS package: Venables and Ripley 2002). All models were checked to assure they met the assumptions for the residuals (DHARMa package: Hartig 2022). We conducted post-hoc tests using pairwise comparisons among means with a Tukey adjustment for multiple comparisons (emmeans package: Lenth 2024). We used the MuMin package to extract coefficients of determination (R2) for seed production (Barton 2024), and McFadden's pseudo R2, which uses the log-likelihood kernels for the intercept-only model and the full estimated model for the probability of flowering (Signorell 2024). Additionally, we performed a chi-square test to determine significant differences in the probability of flowering between sites, and fisher's test to determine significant differences in the probability of flowering across all maternal treatments and sites as well as between maternal treatments within each site. Pairwise fisher comparisons were also used to contrast pairs of site and maternal treatment differences.

Trait differences in performance:

To evaluate the difference in seedling performance based on trait values, we used similar models to those previously described, replacing population and maternal treatment with either individual traits (seed mass, days to emergence, and height at day 5) measured on all plants or with means for each population and maternal treatment (PC scores). For individual trait models, we included a random effect of the population and maternal environment (n=9 possible population by maternal environment treatment combinations). To evaluate survival time during the dry-down (days from last watering to death), we used a linear mixed model (lme4 package: Bates et al. 2015); a binomial logistic mixed model was used for probability of flowering (Brooks et al. 2017). For seed production, due to high variance between populations and a limited and unbalanced sample size (n=58 total; Table S3.2), we used a negative binomial model on the mid and low elevation populations separately (Venables and Ripley 2002). To evaluate how population and maternal environment treatment PC scores affected performance, we used the same models as those used for population and maternal treatment on the top 3 PC axes. In order to meet model assumptions for seed set with a reduced sample size, 2 PC axes were modeled at a time. We used likelihood ratio tests on nested models by removing each trait while keeping the rest to test whether each trait significantly explained variation in performance. As for population and maternal treatment analysis, we checked residuals using the DHARMa package (Hartig 2022) for all models, and coefficients of determination were extracted using the MuMIn package (Barton 2024) and McFadden's pseudo R2 (Signorell 2024). All analyses were done in R version 4.4.1 "Race for Your Life".

RESULTS

We observed both expected and unexpected relationships between traits related to resource acquisition and stress tolerance. Belowground, we found that root N, a resource acquisitive trait, was inversely related to RTD, a stress tolerance trait (r = 0.33, Figure S3.1) and SRL (a resource acquisitive trait) was inversely related to root diameter (r = -0.55) along the microbial collaboration gradient. Further, RTD and SRL were negatively correlated (r = -0.84), suggesting a trade-off. We found positive associations between seed provisioning, aboveground, and belowground traits: seed mass and height (r = 0.49), seed mass and root length (r = 0.54), height and root length (r = 0.76; Figure S3.1). However, we did not see evidence of shared stress tolerance strategies between above and belowground traits. SLA was not correlated with RTD; in fact, SLA was not significantly correlated with any root trait. Due to the strong correlations, we did not include root tissue density (RTD), root N, root mass, root tip number, height at day 5, total biomass, leaf N, or average leaf area in the principal component analysis.

Our principal component analysis demonstrated that individuals varied along multiple axes of root traits; such as root tissue durability (root diameter and SRL), root system size (root length), relative allocation to roots (RMF), as well as root foraging effort (root tips per length; Figure 3.1, Table S3.3). The top four axes all related to root function and explained the vast majority of variance observed, once strongly collinear traits were removed (combined 75.8%; Figure 3.1). The primary PC axis related high root tissue durability with higher seed mass and accounted for 29% of the total variation. The second axis related to plant size, where high PC2 values meant short root length, smaller seed mass, and less root mass fraction. PC3 separated quickly emerging plants with higher root allocation from slower emerging plants with higher aboveground allocation. Finally, an active foraging strategy is represented by high PC4 values with high root tips per unit root length (Figure 3.1, Table S3.3). SLA loaded highly on axis 5, and days to seedling emergence on axis 6 (Table S3.3).

The low elevation population generally differed more in functional traits than the mid and high elevation populations. The low elevation population developed less durable root systems with smaller root diameter and higher SRL compared to the other two populations (PC1; Figure 3.1, Table S3.4). Differences in PC2 depended on maternal environment treatment (PC2; Figure 3.2, Table S3.4). The low elevation tended to have reduced allocation to root tissues than the high elevation population (RMF; higher PC3, post-hoc contrast p=0.06; Figure 3.1, Figure 3.2, Table S3.4). As we observed no differences in root foraging effort (PC4) between any populations, and this axis explained a small portion of total variation (Table S3.4), we used PC1 through 3 for subsequent analyses. While trends appear similar among single traits (e.g., SLA) and PC axes (Figure S3.4), only PC axes were statistically contrasted among populations and maternal environment treatments.

Root traits varied in response to maternal watering treatments, showing different effects of high and low water availability at the high elevation population. At the highest elevation population, individuals from the maternal water addition treatment had more acquisitive roots than the maternal drought treatment (low PC1 values; p=0.04, Figure 3.2, Table S3.4), but were not different from the control treatment. In contrast, the maternal environment treatment did not influence root resource acquisition traits in pairwise comparisons within low or mid-elevation populations (Figure 3.2, Table S3.4). The maternal environment treatment interacted with the population to impact root system size (root length, RMF; PC2). The maternal drought and control plants from the low elevation population had shorter root length, and higher RMF than the well-watered plants at the high elevation population and control at the mid-elevation population (PC2, Figure 3.2, Table S3.4). In both instances, less maternal water available shifted seedling root traits to be more conservative through increasing seedling root durability or reduction in root system size. There was no effect of maternal environment treatment on PC3 values at any elevation (Table S3.4). Results were similar when comparing site and maternal treatment seedling traits using PERMANOVA (Table S3.5).

We found small differences in seedling survival between populations but no differences between maternal environment treatments (Figure 3.3a, Table 3.2). The individuals from the high elevation population survived longer under a dry-down than those from the mid-elevation population (post-hoc comparison $p < 0.001$; Figure 3.3a). The plants from the low elevation population, despite having significantly smaller initial mean seed mass (Figure S3.4), did not significantly differ from the mid or high populations in survival (Figure 3.3a, Table 3.2). Populations and maternal environments with lower PC3 scores (higher RMF) had slightly longer survival without water (Figure 3.4a, Table 3.3). Seedling performance was poorly predicted by individual trait values, however, a taller height at day 5 slightly increased survival (Figure S3.2, Table S3.6).

The probability of flowering differed across populations and maternal environment treatments, while differences in seed set varied only across populations. The highest flowering probabilities were observed for the low elevation population, followed by the mid-elevation population, with the highest elevation population having the lowest probability of flowering (Figure 3.3b, Table 3.2, Table S3.7). Maternal treatments were marginally different within both the low ($p=0.06$) and high ($p=0.08$) elevation sites, however, not at the mid-elevation site (Table S3.7). Maternal drought tended to increase flowering probability at low elevation, while it tended to decrease the probability of first year flowering for the high elevation population (Figure 3.3b, Table S3.7). Pairwise significant differences in flowering show that the low elevation control and drought maternal treatments were more likely to flower than all maternal environment treatments from the high elevation population, and from the drought maternal environment treatment at mid-elevation (Table S3.7). The mid-elevation population water and control treatment plants were more likely to flower than the high-elevation drought treatment plants (Table S3.7, Figure 3.3b). Further, the low elevation population had higher seed production than the other two populations (Figure 3.3c, Table 3.2), though no significant effects of maternal environment treatment were observed (Figure 3.3c, Table 3.2). Low PC1 (thin, less durable root growth) scores were positively related to the probability of flowering and seed set (Table 3.3, Figure 3.4b, Figure 3.4c). Individual traits failed to explain any differences in flowering probability, while height at day 5 explained minimal variation in seed production at the mid-elevation site (Table S3.6).

DISCUSSION

In this study we determined if differences in seedling traits correspond to plant performance across different source populations and maternal environments. We found that root traits varied between populations where the low elevation population had highly acquisitive roots (low RTD, high SRL). This translated into higher first year reproduction for plants at low elevations, but not higher survival, which was similar across populations. We found that water availability differentially influenced reproduction across populations – increasing reproduction at the drier population (lower elevation) and decreasing reproduction at the wetter population (high elevation). These results suggest that where water is limited, plants shift to have higher belowground resource acquisition, consistent with a greater sensitivity to precipitation in drier areas. Maternal effects may contribute to understanding differences in how populations respond to drought, particularly in terms of first year reproduction.

Trait variation across populations and maternal environments

Trait trade-offs observed at the global scale (Weigelt et al. 2021) did not occur across populations of *Elymus elymoides*. While SLA is a pivotal trait for identifying growth strategies across species, we found that SLA explained little variation between individuals in our study. This echoes findings of limited variation of SLA values between populations and species in other grasslands (Roybal and Butterfield 2019). Further, while we found leaf N positively correlated with SLA (Carmona et al. 2021), we found no evidence that aboveground resource use traits (SLA, leaf N) were correlated with any belowground traits in contrast to predictions from global frameworks. Our belowground findings further differed in that we found no support for a difference between resource acquisition and collaboration (Bergmann et al. 2020), but a single axis combining SRL, diameter, root N, and RTD. This supports work by Funk et al. (2021) which posits that dryland herbaceous perennial root traits are variable along a single axis of root durability and mycorrhizal collaboration. Collectively, our data suggests that global trait frameworks do not apply generally at the species level, even across populations with different precipitation environments as observed by some (Avila Lovera et al. 2021, but see Mason and Donovan 2015).

Species may possess a combination of resource acquisitive or stress tolerance traits to maximize fitness in response to water limitation. Our findings of acquisitive traits in the low elevation population may indicate a threshold rainfall amount below which species rely on a resource acquisition strategy, whereas above this threshold, populations may deploy stress tolerance traits to increase performance. While plants with stress tolerance traits may have high fitness in dryland environments (Bradford and Hsiao 1982, Muller-Landau 2010), resource acquisitive traits may enable species to escape or avoid drought entirely (Perez-Ramos et al. 2013). For instance, in a study of a dryland perennial grass species, stress tolerance traits such as small plant size and high RMF increased fitness, as did resource-acquisitive traits of fast emergence, high root tip number, high SRL, and long root length (Leger et al. 2021). Our focus on seedlings may have highlighted differences in resource acquisition. While adult perennials have more stress tolerant strategies than annuals (e.g., high root diameter, Funk et al. 2021), this may not be true for early growth stages when perennial seedlings are establishing. Seedlings must acquire enough resources to endure competition and resource limitation; thus, a resource acquisitive strategy may increase early survival in populations that are strongly water limited.

We found that the maternal environment treatment influenced seedling traits related to root durability where, at high elevation, plants exposed to drought produced seedlings with greater stress tolerance traits than well-watered plants. This result concurs with Riginos et al. (2007) who found maternal effects in mesic, but not dry, populations; however, our finding that seedlings from drought-stressed parents had more durable root traits contrasts from Herman et al. (2012), who found that seedlings from drought-stressed parents had more acquisitive traits (early germination, longer roots). It appears that maternal influence is site-dependent, which aligns with the theory that maternal effects will only occur if cues reliably provide information about future conditions (Gallaway and Etterson 2007). For instance, when experiencing high precipitation all *Biscutella didyma* plants increased seed dormancy in their offspring, yet the effect was stronger in drier populations where the cue is reliable. In dry populations, maternal precipitation correlates strongly with next-year seedling density, and high densities can reduce fitness (Lampei et al. 2017, but see Riginos et al. 2007). In our system, precipitation cues may lack reliability at lower elevations; whereas at high elevation, wet years signal high competition in the following year, thus providing a reliable cue that higher resource acquisitive traits may be beneficial. Alternatively, if the low elevation is already optimizing for high resource acquisition, they may not be able to respond further, regardless of the reliability of cues from the maternal environment. Finally, our seedlings from mid and low elevation populations may not have exhibited trait differences between maternal treatments because they were grown in an ambient environment. Stressors; such as drought, herbivory, competition (Ehlers et al. 2018), as well as strong inter-annual variation in precipitation; amplify the expression of maternal effects, which may be less detectable in ambient conditions (Herman et al. 2012).

Performance and trait-performance relationships varied among populations

Water available to the maternal plant impacted whether offspring flowered in their first year of life; however, the effects varied depending on the population and traits. As expected, we found the highest probability of flowering occurred in the low elevation population with the driest environment (Mojzes et al. 2021, Nguyen et al. 2016). The low elevation population also had the most resource acquisitive traits, which may align with a fast life history strategy (early maturity, high seed production; Harvey and Zammuto 1985, Franco and Silvertown 1996, Karlsson and Méndez 2005). Here, maternal drought increased the probability of flowering, which may be a beneficial transgenerational plastic response preparing offspring for fast, resource acquisitive growth and high reproduction. In a dryland environment where precipitation is highly variable, a highly resource acquisitive strategy may benefit perennial species (Raunkiaer 1934, Blanco-Sánchez et al. 2022). This is similar to drought escape strategies observed frequently in annual species in drylands (Schaffer 1975, Kooyers 2015) as well as crops (Berger et al. 2016). In contrast, the generally low probability of flowering observed for the high elevation population may be attributed to trade-offs in resource allocation focused on growth, i.e., durable tissues in lieu of reproduction in order to maximize lifetime fitness (Pugliese 1988). For the high elevation population, more maternal water increased flowering. This coincides with work showing well-watered maternal plants produce well provisioned seeds (Ehlers et al. 2018), although we know here that seed mass did not explain this pattern. Further, it is unexpected for effects from seed provisioning to extend to this stage of development (Ellison and Thompson 1987, Bischoff et al. 2008), particularly in a greenhouse environment, although there is some evidence that well provisioned seeds produce more offspring (Larios et al. 2014). The consistency of water experienced by maternal plants may allow offspring to deploy a less conservative strategy if they do not need to buffer against unpredictable conditions. Across all populations and treatments, the probability of flowering increased with more acquisitive mean root traits (high SRL, low diameter). This finding agrees with trait frameworks where greater acquisitive traits correlate with greater reproductive output, further supporting the idea that maternal environment effects can shift offspring between slightly faster or slower life history strategies based on the population specific environmental cues.

Seed set was higher for the low elevation population, and generally for combined maternal environment treatment and population groups with more resource acquisitive root traits. The effects of maternal environment on seed set were more nuanced. Despite the large differences in the probability of flowering based on maternal environment, if plants did flower, seed production, i.e., seed number and total seed mass, did not vary based on maternal environment treatment. This suggests a decoupling of mechanisms for the initiation of flowering and the process of seed development. This differs from Matesanz's (2022) finding that offspring from droughted maternal plants produced less reproductive mass by reducing seed size. However, our finding agreed with trait frameworks where more acquisitive traits may correlate with greater reproductive output. While maternal environment treatment did not determine seed set, the number of inflorescences produced varied greatly, where more inflorescences produced correlated with lower RMF for the individual. This might suggest a cost of reproduction such that plants with high reproduction have fewer resources available (i.e., carbon stored belowground) for the following year.

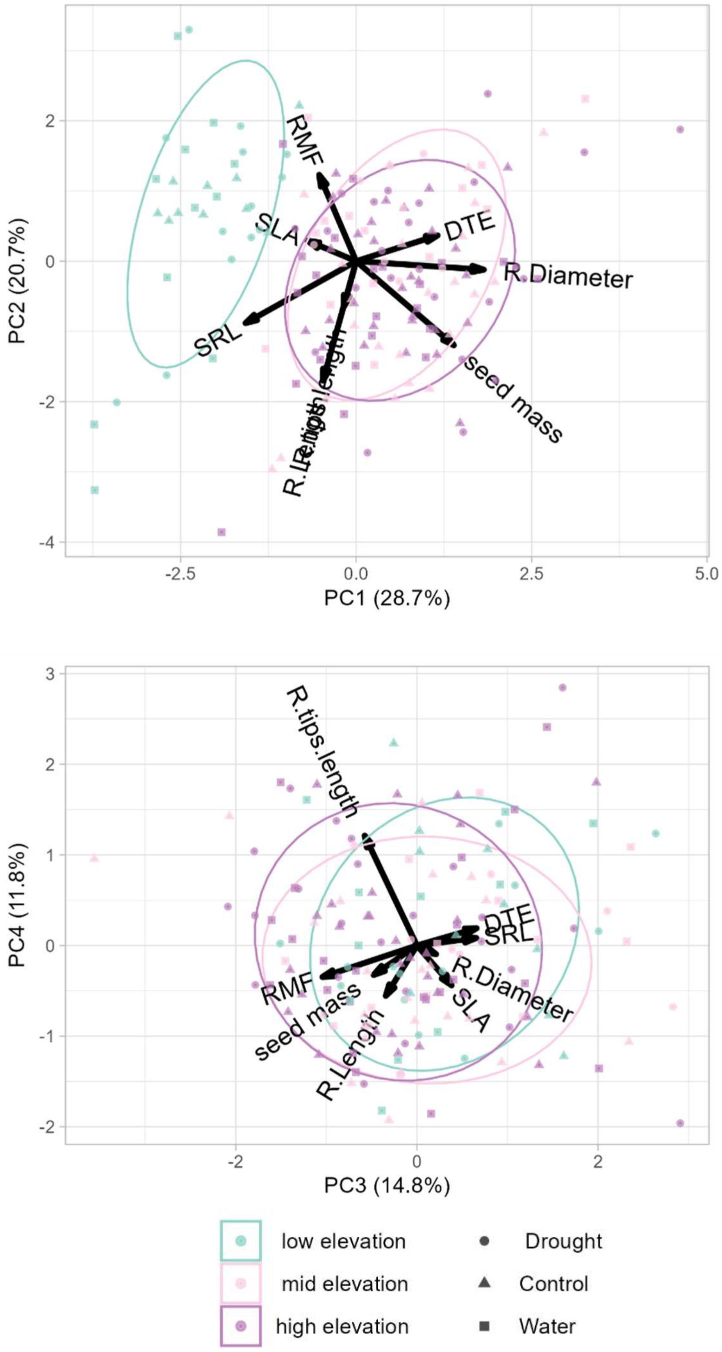
Despite existing studies that show maternal environments may influence the survival probabilities of the offspring (Ehler et al. 2018, Galloway and Etterson 2007, Riginos et al. 2007, Hereford and Moriuchi 2005, Zolfaghari et al. 2022, but see Metz et al. 2015), we did not find any impact of maternal environment, and minimal effect of population, on offspring survival time without water (dry-down). Overall strong resilience to periods of time without water of all three populations may be driven by adaptation to the bimodal precipitation in the CO plateau, where seedlings endure an extended dry foresummer for successful establishment. We observed that different trait combinations, such as larger aboveground size and stress-tolerant root traits (high elevation population) or smaller, resource-acquisitive root traits (low elevation population), are similarly resilient to drought. Alternative designs, where plants can achieve similar performance using different combinations of trait values, may explain why we found weak correlations between drought tolerance traits and some fitness metrics, similar to other studies (e.g., Dias et al. 2020, Worthy et al. 2020). Further, our expectation was that seeds with higher seed mass, which more often survive seedling establishment (Hulme 1998, Muller-Landau 2010, Leger et al. 2020, Harrison and LaForgia 2019), would persist longer than seeds with smaller seed mass; however, we found no effect of seed mass on survival. This highlights the potential complexity in predicting species and community responses to drought, as different trait combinations may similarly withstand drought challenges, leading to weak correlations between drought tolerance traits and fitness metrics.

Conclusions

Identifying which traits enable seedlings to endure stressful environments can improve efforts to understand how individuals and populations will respond to drought. Seedling survival is a limiting factor in dryland restoration and seedling mortality will remain high even with the most advantageous suite of traits (Pilliod et al. 2017, Atwater et al. 2015), however, we found minimal differences among populations and treatments in seedling survival. Seedling traits may also provide a way to predict which populations will be high seed producers. In our study, individuals from populations with resource acquisitive root traits were more likely to flower and have high seed production. Further, maternal environments will influence if plants reproduce in their first year in some populations. Further studies across a broader range of environmental conditions are needed to understand when low maternal water availability shifts from increasing to decreasing the probability of flowering. Additionally, further studies are needed to determine if this effect is truly adaptive. Broadly, our finding that seedlings from all populations were able to survive without water for similar lengths of time is promising and reinforces the idea that different trait combinations can achieve high performance.

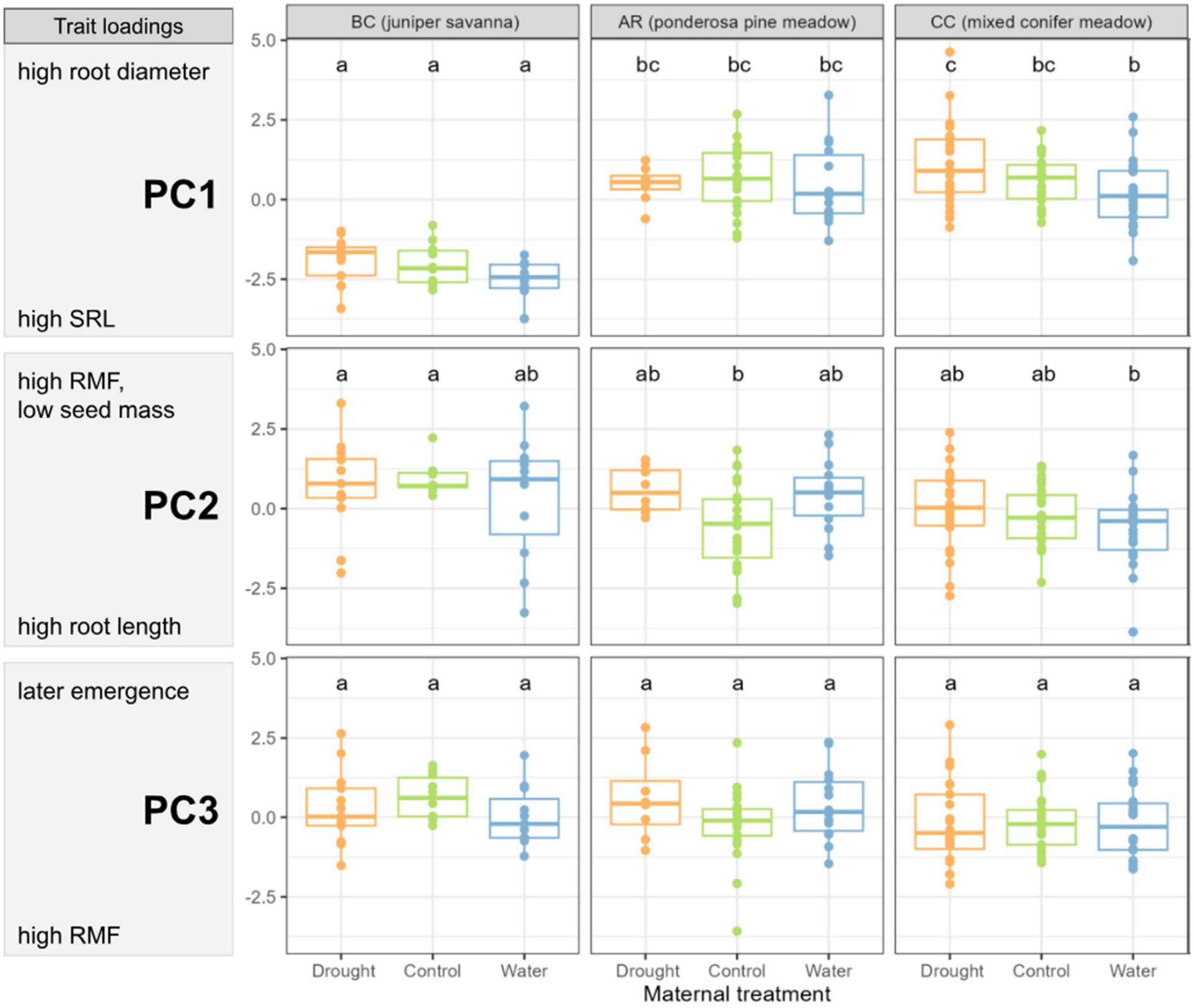
FIGURES AND TABLES

Figure 3.1: PCA of seedling traits



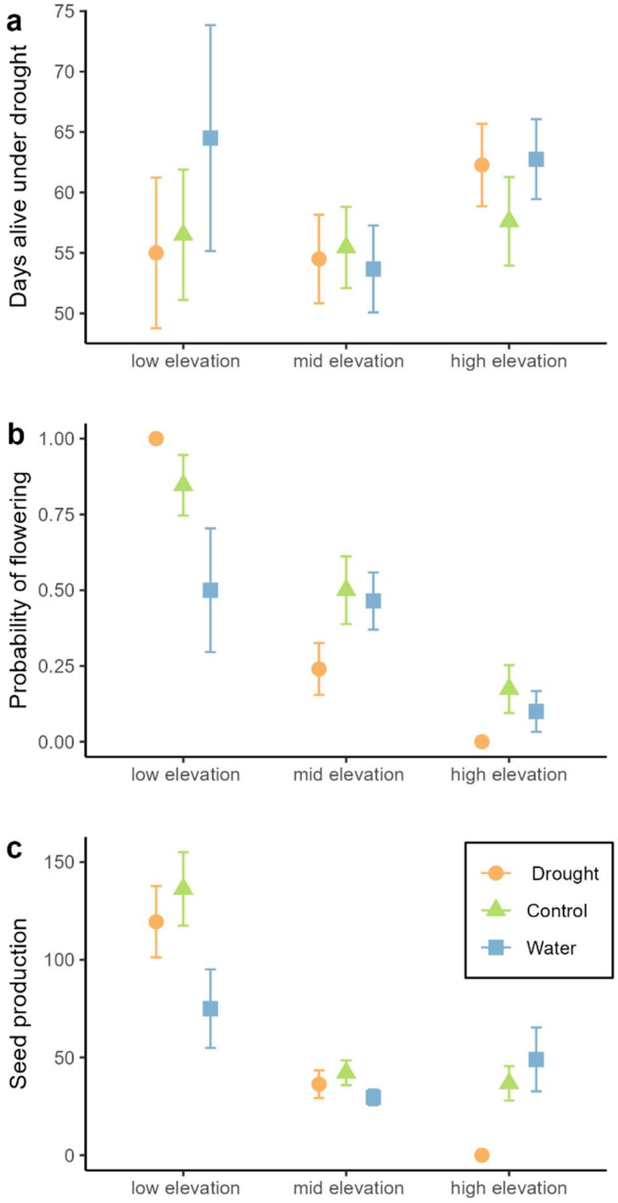
Legend: Principal components analysis where points represent individual seedlings sampled at 10 days, and arrows represent vectors indicating strength and direction of trait loadings. For each population an ellipse indicates the confidence interval for the range of potential values. The most arid, low elevation site (BC – juniper savannah) is in teal; the mid-elevation population is in pink (AR – ponderosa pine meadow); and the wettest, high elevation population is in purple (CC – mixed conifer meadow). Maternal environment treatment is indicated by different shapes where circles are drought, triangles are control, and squares are well-watered maternal treatments.

Figure 3.2: Differences across population and maternal environment treatment PC scores



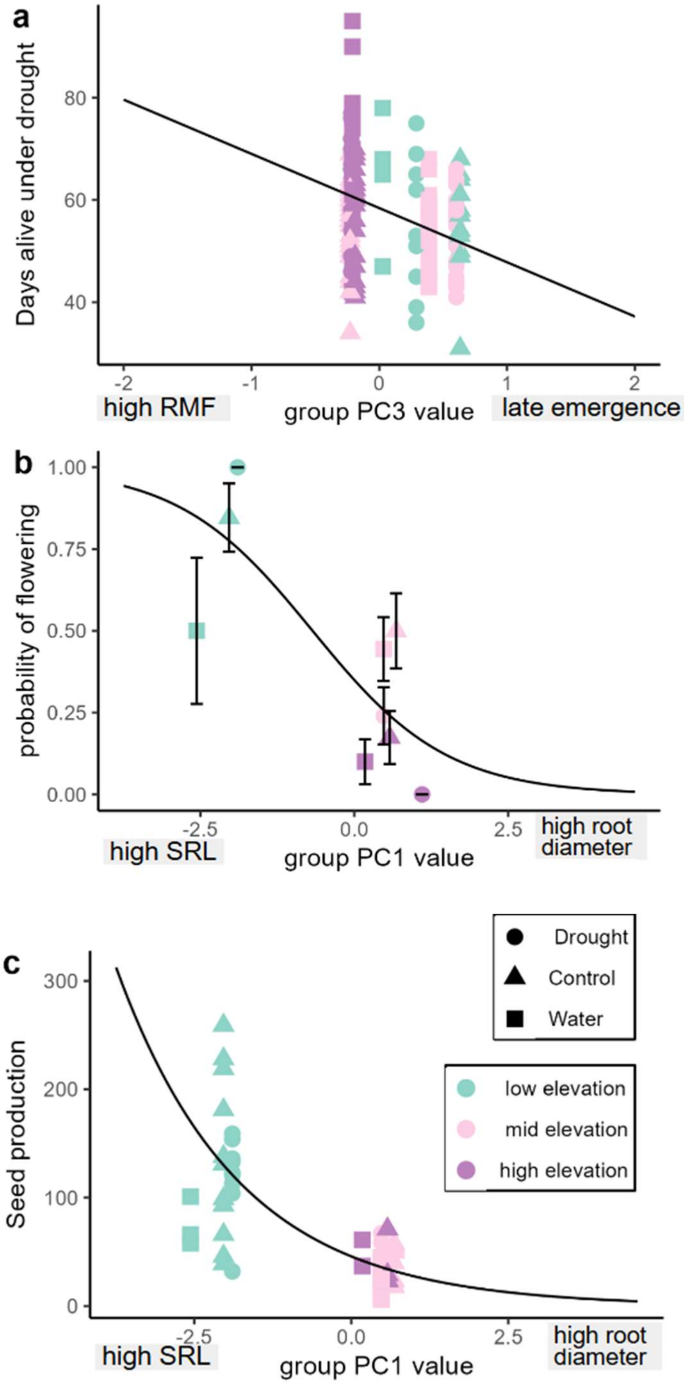
Legend: PC loadings, i.e., trait values for each population (elevation) and maternal treatment group (drought = orange, control = green, well-watered = blue) from ANOVAs of trait values (Table S3). Upper panel shows seedling values for PC1; letters shared by groups indicate that those group means are not significantly different from each other in a pairwise Tukey comparisons across all treatments and populations. The middle and lower panels show PC2 and PC3 respectively.

Figure 3.3: Performance by population and maternal environment treatment



Legend: Performance metrics for each population (elevation) and maternal treatment group (drought = orange circle; control = green triangle; well-watered = blue square) from performance models. Points are estimated marginal means from models in Table 3.2. Bars show standard error of the estimated marginal means extracted from the model. Panel a shows seedling survival days under drought from the dry-down performance group. Panels b and c show reproductive performance group plants. Panel b is back transformed from the logit scale to show the percent of individuals that flowered. There are no SE bars for the low elevation drought group (9 out of 9 flowered) nor high elevation drought group (0 out of 25 flowered). Panel c shows expected seed production for flowering plants; the high elevation drought group had no flowering individuals.

Figure 3.4: Performance by maternal environment treatment and population level traits



Legend: Performance metrics associated with PC values of combined maternal treatment and population level mean seedling trait values. For panel a (dry-down performance) and c (seed production), points represent performance of individual plants, while panel b shows the estimated marginal means with SE bars for the proportion of plants that flowered (same y axis as Figure 3.3b). The lines indicate the predicted performance response to PC3 (panel a) and PC1 (panels b and c) from models in Table 3.3. The high elevation population is shown in purple, mid-elevation in pink, and low elevation in teal. Maternal treatments are drought (circles), control (triangles), and well-watered (squares). The dominant traits from each PC axis are noted in gray boxes along the x axis.

Table 3.1

Trait	Units	Relevance of trait
Seed mass	grams	High seed mass is associated with good seed provisioning and early seedling competitive ability. Seed mass trades off with seed number when resources are limited.
Days to emergence (DTE)	days	Fewer days to emergence is associated with a strategy of fast growth; quicker emergence can give individuals a competitive advantage when conditions are good.
Specific leaf area (SLA)	cm /mg	High SLA leaves may be produced in a fast response to a resource pulse and high carbon assimilation, however, are less durable than leaves with a low SLA.
Root mass fraction (RMF)	g/g	High RMF indicates high root allocation and is associated with resource conservation. Additional aboveground biomass increases the potential for carbon assimilation and water loss, while higher belowground biomass may store resources and provide access to nutrients or water.
Root length (R. Length)	m	High root length may increase the ability of a plant to seek out water and nutrient sources.
Specific root length (SRL)	m/g	High SRL roots are associated with a “do it yourself” mycorrhizal collaboration gradient. Further, high SRL is associated with resource acquisition, including a fast growth rate and less durable roots.
Root diameter (R. Diameter)	mm	Similar to SRL, high diameter may indicate a collaborative relationship with mycorrhizae and may also be associated with root durability.
Root tips per length (R. tips.length)	count / cm	Root tips increase water uptake, local use of resources, and soil adhesion.

Legend: Traits included in the principal component analysis and correlation analysis collected from 10 day old seedlings (except seed mass) from the trait group. Abbreviations are given in parentheses.

Table 3.2

	Model family	**Variable**	**F value or deviance, p-value**	**R squared**
Survival Response: Days alive without water	Linear model (lm)	**Population** Maternal environment Interaction	10.12, **p<0.001** 0.75, p=0.47 1.72, p=0.15	0.10
Probability of flowering Response: Flowered (1) or did not flower (0)	Logistic, binomial (glm)	**Population** Maternal environment **Interaction**	52.42, **p<0.001** 4.84, p=0.11 12.78, **p=0.01**	0.32
Seed production Response: Expected number of seeds (nearest integer)	Generalized linear negative binomial model, (glm.nb)	**Population** Maternal environment Interaction	96.1**, p<0.001** 4.0, p=0.13 3.15, p=0.37	0.65

Legend: Performance metric models by population and maternal environment treatments. Mcfadden's pseudo R-squared is used for the model of the probability of flowering. Significant predictor variables (p-value < 0.05) are bolded.

Table 3.3

	Model family	Variable	Estimate	t or z value and p-value	R2
Survival Response: Days alive during drought	Linear model (lm)	Intercept	58.60		0.05
		PC1	-0.84	-0.99, p= 0.32	
		PC2	-2.30	0.77, p=0.44	
		PC3	-10.60	-2.43, **p=0.02**	
Probability of flowering Response: Flowered (1) or did not flower (0)	Binomial logistic (glm) with logit link	Intercept	-0.76		0.17
		PC1	-0.92	**-4.07, p<0.001**	
		PC2	-0.87	-0.91, p=0.36	
		PC3	2.14	1.61, p=0.11	
Seed production Response: Expected number of seeds produced by a plant, rounded to the nearest integer	Negative binomial generalized linear model (glm.nb)	Intercept	3.88		0.58
		PC1	-0.51	**-7.13, p<0.001**	
		PC2	-0.16	0.94, p=0.35	

Legend: Performance metric models by traits (population and maternal treatment means). For these models the predictive values were the means of the PC values for each combined population and maternal environment treatment, i.e., low elevation-drought, mid-elevation-control, etc. Significant predictor variables (p-value <0.05) are bolded. Mcfadden's pseudo-R-squared is used for the model of the probability of flowering.

References

Ackerly, D. D., and W. K. Cornwell. 2007. A trait-based approach to community assembly: Partitioning of species trait values into within- and among-community components. Ecology Letters 10:135–145.

Agneray, A. C., T. L. Parchman, and E. A. Leger. 2022. Phenotypes and environment predict seedling survival for seven co-occurring Great Basin plant taxa growing with invasive grass. Ecology and Evolution 12: 5, 8870.

An, H., C. C. Baskin, and M. Ma. 2022. Nonlinear response of the soil seed bank and its role in plant community regeneration with increased grazing disturbance. Journal of Applied Ecology 59:10, 2593-2603.

Angert, A. L., L. G. Crozier, L. J. Rissler, S. E. Gilman, J. J. Tewksbury, and A. J. Chunco. 2011. Do species' traits predict recent shifts at expanding range edges? Ecology Letters 14: 7, 677–689. https://doi.org/10.1111/j.1461-0248.2011.01620.x

Arrhenius, O.. 1921. Species and area. Journal of Ecology 9: 95–99.

Arroyo, A. I., Y. Pueyo, and H. Saiz. 2015. Plant – plant interactions as a mechanism structuring plant diversity in a Mediterranean semi-arid ecosystem. Ecology and Evolution 5:22, 5305-5317.

Atwater, D. Z., J. J. James, and E. A. Leger. 2015. Seedling root traits strongly influence field survival and performance of a common bunchgrass. Basic and Applied Ecology 16: 2, 128–140. https://doi.org/10.1016/j.baae.2014.12.004

Ávila-Lovera E., G. R. Goldsmith, K. M. Kay, and J. L. Funk. 2021. Above- and below-ground functional trait coordination in the Neotropical understory genus Costus. AoB PLANTS 14

Bartoń K.. 2024. MuMIn: Multi-Model Inference. R package version 1.48.4, <https://CRAN.R-project.org/package=MuMIn>.

Baskin, C. C., and J. M. Baskin. 2006. The natural history of soil seed banks of arable land. Weed Science 54:3, 549-557.

Baskin, C. C. 2003. Breaking physical dormancy in seeds – focusing on the lens. New Phytologist 158: 229–232.

Baskin, J. M., and C. C. Baskin. 1978. The seed bank in a population of an endemic plant species and its ecological significance. Biological Conservation 14:2, 125-130.

Baskin, J. M., and C. C. Baskin. 2004. A classification system for seed dormancy. Seed Science Research 14: 1, 1–16. https://doi.org/10.1079/ssr2003150

Baskin, J. M., C. C. Baskin, and L. Xiaojie. 2000. Taxonomy, anatomy and evolution of physical dormancy in seeds. Plant Species Biology 15: 139–152.

Basto, S., K. Thompson, and M. Rees. 2015a. The effect of soil pH on persistence of seeds of grassland species in soil. Plant Ecology 216:1163–1175.

Basto, S., K. Thompson, G. Phoenix, V. Sloan, J. Leake, and M. Rees. 2015b. Long-term nitrogen deposition depletes grassland seed banks. Nature Communications 6:1–6.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 67: 1, 1-48. doi:10.18637/jss.v067.i01.

Baughman, O. W., A. C. Agneray, M. L. Forister, F. F. Kilkenny, E. K. Espeland, R. Fiegener, M. E. Horning, R. C. Johnson, T. N. Kaye, J. Ott, J. B. St. Clair, and E. A. Leger. 2019. Strong patterns of intraspecific variation and local adaptation in Great

Basin plants revealed through a review of 75 years of experiments. Ecology and Evolution 9: 11, 6259–6275. https://doi.org/10.1002/ece3. 5200

Bekker, R. M., J. P. Bakker, U. Grandin, R. Kalamees, P. Milberg, P. Poschlod, K. Thompson, and J. H. Willems. 1998. Seed size, shape and vertical distribution in the soil: indicators of seed longevity. Functional Ecology 12:834–842.

Benvenuti, S. 2007. Natural weed seed burial: effect of soil texture, rain and seed characteristics. Seed Science Research 17: 211–219.

Berger, J., J. Palta, J., and V. Vadez. 2016. Review: An integrated framework for crop adaptation to dry environments: Responses to transient and terminal drought. Plant Science 253: 58–67. https://doi.org/10.1016/j.plantsci.2016.09.007

Bergmann, J., A. Weigelt, F. Van Der Plas, D. C. Laughlin, T. W., Kuyper, N. Guerrero-Ramirez, O. J. Valverde-Barrantes, H. Bruelheide, G. T. Freschet, C. M. Iversen, J. Kattge, M. L Mccormack, I. C. Meier, M. C. Rillig, C. Roumet, M. Semchenko, C. J. Sweeney, J. Van Ruijven, L. M. York, and L. Mommer. 2020. The fungal collaboration gradient dominates the root economics space in plants. In Science Advances 6. http://advances. sciencemag.org/

Bischoff A, T. Steinger, and H. Müller-Schärer. 2008. The importance of plant provenance and genotypic diversity of seed material used for ecological restoration. Restoration Ecology 18: 338–348.

Blanco-Sánchez, M., M. Ramos-Muñoz, B. Pías, J. A. Ramírez-Valiente, L. Díaz-Guerra, A. Escudero, and S. Matesanz. 2022. Natural selection favours drought escape and an acquisitive resource-use strategy in semi-arid Mediterranean shrubs. Functional Ecology 36: 9, 2289-2302.

Blumenthal, D. M., D. R. LeCain, L. M. Porensky, E. A. Leger, R. Gaffney, T. W. Ocheltree, and A. M. Pilmanis. 2021. Local adaptation to precipitation in the perennial grass Elymus elymoides: Trade-offs between growth and drought resistance traits. Evolutionary Applications 14: 2, 524–535. https://doi.org/10.1111/eva.13137

Boelt, B., S. Shrestha, Z. Salimi, J. R. Jorgensen, M. Nicolaisen, and J. M. Carstensen. 2018. Multispectral imaging - A new tool in seed quality assessment? Seed Science Research 28: 222–228.

Borer, E. T., E. W. Seabloom, D. S. Gruner, and W. S. Harpole. 2014. Herbivores and nutrients control grassland plant diversity via light limitation. Nature Letters.

Bradford, K. J., and T. C. Hsiao. 1982. Physiological responses to moderate water stress. In Physiologica plant ecology II water relations and carbon assimilation, 263–324. Berlin, Germany: Springer-Verlag.

Bronstein, J. L. 2009. The evolution of facilitation and mutualism. Journal of Ecology:1160–1170.

Brooker, R. W., F. T. Maestre, R. M. Callaway, C. L. Lortie, L. A. Cavieres, G. Kunstler, P. Liancourt, K. Tielbörger, J. M. J. Travis, F. Anthelme, C. Armas, L. Coll, E. Corcket, S. Delzon, E. Forey, Z. Kikvidze, J. Olofsson, F. Pugnaire, C. L. Quiroz, P. Saccone, K. Schiffers, M. Seifan, B. Touzard, and R. Michalet. 2008. Facilitation in plant communities : the past , the present , and the future. Journal of Ecology:18–34.

Brooks M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C.W. Berg, A. Nielsen, H. J. Skaug, M. Maechler, and B. M. Bolker. 2017. glmmTMB Balances Speed and

Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. The R Journal 9:2, 378-400. doi:10.32614/RJ-2017-066.

Browning, D. M., K. A. Snyder, and J. E. Herrick. 2019. Plant phenology: taking the pulse of rangelands. Rangelands 41:3, 129-134.

Brushmann, I., and C. Hobohm. 2013. Factors that create and increase endemism. In Endemism in vascular plants. Dordrecht: Springer Netherlands: 51-68.

Bruno, J. F., J. J. Stachowicz, and M. D. Bertness. 2003. Inclusion of facilitation into ecological theory. Trends in Ecology and Evolution 18:119–125.

Bullock, J. M., and R. T. Clarke. 2000. Long distance seed dispersal by wind: measuring and modelling the tail of the curve. Oecologia 124: 506-521.

Butterfield, B. J. 2009. Effects of facilitation on community stability and dynamics : synthesis and future directions. Journal of Ecology:1192–1201.

Butterfield, B. J., and S. M. Munson. 2016. Temperature is better than precipitation as a predictor of plant community assembly across a dryland region. Journal of Vegetation Science 27:5, 938-947.

Butterfield, B. J., S. M. Copeland, S. M. Munson, C. M. Roybal, and T. E. Wood. 2017a. Prestoration: using species in restoration that will persist now and into the future. Restoration Ecology 25: S155–S163. https://doi.org/10.1111/rec.12381

Butterfield, B. J., J. B. Bradford, S. M. Munson, and J. R. Gremer. 2017b. Aridity increases below-ground niche breadth in grass communities. Plant Ecology 218:385–394.

Callaway, R. M., 2007. Positive interactions and interdependence in plant communities. Springer Science & Business Media.

Carmona, C. P., C. G. Bueno, A. Toussaint, S. Träger, S. Díaz, M. Moora, A. D. Munson, M. Pärtel, M. Zobel, and R. Tamme. 2021. Fine-root traits in the global spectrum of plant form and function. Nature 597:7878, 683–687. https://doi.org/10.1038/s41586-021-03871-y

Cavieres, L. A., R. W. Brooker, B. J. Butterfield, B. J. Cook, K. Zall, C. J. Lortie, R. Michalet, F. I. Pugnaire, C. Schöb, S. Xiao, F. Anthelme, R. G. Bjork, K. J. M. Dickenson, B. H. Cranston, R. Gavilan, A. Gutierrez-Giron, R. Kanka, J.-P. Maalouf, A. F. Mark, J. Noroozi, R. Parajuli, G. Pheonix, A. M. Reid, W. M. Ridenour, C. Rixen, S. Wipf, L. Zhao, A. Escudero, B. Zaitchik, E. Lingua, E. Aschehoug, and R. M. Callaway. 2014. Facilitative plant interactions and climate simultaneously drive alpine plant diversity. Ecology Letters 17:193–202.

Chaves, M. M., and M. M. Oliveira. 2004. Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. Journal of experimental botany 55: 407, 2365-2384.

Chesson, P., L. E. Gebauer, S. Schwinning, N. Huntly, K. Wiegand. 2004. Resource pulses, species interactions, and diversity maintenance in arid and semi-arid environments. Oecologia 141: 236-253.

Clary, W. P.. 1975. Ecotypic adaptation in Sitanion hystrix. Ecology 56: 6, 1407–1415. https://doi.org/10.2307/1934707

Comas, L. H., S. R. Becker, V. M. V. Cruz, P. F. Byrne, and D. A. Dierig. 2013. Root traits contributing to plant productivity under drought. In Frontiers in Plant Science 4: Frontiers Research Foundation. https://doi.org/10.3389/fpls.2013.00442

Comas, L. H.,H. S. Callahan, and P. E. Midford. 2014. Patterns in root traits of woody species hosting arbuscular and ectomycorrhizas: implications for the evolution of belowground strategies. Ecology and evolution 4,15:2979-2990.

Cooksley, H., L. Dreyling, K. J. Esler, S. Griebenow, G. Neumann, A. Valentine, M. Schleuning, and F. M. Schurr. 2024. Functional traits shape plant–plant interactions and recruitment in a hotspot of woody plant diversity. New Phytologist 241: 3, 1100-1114.

Copeland, S. M., S. M. Munson, D. S. Pilliod, J. L. Welty, J. B. Bradford, and B. J. Butterfield. 2018. Long-term trends in restoration and associated land treatments in the southwestern United States. Restoration Ecology 26:311–322.

Cornelissen, J. H., S. Lavorel, E. Garnier, S. Díaz, N. Buchmann, D. E. Gurvich, P. B. Reich, H. Ter Steege, H. D. Morgan, M. G. A., Van Der Heijden, and J. G. Pausas. 2003. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. Australian journal of Botany 51:4, 335-380.

Daehler, C. C. 2003. Performance comparisons of co-occurring native and alien invasive plants: implications for conservation and restoration. Annual Review of Ecology, Evolution, and Systematics 34: 1, 183-211.

Dairel, M., and Fidelis, A. 2020. The presence of invasive grasses affects the soil seed bank composition and dynamics of both invaded and non-invaded areas of open savannas. Journal of Environmental Management 276: 111291.

Dalil, B.. 2014. Response of Medicinal Plants to Seed Priming: A Review. X 4: 2.

Dalling, J. W., A. S. Davis, B. J. Schutte, and A. Elizabeth Arnold. 2011. Seed survival in soil: Interacting effects of predation, dormancy and the soil microbial community. Journal of Ecology 99: 89–95.

Damschen, E. I., S. Harrison, D. D. Ackerly, B. M. Fernandez-Going, and B. L. Anacker. 2012. Endemic plant communities on special soils: Early victims or hardy survivors of climate change?

Davis, A. S. 2007. Nitrogen Fertilizer and Crop Residue Effects on Seed Mortality and Germination of Eight Annual Weed Species. Weed Science 55: 123–128.

DeMalach, N., J. Kigel, and M. Sternberg. 2021. The soil seed bank can buffer long-term compositional changes in annual plant communities. Journal of Ecology 109: 1275–1283.

DeMarche, M. L., A. L. Angert, and K. M. Kay. 2020. Experimental migration upward in elevation is associated with strong selection on life history traits. Ecology and evolution 10:2, 612-625.

Dias, A. T. C. , B. H. Rosado, F. De Bello, N. Pistón, and E. A. De Mattos. 2020. Alternative plant designs: consequences for community assembly and ecosystem functioning. Annals of Botany 125: 3, 391-398.

Díaz, M. 1994. Variability in seed size selection by granivorous passerines: effects of bird size, bird size variability, and ecological plasticity. Oecologia 99: 1-6.

Díaz, S., J. Kattge, J. H. Cornelissen, I. J. Wright, S. Lavorel, S. Dray, B. Reu, M. Kleyer, C. Wirth, I. Colin Prentice, E. Garnier, G. Bönisch, M. Westoby, H. Poorter, P. B. Reich, A. T. Moles, J. Dickie, A. N. Gillison, A. E. Zanne, … and L. D. Gorné. 2016.

The global spectrum of plant form and function. Nature 529: 7585, 167–171. https://doi.org/10.1038/nature16489

Donohue, K. and J. Schmitt. 1999. The genetic architecture of plasticity to density in Impatiens capensis. Evolution 53: 5, 1377-1386.

Dray S. and J. Josse. 2015Principal component analysis with missing values: a comparative survey of methods. Plant Ecology. 216: 657-67.

Drenovsky, R. E., M. L. Thornhill, M. A. Knestrick, D. M. Dlugos, T. J. Svejcar, and J. J. James. 2016. Seed Production and Seedling Fitness are Uncoupled from Maternal Plant Productivity in Three Aridland Bunchgrasses. Rangeland Ecology and Management 69: 3, 161–168. https://doi.org/10.1016/j. rama. 2016.01.003

Du, Z., J. Wang, H. An, H. Zhang, and G. Chen. 2023. Responses of soil seed banks to drought on a global scale. Science of the Total Environment 864.

Edwards E. J., and C. J. Still. 2008. Climate, phylogeny and the ecological distribution of C4 grasses. Ecology Letters 11: 266–276.

Ehlers, B. K., M. Holmstrup, I. K. Schmidt, J. G. Sørensen, and T. Bataillon. 2018. Joint impact of competition, summer precipitation, and maternal effects on survival and reproduction in the perennial Hieracium umbellatum. Evolutionary Ecology 32: 5, 529–545. https://doi.org/10.1007/s10682-018-9953-4

Ehrlén, J., and J. M. Van Groenendael. 1998. The trade-off between dispersability and longevity-an important aspect of plant species diversity. Applied Vegetation Science 1:1, 29-36.

Ellison, R. L. and J. N. Thompson. 1987. Variation in seed and seedling size: the effects of seed herbivores on Lomatium grayi (Umbelliferae). Oikos: 269-280.

Engelbrecht, B. M. J., and T. A. Kursar. 2003. Comparative drought-resistance of seedlings of 28 species of co-occurring tropical woody plants. Oecologia 136: 3, 383–393. https://doi.org/10.1007/s00442-003-1290-8

Epstein H. E., W.K. Lauenroth, I.C. Burke, and D.P. Coffin.1997. Productivity patterns of C3 and C4 functional types in the U.S. Great Plains. Ecology 78: 722–731.

Eskelinen, A., and S. Harrison. 2014. Exotic plant invasions under enhanced rainfall are constrained by soil nutrients and competition. Ecology 95: 682–692.

Eskelinen, A., and S. Harrison. 2015a. Biotic context and soil properties modulate native plant responses to enhanced rainfall. Annals of Botany 116: 963–973.

Eskelinen, A., and S. P. Harrison. 2015b. Resource colimitation governs plant community responses to altered precipitation. Proceedings of the National Academy of Sciences 112: 13009–13014.

Eskelinen, A., E. Elwood, S. Harrison, E. Beyen, and J. R. Gremer. 2021. Vulnerability of grassland seed banks to resource-enhancing global changes. Ecology 102.

ESRI. 2019. ArcGIS Desktop: Release 10.6.1. Redlands, CA: Environmental Systems Research Institute.

Evenari, Mi., D. Kollar, and Y. Guttermann. 1966. Effects of the env-ironment of the mother plant on germination by control of seed coat permeability to water in Ononis sicula Guss. Australian Journal of Biological Sciences 19: 1007-1016.

Farzam, M., and H. Ejtehadi. 2018. Effects of drought and slope aspect on canopy facilitation in a mountainous rangeland. Journal of Plant Ecology 10:626–633.

Fenn, M., R. Haeuber, G. S. Tonnesen, J. S. Baron, S. Grossman-Clarke, D. Hope, D. A. Jaffe, S.Copeland, L. Geiser, H. M. Rueth, and J. O. Sickman. 2003. Nitrogen Emissions, Deposition, and Monitoring in the Western United States. BioScience.

Fenner, M. 1995. Ecology of seed banks. 507-543.

Fenner, M., and K. Thompson. 2005. The ecology of seeds. Cambridge university press.

Flores-Moreno, H., P. B. Reich, E. M. Lind, L. L. Sullivan, E. W. Seabloom, L. Yahdjian ... and E. T. Borer. 2016. Climate modifies response of non-native and native species richness to nutrient enrichment. Philosophical Transactions of the Royal Society B: Biological Sciences, 371: 1694, 20150273.

Funk, J. L., J. E. Larson, and G. Vose. 2021. Leaf traits and performance vary with plant age and water availability in Artemisia californica. Annals of Botany 127: 4, 495–503. https://doi.org/10.1093/aob/mcaa106

Funk, J. L., R. J. Standish, W. D. Stock, and F. Valladares. 2016. Plant functional traits of dominant native and invasive species in mediterranean-climate ecosystems. Ecology 97:75-83.

Gallien, L., D. Zurell, and N. Zimmermann. 2018. Frequency and intensity of facilitation reveal opposing patterns along a stress gradient. Ecology and Evolution:2171–2181.

Galloway, L. F., and J. R. Etterson. 2007. Transgenerational plasticity is adaptive in the wild. Science 318: 5853, 1134–1136. https://doi.org/10.1126/science.1148766

Garbowski, M., B. Avera, J. H. Bertram, J. S. Courkamp, J. Gray, K. M. Hein, R. Lawrence, M. McIntosh, S. McClelland, A. K. Post, I. J. Slette, D. E. Winkler, and C. S. Brown. 2020. Getting to the root of restoration: considering root traits for improved restoration outcomes under drought and competition. Restoration Ecology 28: 6, 1384–1395. https://doi.org/10.1111/rec.13291

Garbowski, M., D. B. Johnston, and C. S. Brown. 2021. Leaf and root traits, but not relationships among traits, vary with ontogeny in seedlings. Plant and Soil 460: 1–2, 247–261. https://doi.org/10.1007/s11104-020-04790-z

Gardarin, A., C. Dürr, M. R. Mannino, H. Busset, and N. Colbach. 2010. Seed mortality in the soil is related to seed coat thickness. Seed Science Research 20: 243–256.

Gastauer, M., N. Mesquita, and J. Fernando. 2017. Facilitation as a driver of plant assemblages in Caatinga. Journal of Arid Environments 142:50–58.

Gholami, S., L. Saadat, and E. Sayad. 2018. Different microhabitats have contrasting effects on the spatial distribution of tree regeneration density and diversity. Journal of Arid Environments 148:1–5.

Gómez-Aparicio, L., R. Zamora, J.M. Gómez, J. A. Hódar, J. Castro, and E. Baraza. 2004. Applying plant facilitation to forest restoration: a meta-analysis of the use of shrubs as nurse plants. Ecological applications 14:4, 1128-1138.

Gong, H., C. Tang, and B. Wang. 2015. Seed Predation and Seed Traits. Plant Species Biology 30: 193-201. https://doi.org/10.1111/1442-1984.12051

Gravuer, K., and A. Eskelinen. 2017. Nutrient and rainfall additions shift phylogenetically estimated traits of soil microbial communities. Frontiers in Microbiology 8.

Gremer, J. R., L. P. Thomas, S. M. Munson, M. C. Duniway, and J. B. Bradford. 2018. Increasing temperature seasonality may overwhelm shifts in soil moisture to favor shrub over grass dominance in Colorado Plateau drylands. Oecologia 188:4, 1195-1207.

Gridzak, R., T. M. Lavender, L. W. Aarssen, and B. S. Schamp. 2024. Competition intensity is linked to the co-occurrence status and height differences of plant species found growing together in an old-field community. Journal of Ecology.

Grime, J.P. 1974. Vegetation classification by reference to strategies. Nature 250: 26–31.

Gross, K. L., G. G. Mittelbach, and H. L. Reynolds. 2005. Grassland invasibility and diversity: responses to nutrients, seed input, and disturbance. Ecology 86: 476-486.

Grubb, P. J., 1998. A reassessment of the strategies of plants which cope with shortages of resources. Perspectives in Plant Ecology, Evolution and Systematics 1: 1, 3-31.

Grubb, P. J., 1977. The maintenance of species-richness in plant communities: the importance of the regeneration niche. Biological reviews 52: 1, 107-145.

Harrison, S., and M. LaForgia. 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: 12, 5576–5581. https://doi.org/10.1073/pnas.1818543116

Hartig F.. 2022. DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.4.6, <https://CRAN.R-project.org/package=DHARMa>.

Hereford, J., and K. S. Moriuchi. 2005. Variation among populations of Diodia teres (Rubiaceae) in environmental maternal effects. Journal of Evolutionary Biology 18: 1, 124–131. https://doi.org/10.1111/j.1420-9101. 2004.00797.x

Herman, J. J., S. E. Sultan, T. Horgan-Kobelski, and C. Riggs. 2012. Adaptive transgenerational plasticity in an annual plant: Grandparental and parental drought stress enhance performance of seedlings in dry soil. Integrative and Comparative Biology 52: 1, 77–88. https://doi.org/10.1093/icb/ics041

Hijmans R. J., S. Phillips, J. Leathwick, and J. Elith. 2023. _dismo: Species Distribution Modeling_. R package version 1.3-14. <https://CRAN.R-project.org/package=dismo>.

Hooper, D. U., E. C. Adair, B. J. Cardinale, J. E. Byrnes, B. A. Hungate, K. L. Matulich, ... and M. I. O'Connor. 2012. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature 486: 105-108.

Howard, K. S. C., D. J. Eldridge, and S. Soliveres. 2012. Positive effects of shrubs on plant species diversity do not change along a gradient in grazing pressure in an arid shrubland. Basic and Applied Ecology 13:159–168.

Huenneke, L. F., S. P. Hamburg, R. Koide, H. A. Mooney, and P. M. Vitousek. 1990. Effects of soil resources on plant invasion and community structure in California serpentine grassland. Ecology 71: 478-491.

Hulme, P. E., 1998. Post-dispersal seed predation and seed bank persistence. Seed Science Research 8: 4, 513-519.

Jankju, M. 2013. Role of nurse shrubs in restoration of an arid rangeland : Effects of microclimate on grass establishment. Journal of Arid Environments 89:103–109.

Jeong, W. H., K. Harada, T. Yamada, J. Abe, and K. Kitamura. 2010. Establishment of new method for analysis of starch contents and varietal differences in soybean seeds. Breeding Science.

Jessen, M. T., H. Auge, W.S. Harpole, and A. Eskelinen. 2023. Litter accumulation, not light limitation, drives early plant recruitment. Journal of Ecology 111:6, 1174-1187.

Jones, Q., and F. R. Earle. 1966. Chemical Analyses of Seeds II: Oil and Protein Content of 759 Species.

Kalisz, S., and M. A. McPeek. 1993. Extinction dynamics, population growth and seed banks: an example using an age-structured annual. Oecologia 95: 314-320.

Karlsson, P. S. and M. Méndez. 2005. The resource economy of plant reproduction. In Reproductive allocation in plants, 1-49. Academic Press.

Kassambara, A.. 2023. ggcorrplot: Visualization of a Correlation Matrix using 'ggplot2'. R package version 0.1.4.1, <https://CRAN.R-project.org/package=ggcorrplot>.

Kikvidze, Z., and G. Nakhutsrishvili. 1998. Facilitation in subnival vegetation patches. Journal of Vegetation Science 9:261–264.

Kimball, S., J. L. Funk, M. J. Spasojevic, K. N. Suding, S. Parker, and M. L. Goulden. 2016. Can functional traits predict plant community response to global change? Ecosphere 7: 12. https://doi.org/10.1002/ecs2.1602

Kleyer, M., R. M. Bekker, I. C. Knevel, J. P. Bakker, K. Thompson, … and B. Peco. 2008. The LEDA Traitbase : a database of life-history traits of the Northwest European flora. Journal of Ecology 96: 1266–1274.

Knapp, A. K., C. J. W. Carroll, E. M. Denton, K. J. La Pierre, S. L. Collins, and M. D. Smith. 2015. Differential sensitivity to regional-scale drought in six central US grasslands. Oecologia 177: 949-957.

Kooyers, N. J.. 2015. The evolution of drought escape and avoidance in natural herbaceous populations. Plant Science 234: 155–162.

Koutzoukis, S., D. A. Pyke, J. Brunson, J. Baggio, C. Calzado-Martinez, and K. E. Veblen. 2023. Goldilocks forbs: survival is highest outside—but not too far outside—of Wyoming big sagebrush canopies. Restoration Ecology 31:6, e13919.

Kraft, N. J., Godoy, O., and J. M. Levine. 2015. Plant functional traits and the multidimensional nature of species coexistence. Proceedings of the National Academy of Sciences 112: 3, 797-802.

Laforgia, M. L., M. J. Spasojevic, E. J. Case, A. M. Latimer, and S. P. Harrison. 2018. Seed banks of native forbs, but not exotic grasses, increase during extreme drought. Ecology 0: 1–8.

Lambers, H., F. Stuart Chapin III, and T. L. Pons. 2008. Plant Physiological Ecology. Page Springer.

Lampei, C., J. Metz, and K. Tielbörger. 2017. Clinal population divergence in an adaptive parental environmental effect that adjusts seed banking. New Phytologist 214: 3, 1230–1244. https://doi.org/10.1111/nph.14436

Larios, E., A. Búrquez, J.X. Becerra, and D. L. Venable. 2014. Natural selection on seed size through the life cycle of a desert annual plant. Ecology 95: 11, 3213-3220.

Larson, J. E., and J. L. Funk. 2016a. Regeneration : an overlooked aspect of trait-based plant community assembly models:1284–1298.

Larson, J. E., and J. L. Funk. 2016b. Seedling root responses to soil moisture and the identification of a belowground trait spectrum across three growth forms. New Phytologist 210: 3, 827–838. https://doi.org/10.1111/nph.13829

Larson, J. E., and K. N. Suding. 2022. Seed bank bias: Differential tracking of functional traits in the seed bank and vegetation across a gradient. Ecology 103.

Larson, J. E., B. L. Anacker, S. Wanous, and J. L. Funk. 2020. Ecological strategies begin at germination: Traits, plasticity and survival in the first 4 days of plant life. Functional Ecology 34: 5, 968–979. https://doi.org/10.1111/1365-2435.13543

Lau, J. A., and J. L. Funk. 2023. How ecological and evolutionary theory expanded the 'ideal weed' concept. Oecologia 203: 251-266.

Lauenroth, W. K., and P. B. Adler. 2008. Demography of perennial grassland plants : survival , life expectancy and life span. Journal of Ecology:1023–1032.

Laughlin, D. C., J. J. Leppert, M. M. Moore, and C. H. Sieg. 2010. A multi-trait test of the leaf-height-seed plant strategy scheme with 133 species from a pine forest flora. Functional Ecology:493–501.

Laughlin, D. C., R. T. Strahan, P. B. Adler, and M. M. Moore. 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 21:3, 411-421.

Laughlin, D. C., J. R. Gremer, P.B. Adler, R. M. Mitchell, and M.M. Moore. 2020. The net effect of functional traits on fitness. Trends in Ecology and Evolution 35:1037-1047.

Leger, E. A., D. Z. Atwater, and J. J. James. 2019. Seed and seedling traits have strong impacts on establishment of a perennial bunchgrass in invaded semi-arid systems. Journal of Applied Ecology 56: 6, 1343–1354. https://doi.org/10.1111/1365-2664.13367

Leger, E. A., S. Barga, A. C. Agneray, O. Baughman, R. Burton, and M. Williams. 2020. Selecting native plants for restoration using rapid screening for adaptive traits:

methods and outcomes in a Great Basin case study. Restoration Ecology 1–9. https://doi.org/10.1111/rec.13260

Leishman, M. R., I. J. Wright, A. T. Moles, and M. Westoby. 2000. The Evolutionary Ecology of Seed Size.

Leishman, M. R., V. P. Thomson, and J. Cooke. 2010. Native and exotic invasive plants have fundamentally similar carbon capture strategies. Journal of Ecology 98: 1, 28-42.

Lenth R.. 2024. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.10.3, <https://CRAN.R-project.org/package=emmeans>.

Levine, J. M. 1999. Indirect Facilitation : Evidence and Predictions from a Riparian Community. Ecology 80:1762–1769.

Li, Y., S. Dong, Q. Gao, Y. Zhang, S. Liu, D. Swift, H. Ganjurjav, G. Hu, X. Wang, Y. Yan, and H. Wu. 2019. The effects of grazing regimes on phenological stages, intervals and divergences of alpine plants on the Qinghai-Tibetan Plateau. Journal of Vegetation Science 30:1, 134-145.

Loydi, A., and S. L. Collins. 2021. Extreme drought has limited effects on soil seed bank composition in desert grasslands. Journal of Vegetation Science 32.

Lozano, Y. M., C. A. Aguilar-Trigueros, I. C. Flaig, and M. C. Rillig. 2020. Root trait responses to drought are more heterogeneous than leaf trait responses. Functional Ecology 34: 11, 2224–2235. https://doi.org/10.1111/1365-2435.13656

MacNeill, G. J., S. Mehrpouyan, M. A. A. Minow, J. A. Patterson, I. J. Tetlow, and M. J. Emes. 2017. Starch as a source, starch as a sink: The bifunctional role of starch in carbon allocation. Oxford University Press.

Maestre, F. T., R. M. Callaway, F. Valladares, and C. J. Lortie. 2009. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. Journal of Ecology:199–205.

Maestre, F. T., S. Bautista, J. Cortina, and J. Bellot. 2001. Potential for using facilitation by grasses to establish shrubs on a semiarid degraded steppe. Ecological Applications 11:6, 1641-1655.

Marbach, I., and A. M. Mayer. 1974. Permeability of Seed Coats to Water as Related to Drying Conditions and Metabolism of Phenolics. Plant Physiology.

Mason, C. M., and L. A. Donovan. 2015. Evolution of the leaf economics spectrum in herbs: Evidence from environmental divergences in leaf physiology across Helianthus : Asteraceae. Evolution 69: 2705-2720.

Matesanz, S., M. Ramos-Muñoz, M. L. Rubio Teso, and J. M. Iriondo. 2022. Effects of parental drought on offspring fitness vary among populations of a crop wild relative. Proceedings of the Royal Society B 289: 1981, p.20220065.

McAuliffe, J. R..1984. Sahuaro-nurse tree associations in the Sonoran Desert: competitive effects of sahuaros. Oecologia 64: 319-321.

McKey, D.1994. Legumes and nitrogen: the evolutionary ecology of a nitrogen-demanding lifestyle. the nitrogen factor, 221-228.

Metz, J., J. von Oppen, and K. Tielbörger. 2015. Parental environmental effects due to contrasting watering adapt competitive ability, but not drought tolerance, in offspring of a semi-arid annual Brassicaceae. Journal of Ecology 103: 4, 990-997.

Meyer, S. E., S. B. Debaene-Gill, and P. S. Allen. 2000. Using hydrothermal time concepts to model seed germination response to temperature, dormancy loss, and priming effects in Elymus elymoides. Seed Science Research 10: 3, 213–223. https://doi.org/10.1017/s0960258500000246

Michalet, R., R. W. Brooker, L. A. Cavieres, Z. Kikvidze, and C. L. Lortie. 2006. Do biotic interactions shape both sides of the humped-back model of species richness in plant communities ? Ecology Letters:767–773.

Mihoč, M. A. K., L. Giménez-Benavides, D. S. Pescador, A. M. Sánchez, L. A. Cavieres, and A. Escudero. 2016. Soil under nurse plants is always better than outside: a survey on soil amelioration by a complete guild of nurse plants across a long environmental gradient. Plant and Soil 408: 31-41.

Mojzes, A., T. Kalapos, and G. Kröel-Dulay. 2021. Drought in maternal environment boosts offspring performance in a subordinate annual grass. Environmental and Experimental Botany 187: 104472.

Moles, A. T., and M. Westoby. 2006. Seed size and plant strategy across the whole life cycle. Oikos 113: 1, 91-105.

Moore, M. M., J. S. Jenness, D. C. Laughlin, R. T. Strahan, J. D. Bakker, H. E. Dowling, and J. D. Springer. 2022. Cover and density of southwestern ponderosa pine understory plants in permanent chart quadrats (2002–2020). Ecology 103: 5, e3661.

Moravcová , L., P. Pyšek, V. Jarošík, V. Havlíčková, and P. Zákravský. 2010. Reproductive characteristics of neophytes in the Czech Republic: traits of invasive and non-invasive species. Preslia 82: 4, 365-390.

Muller-Landau, H. C.. 2010. The tolerance-fecundity trade-off and the maintenance of diversity in seed size. PNAS 107: 9, 4242–4247. https://doi.org/10.1073/pnas.0911637107

Munson, S. M., E. L. Bunting, J. B. Bradford, B. J. Butterfield, and J. R. Gremer. 2019. Plant Production Responses to Precipitation Differ Along an Elevation Gradient and Are Enhanced Under Extremes. Ecosystems 22: 4, 699–708. https://doi.org/10.1007/s10021-018-0296-3

Munson, S. M., J. B. Bradford, B. J. Butterfield, and J. R. Gremer. 2022. Primary production responses to extreme changes in North American Monsoon precipitation vary by elevation and plant functional composition through time. Journal of Ecology 110: 9, 2232–2245. https://doi.org/10.1111/1365-2745.13947

Nakazawa, T., and H. Doi. 2012. A perspective on match/mismatch of phenology in community contexts. Oikos 121:4, 489-495.

Nathan, R. 2006. Long-distance dispersal of plants. Science 313: 5788, 786-788.

Nathan, R., G. G. Katul, G. Bohrer, A. Kuparinen, M. B. Soons, S. E. Thompson, A. Trakhtenbrot, and H. S. Horn. 2011. Mechanistic models of seed dispersal by wind. Theoretical Ecology 4: 113–132.

Navarro-Cano, J. A., M. Goberna, and M. Verdú. 2021. Facilitation enhances ecosystem function with non-random species gains. Oikos 130:12, 2093-2099.

Nawaz, J., M. Hussain, A. Jabbar, G. A. Nadeem, M. Sajid, M. Subtain, and I. Shabbir. 2013. Seed Priming A Technique.

Nguyen, M. A., A. E.Ortega, K. Q. Nguyen, S. Kimball, M. L. Goulden, and J. L. Funk. 2016. Evolutionary responses of invasive grass species to variation in precipitation and soil nitrogen. Journal of Ecology 104: 4, 979–986. https://doi.org/10.1111/1365-2745.12582

Nooden, L. D., K. A. Blakley, and J. M. Grzybowski. 1985. ‘Control of Seed Coat Thickness and Permeability in Soybean’ a Possible Adaptation to Stress. Plant Physiology. issue.

Norberg, A., N. Abrego, F.G. Blanchet, F.R. Adler, B.J. Anderson, J. Anttila, M.B. Araújo, T. Dallas, D. Dunson, J. Elith, and S.D. Foster. 2019. A comprehensive evaluation of predictive performance of 33 species distribution models at species and community levels. Ecological monographs 89: 3, p.e01370.

Oksanen J., G. Simpson, F. Blanchet, R. Kindt, P. Legendre, P. Minchin, R. O'Hara, P. Solymos, M. Stevens, E. Szoecs, H. Wagner, M. Barbour, M. Bedward, B. Bolker, D. Borcard, G. Carvalho, M. Chirico, M. De Caceres, S. Durand, H. Evangelista, R. FitzJohn, M. Friendly, B. Furneaux, G. Hannigan, M. Hill, L. Lahti, D. McGlinn, M. Ouellette, E. Ribeiro Cunha, T. Smith, A. Stier, C. Ter Braak, J. Weedon. 2022. _vegan: Community Ecology Package_. R package version 2.6-4, <https://CRAN.R-project.org/package=vegan>.

Othman, J., J. Bennett, and R. Blamey. 2004. Environmental values and resource management options: a choice modelling experience in Malaysia. Environment and Development Economics 9:6, 803-824.

Parsons, M. C., T. A. Jones, S. R. Larson,I. W. Mott, and T. A. Monaco. 2011. Ecotypic variation in Elymus elymoides subsp. brevifolius in the northern Intermountain West. Rangeland Ecology & Management 64: 6, 649-658.

Peart, M. H. 2016. Experiments on the Biological Significance of the Morphology of Seed-Dispersal Units in Grasses. British Ecological Society. Stable URL : http://www.jstor.org/stable/2259218

Pérez-Ramos, I. M., F. Volaire, M. Fattet, A. Blanchard, and C. Roumet. 2013. Tradeoffs between functional strategies for resource-use and drought-survival in Mediterranean rangeland species. Environmental and Experimental Botany 87: 126-136.

Philippi, T.. 1993. Bet-Hedging Germination of Desert Annuals: Variation Among Populations and Maternal Effects in Lepidium lasiocarpum. The American Naturalist 142: 3.

Pilliod, D. S., J. L. Welty, and G. R. Toevs. 2017. Seventy-five years of vegetation treatments on public rangelands in the Great Basin of North America. Rangelands 39: 1, 1-9.

Pinheiro J, D. Bates, R Core Team. 2023. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-164, https://CRAN.R-project.org/package=nlme.

PRISM Climate Group, Oregon State University, https://prism.oregonstate.edu, data created 4 Feb 2014.

Pueyo, Y., D. Moret-fernández, A. I. Arroyo, A. De Frutos, S. Kéfi, H. Saiz, R. Charte, M. De, L. Giner, and C. L. Alados. 2016. Plant nurse effects rely on combined hydrological and ecological components in a semiarid ecosystem. Ecosphere 7:1–19.

Pugliese, A.. 1988. Optimal resource allocation in perennial plants: a continuous-time model. Theoretical Population Biology 34: 3, 215-247.

R Core Team. 2024. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Ramırez, N., and A. Traveset. 2010. Predispersal seed-predation by insects in the Venezuelan Central Plain: Overall patterns and traits that influence its biology and taxonomic groups. Perspectives in Plant Ecology, Evolution and Systematics.

Raunkiaer, C.. 1934. The life forms of plants and statistical geography. Oxford, UK: Claredon

Reich, P. B.. 2014. The world-wide “fast-slow” plant economics spectrum: A traits manifesto. Journal of Ecology 102: 2, 275–301. https://doi.org/10.1111/1365-2745.12211

Reyl, R. J., M. M, Caldwell and J.H. Manwaring. 1996. Temporal dynamics of soil spatial heterogeneity in sagebrush-wheatgrass steppe during a growing season. Plant and Soil 184: 299-309.

Richardson, A. D., T. Keenan, M. Migliavacca, Y. Ryu, O. Sonnentag, and M. Toomey. 2013. Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. Agricultural and Forest Meteorology: 156-173.

Riginos, C., M. S. Heschel, and J. Schmitt. 2007. Maternal effects of drought stress and inbreeding in Impatiens capensis :Balsaminaceae. American Journal of Botany 94: 12, 1984–1991. https://doi.org/10.3732/ajb. 94.12.1984

Roach, D. A., and R. D. Wulff. 1987. Maternal Effects In Plants. Ann. Rev. Ecol. Svst : 18. www. annualreviews.org

Roybal, C. M., and B. J. Butterfield. 2019. Species-specific trait–environment relationships among populations of widespread grass species. Oecologia, 189: 4, 1017–1026. https://doi.org/10.1007/s00442-019-04372-6

Rutten, G., and E. Allen. 2023. Using root economics traits to predict biotic plant soil-feedbacks. Plant and Soil 485: 71-89.

Ryu, Y., D. Baldocchi, S. Ma, and T. Hehn. 2008. Interannual variability of evapotranspiration and energy exchange over an annual grassland in California. J. Geophys. Res. 113: D09104, http://dx.doi.org/10.1029/2007JD009263.

Saatkamp, A., A. Cochrane, L. Commander, L. K. Guja, B. Jimenez-Alfaro, J. Larson, A. Nicotra, P. Poschlod, F. A. O. Silveira, A. T. Cross, E. L. Dalziell, J. Dickie, T. E. Erickson, A. Fidelis, A. Fuchs, P. J. Golos, M. Hope, W. Lewandrowski, D. J. Merritt, B. P. Miller, R. G. Miller, C. A. Offord, M. K. J. Ooi, A. Satyanti, K. D. Sommerville, R. Tangney, S. Tomlinson, S. Turner, and J. L. Walck. 2019. A research agenda for seed-trait functional ecology. New Phytologist 221: 4, 1764–1775. https://doi.org/10.1111/nph.15502

Saatkamp, A., P. Poschlod, and D. L. Venable. 2014. The Functional Role of Soil Seed Banks in Natural Communities: 263–295.

Schafer, M., and P. M. Kotanen. 2003. The influence of soil moisture on losses of buried seeds to fungi. Acta Oecologica 24:255–263.

Schaffer, W.M.. 1975. Selection for optimal life histories in plants. Ecology and evolution of communities,142-156.

Scheiner, S. M.. 2003. Six types of species-area curves. Global ecology and biogeography 12: 6, 441-447.

Shaw, J.. 2018. Ammophila arenaria as a Nurse Plant: Implications for Management of an Invasive Species. Ecological Restoration 36:3, 171-173.

Shreve, F.. 1931. Physical conditions in sun and shade. Ecology 12: 96-104.

Signorell A.. 2024. DescTools: Tools for Descriptive Statistics. R package version 0.99.55, <https://CRAN.R-project.org/package=DescTools>.

Soliveres, S., D. J. Eldridge, F. T. Maestre, M. A. Bowker, M. Tighe, and A. Escudero. 2011. Microhabitat amelioration and reduced competition among understorey plants as drivers of facilitation across environmental gradients : Towards a unifying framework. Journal of PPEES Sources 13:247–258.

Spasojevic, M. J., and K. N. Suding. 2012. Inferring community assembly mechanisms from functional diversity patterns: the importance of multiple assembly processes. Journal of Ecology 100:3, 652-661.

Sperry, J. H., D. O'Hearn, D.R. Drake, A. M. Hruska, S. B. Case, J. Vizentin-Bugoni, ... and C. E. Tarwater. 2021. Fruit and seed traits of native and invasive plant species in Hawai 'i: implications for seed dispersal by non-native birds. Biological Invasions 23: 1819-1835.

Stable isotope facility guidelines. 2022. University of California, Davis. https://stableisotopefacility.ucdavis.edu/sample-preparation-carbon-and-nitrogen-solids

Steenberg, W. F. and C. H. Lowe. 1977. Ecology of the saguaro H. Reproduction, germination, establishment, growth, and survival of the young plant. US Government Printing Office, Washington, DC.

Sultan, S. E., and F. A. Bazzaz. 1993. Phenotypic plasticity in Polygonum persicaria. I. Diversity and uniformity in genotypic norms of reaction to light. Evolution 47: 4, 1009–1031. https://doi.org/10.1111/j.1558-5646.1993. tb02132.x

Suttle, K. B., M. A. Thomsen, and M. E. Power. 2007. Species interactions reverse grassland responses to changing climate. Science 315: 640–642.

Tautenhahn, S., C. Grün-Wenzel, M. Jung, S. Higgins, and C. Römermann. 2019. On the relevance of intraspecific trait variability—A synthesis of 56 dry grassland sites across Europe. Flora: Morphology, Distribution, Functional Ecology of Plants 254: 161–172. https://doi.org/10.1016/j. flora.2019.03.002

Tecco, P. A., S. Díaz, M. Cabido, and C. Urcelay. 2010. Functional traits of alien plants across contrasting climatic and land-use regimes: do aliens join the locals or try harder than them? Journal of Ecology 98: 1, 17-27.

Temperton, V., R. Hobbs, T. Nuttle, and S. Halle. 2004. Assembly Rules and Restoration Ecology: Bridging the Gap Between Theory and Practice New from Island Press.

Tewksbury, J. J., and J. D. Lloyd. 2001. Positive interactions under nurse-plants : spatial scale , stress gradients and benefactor size. Oecologia:425–434.

Thompson, K. 1987. Seeds and seed banks. New Phytologist 106: 23–34.

Thompson, K., S. R. Band, and J. G. Hodgson. 1993. Seed Size and Shape Predict Persistence in Soil. Ecology.

Tielborger K., and A. Valleriani. 2005. Can seeds predict their future? Germination strategies of density-regulated desert annuals. Oikos 111: 2, 235-244.

Tigano, A., and V. L. Friesen. 2016. Genomics of local adaptation with gene flow. Molecular ecology 25: 10, 2144-2164.

Traveset, A., J. Rodri, and G. Rez. 2008. Seed Trait Changes in Dispersers' Guts and Consequences for Germination and Seedling Growth. Ecology.

Usinowicz, J., and J. M. Levine. 2018. Species persistence under climate change : a geographical scale. Ecology Letters:1589–1603.

Vasques, A., E. Chirino, A. Vilagrosa, V. R. Vallejo, and J. J. Keizer. 2013. The role of seed provenance in the early development of Arbutus unedo seedlings under contrasting watering conditions. Environmental and Experimental Botany 96: 11–19. https://doi.org/10.1016/j. envexpbot.2013.08.004

Venable D. L. 1986. Dormancy, Dispersal, and the Evolution of Seed Size. American Journal of Botany 73: 5,659-659.

Venables, W. N. and B. D. Ripley. 2002. Modern Applied Statistics with S. Fourth Edition. Springer, New York. ISBN 0-387-95457-0

Violle, C., M. L. Navas, D. Vile, E. Kazakou, C. Fortunel, I. Hummel, and E. Garnier. 2007. Let the concept of trait be functional! Oikos 116: 5, 882–892. https://doi.org/10.1111/j.2007.0030-1299.15559.x

Volaire, F.. 2018. A unified framework of plant adaptive strategies to drought: crossing scales and disciplines. Global change biology 24:7, 2929-2938.

Wagner, M., and N. Mitschunas. 2008. Fungal effects on seed bank persistence and potential applications in weed biocontrol: A review.

Wang, S., C. Wang, J. Duan, X. Zhu, G. Xu, C. Luo, and M. Du. 2014. Timing and duration of phenological sequences of alpine plants along an elevation gradient on the Tibetan plateau. Agricultural and Forest Meteorology: 189–228. https://doi.org/10.1016/j. Agrformet.2014.01.021

Weemstra, M., L. Mommer, E. J. W. Visser, J. van Ruijven, T. W. Kuyper, G. M. J. Mohren, and F. J. Sterck. 2016. Towards a multidimensional root trait framework: a tree root review. In The New phytologist 211: 4, 1159–1169. Blackwell Publishing Ltd. https://doi.org/10.1111/nph.14003

Weigelt, A., L. Mommer, K. Andraczek, C. M. Iversen, J. Bergmann, H. Bruelheide, Y. Fan, G. T. Freschet, N. R. Guerrero-Ramírez, J. Kattge, T. W. Kuyper, D. C. Laughlin, I. C. Meier, F. van der Plas, H. Poorter, C. Roumet, J. van Ruijven, F. M. Sabatini, M. Semchenko, … and M. L. McCormack. 2021. An integrated framework of plant form and function: the belowground perspective. In New Phytologist 232: 1, 42–59. John Wiley and Sons Inc. https://doi.org/10.1111/nph.17590

Western Regional Climate Center. 2021. RAWS Knoxville Creek Weather Station. University of Nevada, Desert Research Institute. https://wrcc.dri.edu/cgi-bin/rawMAIN.pl?caCKNO

Westoby, M., and I. J. Wright. 2006. Land-plant ecology on the basis of functional traits. Trends in Ecology and Evolution 21:5, 261-268.

Westoby, M., D. S. Falster, A. T. Moles, P. A. Vesk, and I. J. Wright. 2002. Plant Ecological Strategies: Some Leading Dimensions of Variation Between Species. Annual Review of Ecology and Systematics 33: 1, 125–159. https://doi.org/10.1146/annurev. ecolsys.33.010802.150452

Wilcots, M. E., B. N. Taylor, E. K. Kuprewicz, and D.N. Menge. 2019. Small traits with big consequences: how seed traits of nitrogen-fixing plants might influence ecosystem nutrient cycling. Oikos 128: 5, 668-679.

Wilson, M. F. 1993. Dispersal mode, seed shadows, and colonization patterns.

Worthy, S. J., D. C. Laughlin, J. Zambrano, M. N. Umaña, C. Zhang, L. Lin, M. Cao, and N. G. Swenson. 2020. Alternative designs and tropical tree seedling growth performance landscapes. Ecology 101: 6. https://doi.org/10.1002/ecy.3007

Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. C. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P. K. Groom, J. Gulias, K. Hikosaka, B. B. Lamont, T. Lee, W. Lee, C. Lusk, … and R. Villar. 2004. The worldwide leaf economics spectrum. Nature 428: 6985, 821-827.

Xavier, R. O., A. V. Christianini, G. Pegler, M. B. Leite, and D. M. Silva-Matos. 2021. Distinctive seed dispersal and seed bank patterns of invasive African grasses favour their invasion in a neotropical savanna. Oecologia 196: 155-169.

Zhou, G., X. Zhou, Y. Nie, S. H. Bai, L. Zhou, J. Shao, W. Cheng, J. Wang, F. Hu, and Y. Fu. 2018. Drought-induced changes in root biomass largely result from altered root morphological traits: Evidence from a synthesis of global field trials. Plant Cell and Environment 41: 11, 2589–2599. https://doi.org/10.1111/pce.13356

Zhou, M., J. Wang, W. Bai, Y. Zhang, and W. H. Zhang. 2019. The response of root traits to precipitation change of herbaceous species in temperate steppes. Functional Ecology 33: 10, 2030–2041. https://doi.org/10.11111365-2435.13420

Ziffer-berger, J., P. J. Weisberg, M. E. Cablk, and Y. Osem. 2014. Spatial patterns provide support for the stress-gradient hypothesis over a range-wide aridity gradient. Journal of Arid Environments 102:27–33.

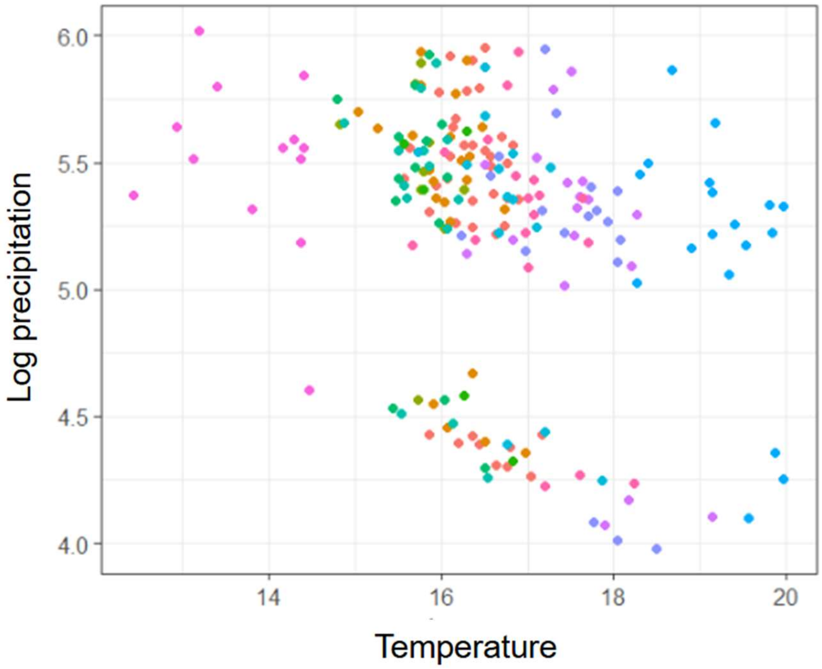
Zirbel, C. R., and L. A. Brudvig. 2020. Trait–environment interactions affect plant establishment success during restoration. Ecology 101: 3. https://doi.org/10.1002/ecy.2971

Zolfaghari, R., F. Dalvand, P. Fayyaz, and A. Solla. 2022. Maternal drought stress on Persian oak (Quercus brantii Lindl.) affects susceptibility to single and combined drought and biotic stress in offspring. Environmental and Experimental Botany 194. https://doi.org/10.1016/j.envexpbot.2021.104716

Supplement

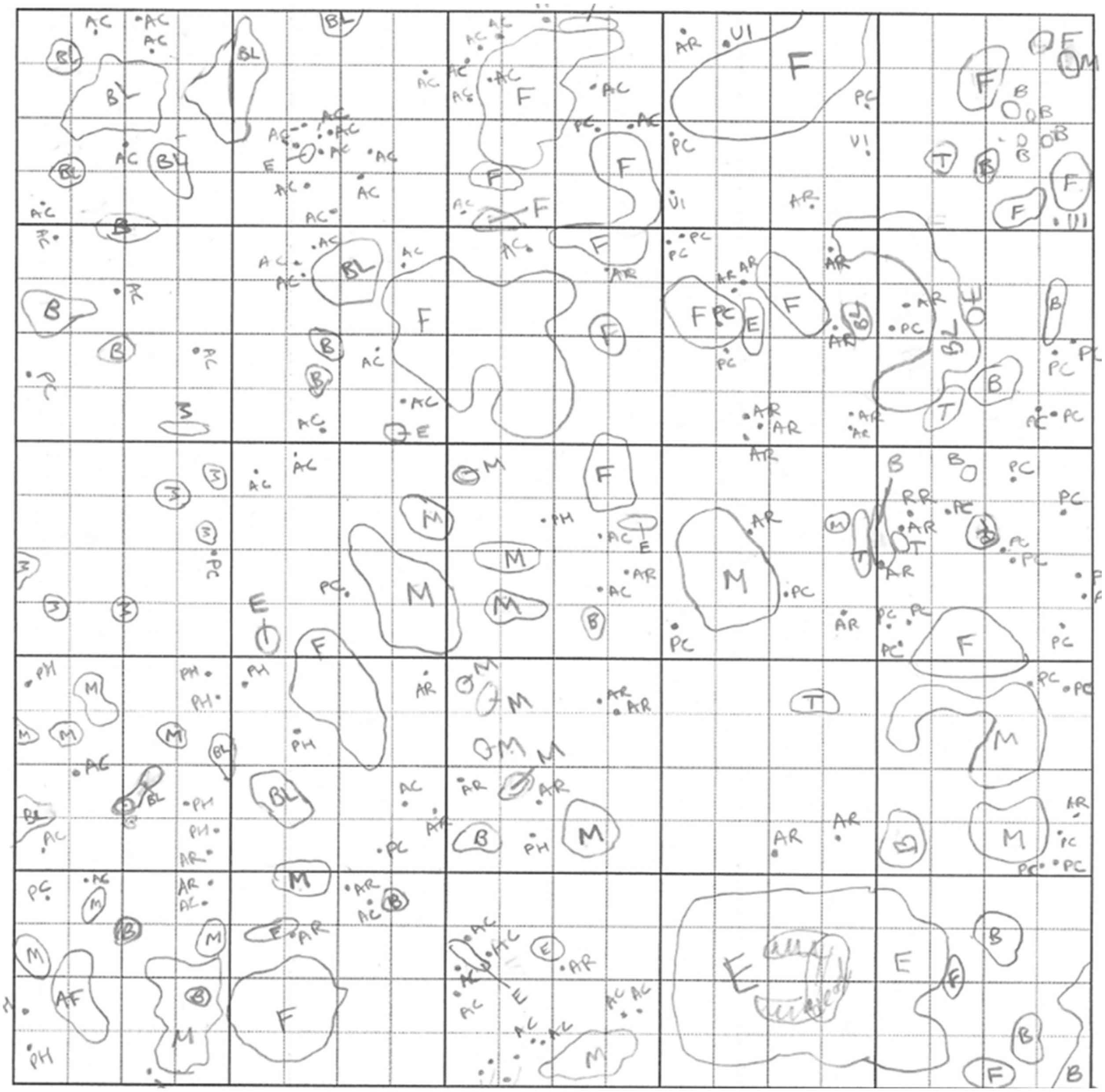
CHAPTER 1

Figure S1.1: Temperature and precipitation across the dataset



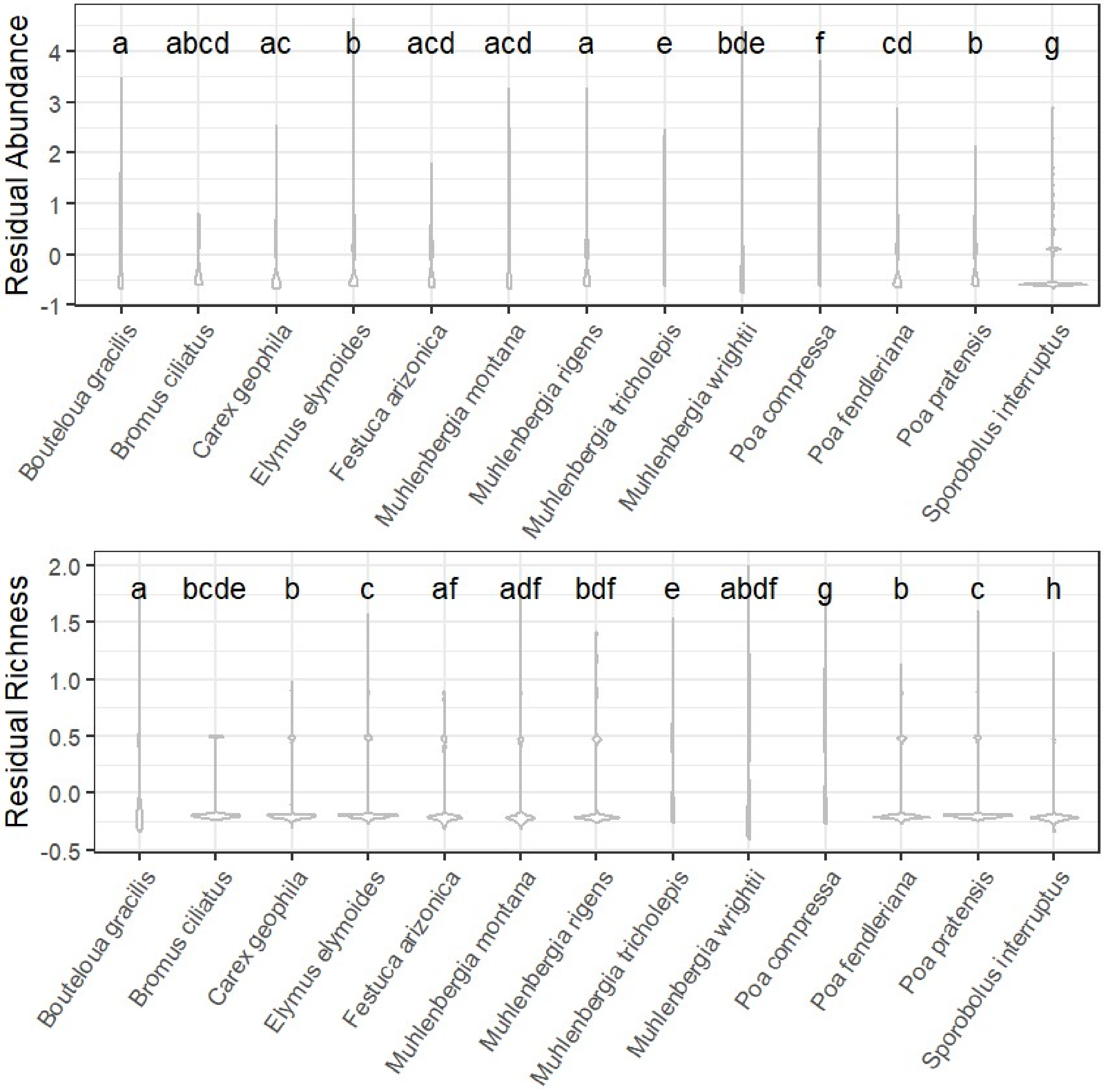
Legend: Each point represents the annual log precipitation and temperature for a year and site. Points of the same color indicate different years of data from the same site.

Figure S1.2: Chart-quadrat map



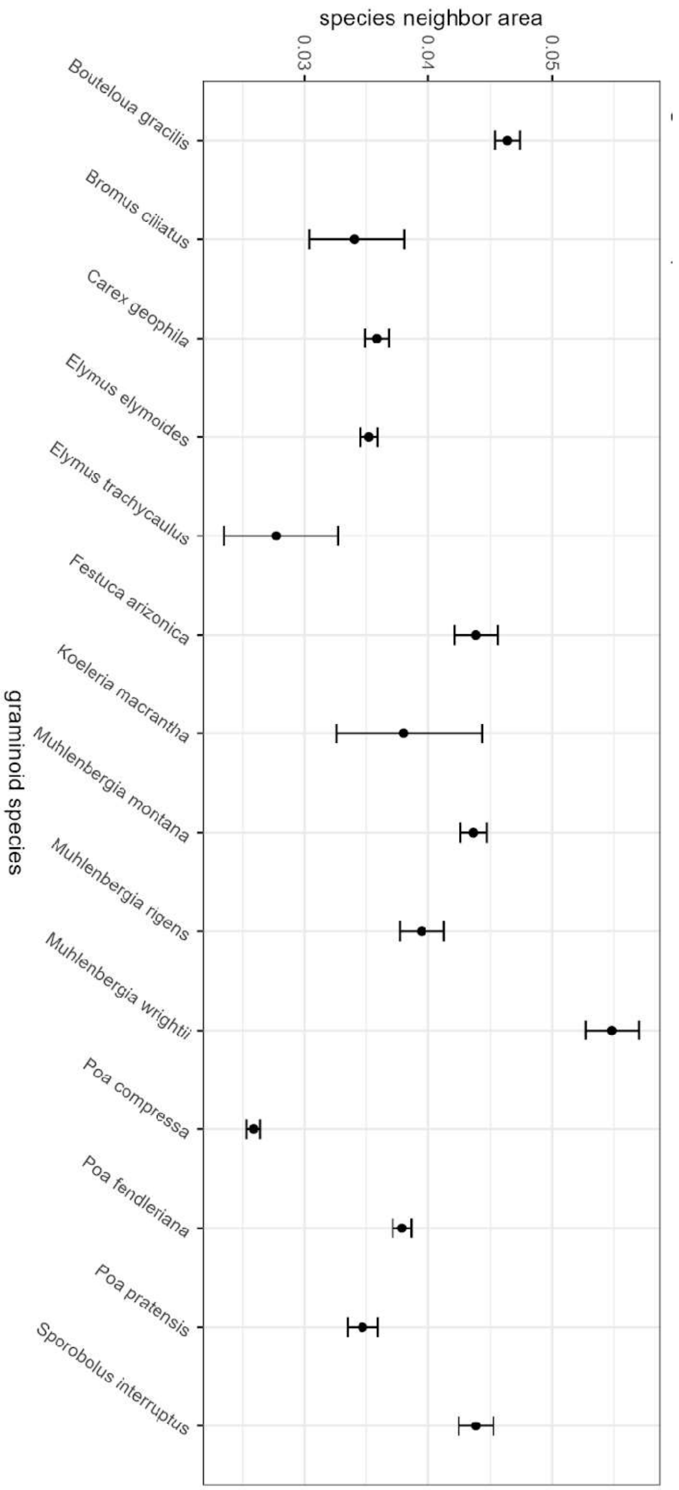
Legend: This is a sample chart-quadrat map. Chart-quadrat mapping is used to locate every forb and graminoid within the 1 square meter plot each year, allowing individuals to be tracked over time and collects small-scale spatial data for species observed. Graminoid basal area is drawn as polygons while forbs and bushes are indicated by points. Each species has a species code that is specific to that data sheet (not included in image), e.g., F here is for *Festuca arizonica*. Maps are traced in the field, then hand drawn to reduce the total size, and digitized using ArcGIS.

Figure S1.3: Distributions of neighborhood abundance and richness for the graminoid species



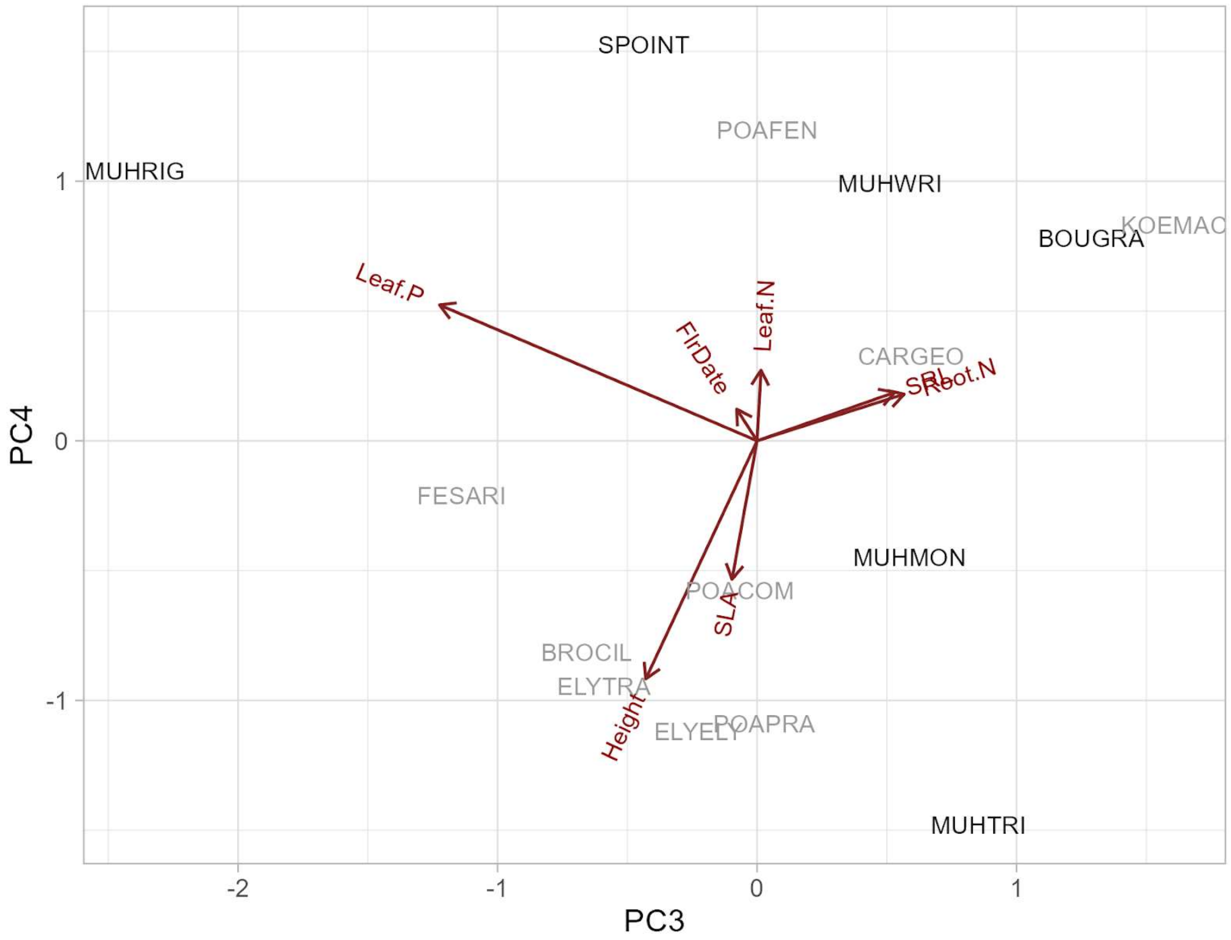
Legend: Violin plots showing the distribution of the residual richness or abundance of the neighborhoods for each graminoid species, i.e., vertical component indicates range of values and width indicates the relative abundance of data points with that value. Residual richness is the variation observed that is not accounted for by the species area model (Table S1.1). Species are ordered alphabetically. Different letters denote a significant difference in a post-hoc pairwise comparisons between species abundance or richness distributions.

Figure S1.4: Neighborhood sizes by graminoid species



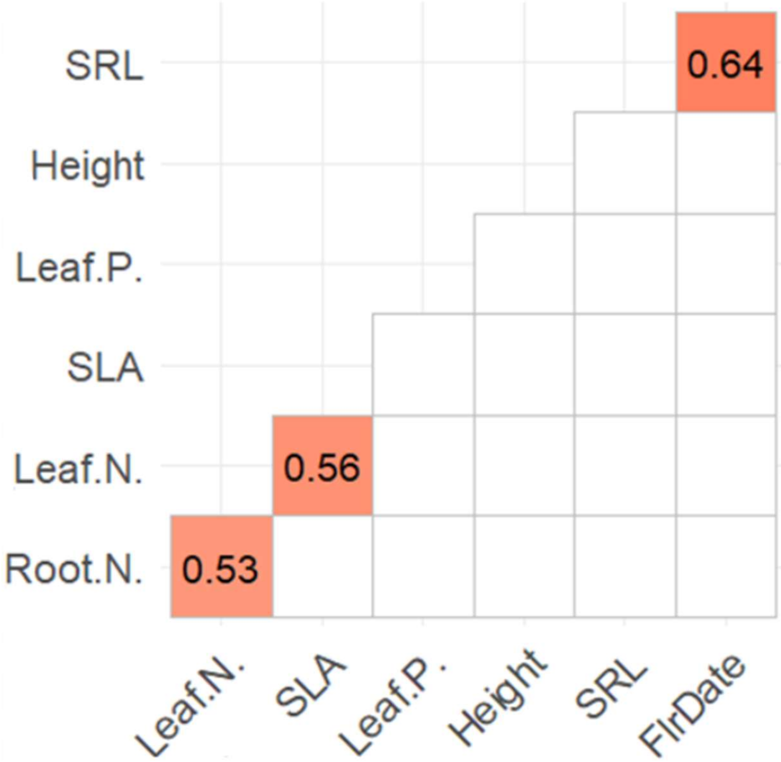
Legend: This figure shows the estimated marginal mean and SE of the neighborhood size associated with each graminoid species. Values are from the binomial model of neighbor presence absence (Table S1.2, Figure 1.1).

Figure S1.5: Principal component axes 3 and 4 of graminoid traits



Legend: Third and fourth Principal Component (PC) axes for the dominant graminoid species included in the study (Table 1.1). Arrows indicate direction (positive or negative) and strength (length of arrow) of trait loadings for each axis (Table S1.5). Traits included in the analysis are: Specific root length (SRL), Root nitrogen content (Root N), Specific leaf area (SLA), Leaf phosphorus content (Leaf P), Leaf nitrogen concentration (Leaf N), Height, and Flowering date (FlrDate). Species abbreviations (Table 1.1) are positioned to indicate trait loadings along the PC axes for each species. Species abbreviations are colored according to photoperiod, as either C3 (gray) or C4 (black).

Figure S1.6: Graminoid trait correlation matrix



Legend: Pairwise correlations for adult graminoid traits included in the principal component analysis with trait data from Laughlin et al. 2010. White squares are not significant correlations; number indicates the strength of the correlation, while color indicates if it is a positive (red) or negative (blue) relationship. Trait abbreviations: specific root length (SRL), leaf Phosphorus and Nitrogen (leaf P, leaf N), root Nitrogen (root N), specific leaf area (SLA), peak flowering (FlrDate).

Table S1.1

	Estimate	Std. Error	t-value	p-value
Intercept	0.491	0.051	9.541	<0.001
Log (neighborhood area)	0.086	0.016	5.500	<0.001

Legend: Power model of a species - area relationship calculated from all graminoids, where the response is the log(species richness) for each individual graminoid polygon, and the predictive variable was the log(basal area of the individual graminoid polygon).

Table S1.2

	Estimate	Standard error	Log-likelihood (full model = -9797.1)	Chi-square (comparison with full model)	p-value
Graminoid species	-	-	-9987.1	379.95	<0.001
log(temperature)	-1.602	0.609	-9801.2	8.30	0.004
log(precipitation)	0.044	0.045	-9797.6	0.96	0.327
log(graminoid size)	0.168	0.015	-9864.4	134.51	<0.001
Random effect:	Variance	SD			
Site	0.178	0.422			

Legend: Model parameters values where the response variable was the presence or absence of any neighboring forbs, the fixed effects are species identity, log(temperature), log(precipitation), log(individual graminoid basal area), and a random effect for Site. Temperature and precipitation data is the annual precipitation for the site and year associated with the individual graminoid. Quadrat was not included as a random effect because the model was not able to converge when this factor was included. Precipitation had no significant impact on the likelihood that an individual graminoid would have any neighboring forbs, while temperature and larger area increased the likelihood.

Table S1.3

Graminoid species by	Kruskal-Wallis chi-squared	Degrees of freedom	p-value
Residual richness	896.46	12	<0.001
Residual abundance	713.53	12	<0.001

Legend: Kruskal-Wallis rank sum test to determine if there are differences in richness and abundance of all forb seedlings in the neighborhoods of graminoids that are different species. Analysis is run with 13 species. A p-value less than 0.05 indicates a significant difference between at least one species pair. Residual richness or abundance is the variation observed that is not accounted for by the species area model (Table S1.1).

Table S1.4

	Estimate	Std. Error	log-likelihood (full model = -14388)	Chi-square (comparison with full model)	p-value
Intercept	5.067	1.554	NA	NA	
Type of forbs: spring or summer	0.562	0.046	NA	NA	
Log (graminoid flowering date)	-0.438	0.134	NA	NA	
log(temperature)	-2.09	0.518	-14399	20.63	<0.001
log(precipitation)	0.089	0.040	-14391	5.23	0.022
log(graminoid size)	0.187	0.012	-14516	256.48	<0.001
Interaction between type and graminoid flowering date	2.034	0.173	-14458	139.27	<0.001
Random effect:	Variance	SD			
Site	0.089	0.296			

Legend: Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) to determine if spring and summer forbs have different probabilities of occurring next to graminoid species. Model response variable is the presence of neighbors (0/1), with fixed parameters for the type of seedling (spring or summer), log(graminoid flowering data), log(temperature) and log(precipitation), the graminoid basal area, and the interaction between graminoid flowering date and seedling type. A random effect of Site is included. Significant p values ($p<0.05$) indicate that the model including this parameter was significantly better than the model without this parameter in a nested log-likelihood comparison.

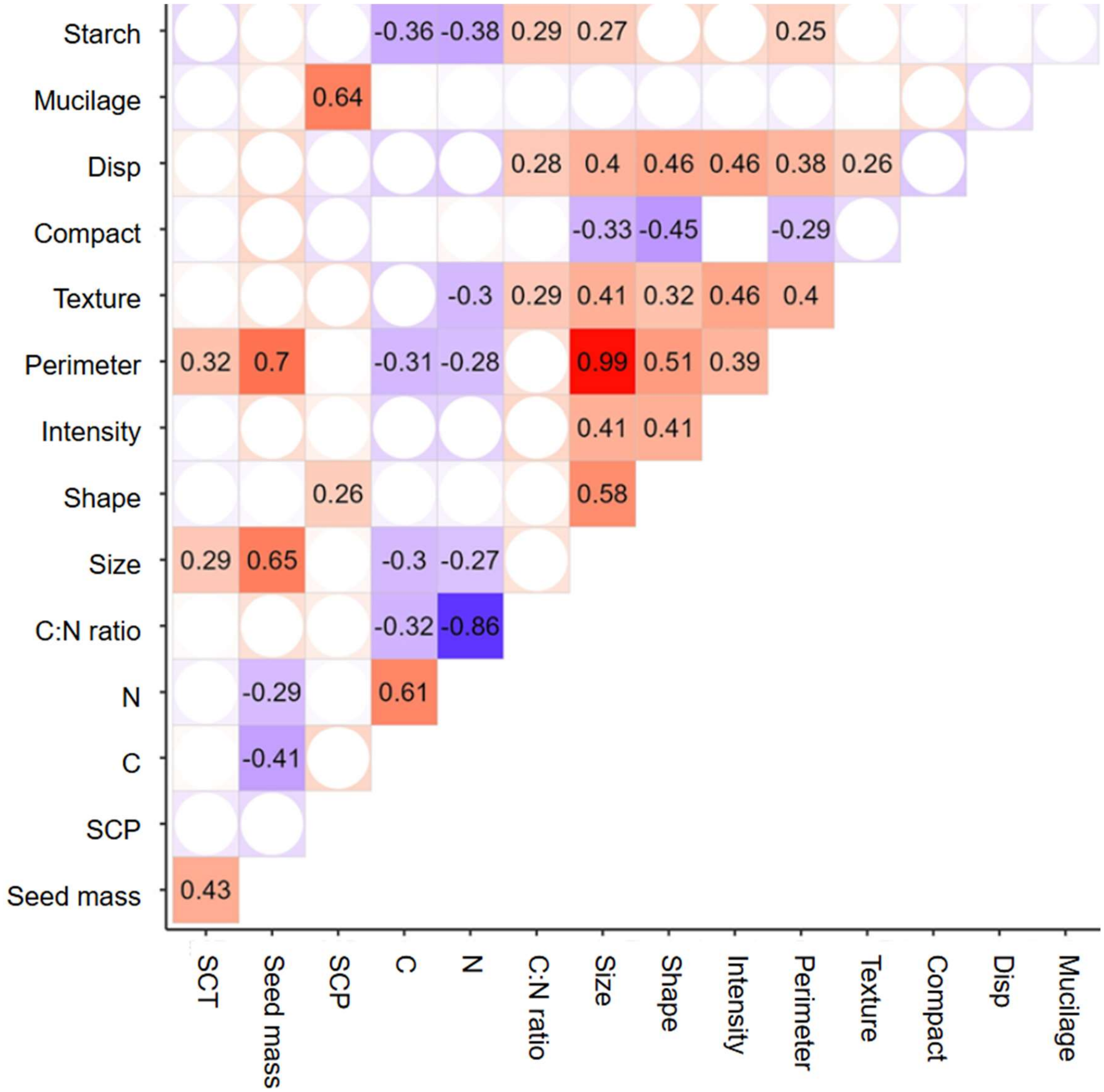
Table S1.5

Trait	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Height	-0.351	-0.270	-0.283	-0.735	-0.382	-0.140	0.131
Leaf N.	-0.393	0.516	0.010	0.218	-0.313	0.251	0.608
Leaf P.	-0.239	0.006	-0.807	0.420	0.070	-0.304	-0.136
Root N.	-0.485	0.178	0.374	0.144	-0.37	-0.214	-0.624
SLA	-0.242	0.572	-0.064	-0.428	0.633	-0.002	-0.160
SRL	-0.416	-0.366	0.350	0.150	0.405	-0.482	0.389
FlrDate	-0.444	-0.409	-0.052	0.098	0.221	0.740	-0.165

Legend: Principal component analysis trait loading values. Values indicate the strength (greater absolute value) and direction (positive or negative) that a single trait aligns with a PC axis. The first three principal component axes comprise 76.3% of the variation observed within graminoid species in this study. The first axis differentiates tall species with high SRL, late flowering and high root N (low PC1 - 35.3% of variation); while the second axis relates to leaf speed and photosynthetic pathways (high PC2 = high SLA, high leaf N ; 24.2% of variation); and the third axes separate graminoids based on leaf P content (low PC3 = high P; 16.8% of variation). Trait abbreviations: specific root length (SRL), leaf Phosphorus and Nitrogen (leaf P, leaf N), root Nitrogen (root N), specific leaf area (SLA), peak flowering (FlrDate).

CHAPTER 2

Figure S2.1: Trait correlations



Legend: Pairwise correlations for all traits measured. White squares are not significant correlations; number indicates the strength of the correlation, while color indicates if it is a positive (red) or negative (blue) relationship. See Table 2.1 and Table S2.1 for abbreviations.

Table S2.1

Trait	Trait category	Units and formulas	Methods
Seed coat reflectance (intensity)	Morphological	VideometerLab multispectral acquisition system (Videometer A/S, Denmark)	VideometerLab Manual
Dispersal morphological categorization	Morphological	Categories include no appendages- coarse surface, no appendages – smooth, balloon, flat, and elongated appendages (further divided into many or few and long or short) Short appendages must be 1/10 the length of the seed to be considered and less than or equal to the length of the seed; long appendages are > length of the seed	Categorical (Kleyer et al. 2008; LEDA trait database methodology)
Seed mucilage presence (mucilage)	Surface barrier	(Presence/Absence) : Removed as highly correlated with SCP	Yang et al. 2021
Seed total nitrogen per gram (N)	Chemical	Total nitrogen per gram of pulverized seed	UC Davis Stable Isotope Laboratory
Perimeter (P)	Morphological	Total length of the perimeter	VideometerLab Manual

Legend: Seed traits included in correlation assessment (Figure S2.1) but not included in principal component analysis. Seed traits measured with trait type, definition of measurement, and methods citation. VideometerLab multispectral acquisition system software (Videometer A/S, Denmark) was used to measure seed coat intensity, perimeter, seed area, rectangular fit, and variance from radial symmetry.

Table S2.2

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11
SCT	-0.13	0.29	0.57	-0.08	-0.05	0.53	0.00	-0.13	0.52	0.05	-0.02
mass	-0.32	0.51	0.24	-0.11	0.17	-0.2	0.13	0.05	-0.38	-0.13	0.57
SCP	-0.01	-0.33	0.09	-0.74	0.23	-0.07	0.35	0.21	0.15	-0.28	-0.04
carbon	0.3	-0.32	0.36	-0.06	-0.04	0.10	0.04	-0.69	-0.4	-0.14	0.09
CN	-0.27	0.08	-0.36	-0.34	-0.48	0.43	0.27	-0.10	-0.31	0.30	-0.04
length	-0.5	0.03	0.23	0.04	0.3	-0.1	-0.02	-0.06	-0.32	0.08	-0.69
starch	-0.23	0.07	-0.52	0.11	0.47	0.28	0.06	-0.42	0.19	-0.37	0.07
shape	-0.37	-0.44	0.05	0.11	0.16	-0.2	0.18	-0.19	0.24	0.59	0.34
dispersal structure	-0.36	-0.11	0.07	0.27	-0.56	-0.3	0.31	-0.10	0.20	-0.49	-0.06
texture	-0.33	-0.14	-0.05	-0.36	-0.19	-0.10	-0.81	-0.13	0.07	-0.11	0.12
compact	0.21	0.46	-0.13	-0.30	-0.07	-0.51	0.09	-0.46	0.26	0.22	-0.20

Legend: Principal component analysis trait loadings. Values indicate the strength (greater absolute value) and direction (positive or negative) that a single trait aligns with a PC axis. See Table 2.1 for trait abbreviations.

Table S2.3

Species origin	Functional type	Life history (annual or perennial)	Number of species	PC1	PC2	PC3
non-native	forb	annual	6	0.69 ^{c}	-0.37 ^{ab}	-0.5
non-native	grass	annual	5	-3.32 ^{a}	0.27 ^{ab}	-1.27
non-native	N-fixing forbs	annual	3	0.08 ^{bc}	2.01 ^{a}	-1.25
native	forb	annual	33	0.47 ^{a}	-0.43 ^{b}	1.112
native	forb	perennial	12	0.41 ^{c}	0.55 ^{b}	0.25
native	grass	annual	1			
native	grass	perennial	2	-2.37 ^{ab}	0.39 ^{ab}	-1.13
native	N-fixing forbs	annual	5	-0.06 ^{bc}	0.86 ^{ab}	0.0

Legend: Mean PC values by life form group. There are no significant differences among any groups for PC3 value. Letters shared by groups indicate that those group means are not significantly different from each other in a pairwise Tukey comparisons across groups.

Table S2.4

Species code	Species name	Species origin	Functional type	PC1	PC2	PC3	PC4
ALLFAL	*Allium falcifolium*	native	Forb	0.96	1.92	0.53	0.36
ANAARV	*Anagallis arvensis*	non-native	Forb	1.94	1.05	-0.74	-0.48
ANCFIL	*Ancistrocarpus filagineus*	native	Forb	1.09	-0.83	0.76	0.3
ATHPUS	*Athysanus pusillus*	native	Forb	-0.37	-0.68	-0.27	0.2
AVEFAT	*Avena fatua*	non-native	Grass	-3.32	2.07	0.05	0.37
BROELE	*Brodiaea elegans*	native	Forb	0.93	2.44	-0.92	0.04
BROHOR	*Bromus hordeaceus*	non-native	Grass	-3.96	-1.01	-2.03	0.21
BROMAD	*Bromus madritensis*	non-native	Grass	-3	-0.59	-1.05	0.25
CALCIL	*Calandrinia ciliata*	native	Forb	1.78	0.89	-1.05	1.4
CALPAU	*Calycadenia pauciflora*	native	Forb	0.08	0.76	0.96	0.2
CASATT	*Castillea attenuata*	native	Forb	2.3	-1.32	0.26	0.17
CENTRI	*Centaurium trichanthum*	native	Forb	4.05	-0.66	-0.8	1.08
CHAGLA	*Chaenactis glabriuscula*	native	Forb	-1.23	-1.92	0.3	0.86
CHLPOM	*Chlorogalum pomeridianum*	native	Forb	-0.13	1.66	1.52	-0.13
CLAGRA	*Clarkia gracilis*	native	Forb	1.51	-1.06	0.85	-0.43
CLAPUR	*Clarkia purpurea*	native	Forb	2.11	-0.2	0.73	0.61
COLSPA	*Collinsia sparsiflora*	native	Forb	-0.58	0.4	-0.73	-1.39
CRYHIS	*Cryptantha hispidula*	native	Forb	1.11	-0.53	1.33	0.58
DICCAP	*Dichelostemma capitatum*	native	Forb	0.26	0.19	-0.52	0.84
ELYELY	*Elymus elymoides*	native	Grass	-3	0.16	-0.95	-0.3
EPIBRA	*Epilobium brachycarpum*	native	Forb	0.35	-1.21	0.74	-0.39
ERINUD	*Eriogonum nudum*	native	Forb	-0.88	0.19	-1.3	-0.13
ERILAN	*Eriophyllum lanatum*	native	Forb	-0.82	-2.67	2.07	-0.8
EUPCRE	*Euphorbia crenulata*	native	Forb	1.41	0.71	0.32	-0.88
FILCAL	*Filago californica*	native	Forb	2.53	-2	-0.97	0.9
FRIPUR	*Fritillaria purdyi*	native	Forb	-0.32	0.23	0.47	-0.18
GALAPA	*Galium aparine*	native	Forb	1.66	-0.48	-1.07	-0.75
GALMUR	*Galium murale*	non-native	Forb	1.34	-0.76	-1.03	-0.73
GILCAP	*Gilia capitata*	native	Forb	0.42	-0.07	0.52	-2.36
GILTRI	*Gilia tricolor*	native	Forb	1.27	-0.98	-1.09	-1.99
HOLVIR	*Holocarpa virgata*	native	Forb	0.29	0.37	2.67	-0.19
HYPGLA	*Hypochaeris glabra*	non-native	Forb	-1.11	-1.01	1.2	0.95
LACSER	*Lactuca serriola*	non-native	Forb	1.25	-1.36	0.58	0
LAGMIN	*Lagophylla minor*	native	Forb	1.1	-1.36	0.45	0.41

LASCAL	*Lasthenia californica*	native	Forb	-0.02	-2.65	0.34	0.98
LEPNIT	*Lepidium nitidum*	native	Forb	0.45	-0.73	0.76	-2.66
LINDIC	*Linanthus dichotomus*	native	Forb	1.72	-0.95	-0.13	-0.05
LOLMUL	*Lolium multiflorum*	non-native	Grass	-2.53	0.65	-1.63	-1.02
LOMMAR	*Lomatium marginatum*	native	Forb	-1.06	1.34	1.54	-1.24
LOTHUM	*Lotus humistratus*	native	N-fixer	0.39	1.83	0.1	0.67
MEDPOL	*Medicago polymorpha*	non-native	N-fixer	0.42	0.26	1.19	1.69
MICCAL	*Micropus californicus*	native	Forb	-1.54	0.02	0.44	-0.91
MICDOU	*Microseris douglasii*	native	Forb	-2.12	-0.58	1.99	-0.14
MIMDOU	*Mimulus douglasii*	native	Forb	-0.04	0.36	-1.29	-0.57
MIMGUT	*Mimulus guttatus*	native	Forb	3.87	-0.87	-0.45	0.99
NASPUL	*Nassella pulchra*	native	Grass	-1.7	0.81	-1.29	0.7
NAVJEP	*Navarretia jepsonii*	native	Forb	-0.3	-0.48	0.61	-0.3
PETPRO	*Petrorhagia prolifera*	non-native	Forb	-0.57	0.42	-3.5	0.89
PHLGRA	*Phlox gracilis*	native	Forb	-0.18	-0.24	-1.06	-1.6
PLAERE	*Plantago erecta*	native	Forb	0.44	0.22	1.12	-2.47
RIGLEP	*Rigiopappus leptocladus*	native	Forb	-1.39	-1.48	0.74	0.92
SIDDIP	*Sidalcea diploscypha*	native	Forb	0.53	0.96	-0.18	-1.8
SILGAL	*Silene gallica*	native	Forb	2.06	0.84	-1.78	1
SISBEL	*Sisyrinchium bellum*	native	Forb	0.88	2.16	0.83	-0.83
TAECAP	*Taeniatherum caput-medusae*	non-native	Grass	-3.8	0.75	-1.71	-0.11
THYCUR	*Thysanocarpus curvipes*	native	Forb	-1.25	0.12	-1.62	-1.35
TRIALB	*Trifolium albopurpureum*	native	N-fixer	0.2	0.6	0.8	1.55
TRIFUC	*Trifolium fucatum*	native	N-fixer	-0.78	1.67	-0.26	1.01
TRIHIR	*Trifolium hirtum*	non-native	N-fixer	0.1	2.33	0.78	1.64
TRIWIL	*Trifolium willdenovii*	native	N-fixer	0.34	0.67	-0.19	0.58
TRIGRA	*Trifolium gracilentum*	native	N-fixer	-0.24	0.82	-0.15	1.21
UROLIN	*Uropappus lindleyi*	native	Forb	-4.27	-2.12	2.29	0.6
VICVIL	*Vicia villosa*	non-native	N-fixer	-0.13	4.39	1.67	0.72
VIODOU	*Viola douglasii*	native	Forb	-0.02	0.73	1.59	-0.55
VULMIC	*Vulpia microstachys*	native	Grass	-3.03	-2.49	-0.39	2.05
ZIGFRE	*Zigadenus fremontii*	native	Forb	1.27	-1.44	-2.13	-1.17

Legend: Summary table of species with the PC values 1-4 for each species.

CHAPTER 3

S3.0 Supplement information on imputed data:

Missing datapoints: For the imputation of dry leaf mass and dry cotyledon mass for 5 individuals (leaf samples were lost to drying oven malfunction), we used the wet mass from all individuals at the site to create a response of dry mass. For cotyledons, we included both full wet leaf mass and cotyledon wet mass as predictive variables (r2=0.53); while for full dry leaf mass, we used wet leaf mass as the only predictive variable (r2=0.93). Model selection was done using AIC. Using the resulting parameters, we calculated values for the 5 missing individuals. Further, we used these values to calculate other traits (SLA, RMR, total biomass). All plants were from the low elevation site.

Cotyledon model

	estimate	standard error	t value	p-value
Intercept	0.0005	0.0001	3.170	0.004
Wet cotyledon mass	0.0971	0.0341	2.846	0.008
Wet leaf mass	0.0203	0.0112	1.819	0.080

Leaf model

	estimate	standard error	t value	p-value
Intercept	0.0006	0.0001	5.36	<0.0001
Wet leaf mass	0.1432	0.0074	19.34	<0.0001

Seed production: Linear predictive model for number of seeds produced based on site, maternal treatment, and length of inflorescences produced.

In order to estimate the seed production from all flowering individuals, we used a linear model including population, maternal treatment, their interaction, and the inflorescence length. The response variable was the number of seeds (although the model done with total seed mass per inflorescence was very similar - not shown). We used the estimates from this model and the true inflorescence length of every plant (sum of all inflorescences produced) to impute a number of seeds for each flowering plant. The number of expected seeds was rounded to the nearest full integer in order to use this dataset in a negative binomial model. For the model, the residual standard error was 5.01, with 25 degrees of freedom, and an adjusted R-squared of 0.61.

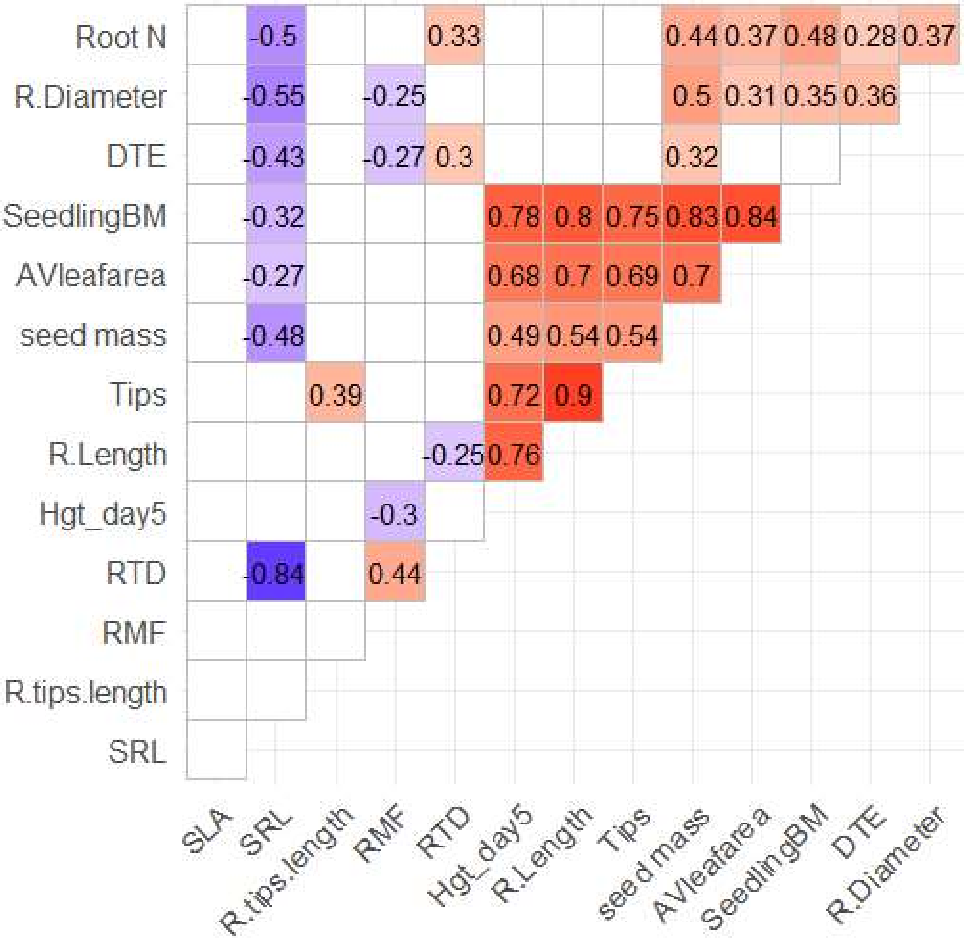
Analysis of Variance Table

	Degrees of freedom	Sum Sq	Mean Sq	F value	p-value
population (elevation)	2	798.02	399.01	15.896	<0.0001
maternal environment treatment	2	41.74	20.87	0.831	0.447
inflorescence length	1	556.43	556.43	22.168	<0.0001
population: maternal treatment	3	102.06	34.02	1.355	0.279
residual	25	627.52	25.10		

Seed production model summary:

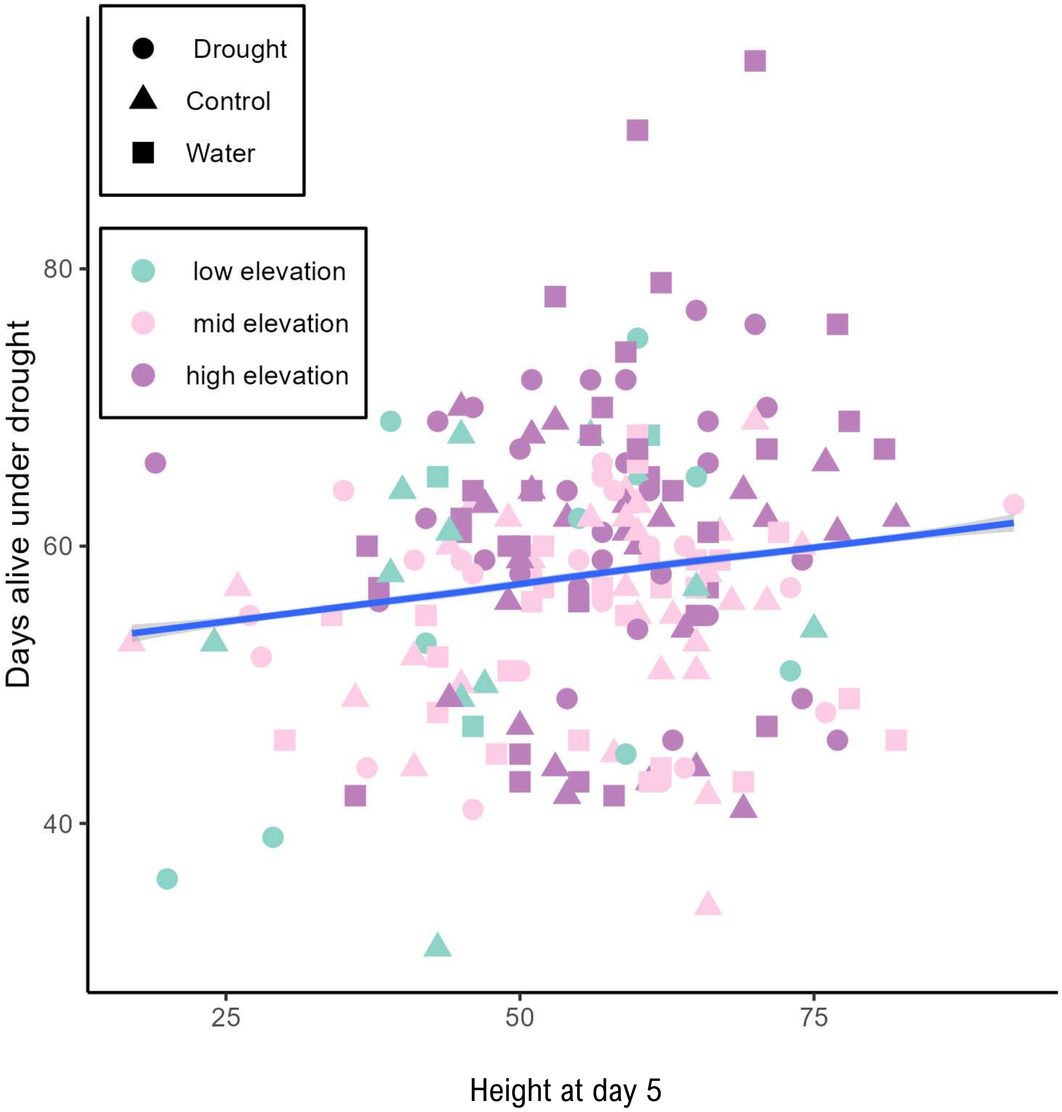
	estimate	standard error	t value	p-value
mid-elevation, control (Intercept)	3.519	4.270	0.824	0.418
low elevation	5.908	3.189	1.852	0.076
high elevation	5.656	3.668	1.542	0.136
drought maternal treatment	-1.890	3.189	-0.59	0.558
water maternal treatment	-3.830	3.167	-1.21	0.238
infl. length	2.154	0.519	4.148	0.000
low:drought	0.462	4.486	0.103	0.919
high:drought	NA	NA	NA	NA
low:water	2.604	4.968	0.524	0.605
high:water	11.040	5.543	1.991	0.060

Figure S3.1: Trait correlations for all traits



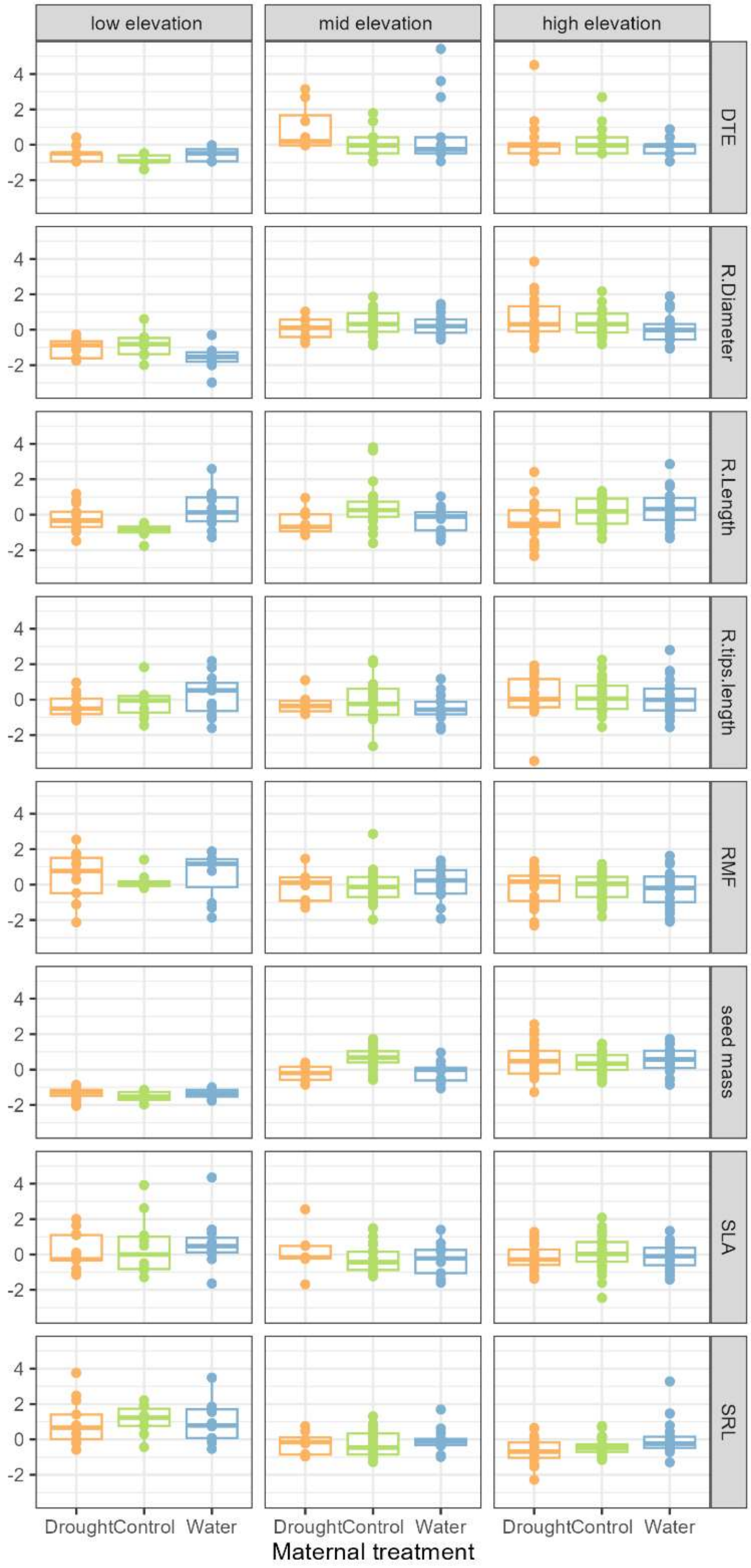
Legend: Pairwise correlations for all traits measured. White squares are not significant correlations; number indicates the strength of the correlation, while color indicates if it is a positive (red) or negative (blue) relationship. See Table 3.1 and Table S3.1 for abbreviations.

Figure S3.2: Individual seedling height and dry-down survival



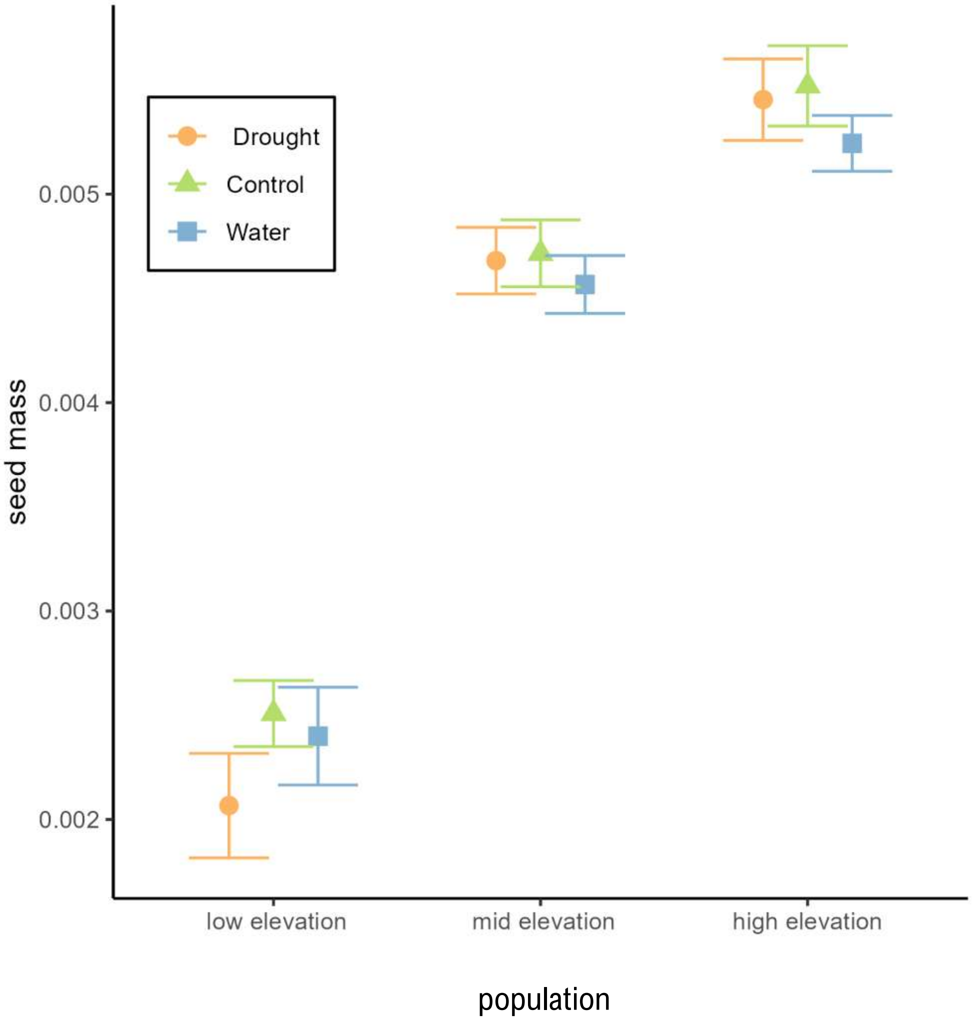
Legend: The relationship for dry-down performance plants where each dot represents an individual plant's height at day 5 and days that individual remained alive during the dry-down. The low elevation population is shown in teal, mid-elevation is pink, and the high elevation is purple. Shapes represent maternal environment treatments, where drought plants are circles, control plants are triangles, and well-watered plants are squares. The line represents a significant slope change with trait values (see Table S3.6).

Figure S3.3: Seedling trait distributions



Legend: Boxplots show maternal environment treatment and population means and distribution of seedling trait data collected at 10 days. PC values were used for analyses. Drought maternal environment treatments are shown in orange circles, control in green triangles, and water in blue squares. See Table 3.1 and Table S3.1 for abbreviations.

Figure S3.4: Seed mass of field collected plants



Legend: Points are the initial average seed mass for plants from each maternal environment treatment with standard error bars for all plants in the dry-down experimental performance group. Drought maternal environment treatments are shown in orange circles, control in green triangles, and water in blue squares.

Table S3.1

Trait	Units	Relevance of trait
Height at day 5 (Hgt_day5)	mm	Indication of early seedling growth rate as well as plant size.
Total dry seedling biomass (SeedlingBM)	g	Indication of total plant size.
Average leaf area (AVleafarea)	cm^2	Indication of leaf size.
Leaf nitrogen (leaf N)	%	Associated with photosynthetic ability of leaf and leaf durability.
Root tissue density (RTD)	g/cm^3	Relates to root conservation and durability; higher RTD is associated with higher durability.
Root dry mass (R.mass)	g	Indication of root system size.
Root tips (Tips)	count	Measure of root interaction with the soil; more root tips are associated with greater intake of water and nutrients from the soil.
Root nitrogen (Root N)	%	Relates to root conservation and durability; higher root N is associated with lower durability.

Legend: Traits analyzed for correlations but not included in the principal component collected from 10-day old seedlings from the trait group. Abbreviations are given in parentheses. Height was measured as the distance from soil to highest leaf tip. Due to limited tissue available from each seedling, leaf N and root N samples were pooled across multiple individuals from the same population and maternal treatment.

Table S3.2

	Survival			Reproductive performance (flowered/total)			Trait data collection		
	drought	control	water	drought	control	water	drought	control	water
Low elevation (BC)	9	12	4	9/9	11/13	3/6	13	12	11
Mid-elevation (AR)	26	31	27	6/24	10/20	12/27	8	23	14
High elevation (CC)	30	26	32	0/25	4/23	2/20	25	29	22

Legend: Final sample sizes for each population and maternal treatment for the 3 groups: seedling trait group, dry-down seedlings, and reproductive performance plants. Reproductive performance sample size for the probability of flowering includes all plants, while sample size for seed production is limited to only the plants that flowered. Sample sizes varied greatly due to different germination rates among populations and due to random chance via sampling design.

Table S3.3

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
DTE	0.366	0.136	0.392	0.127	-0.229	0.779	-0.122	0.063
Seed mass	0.438	-0.444	-0.285	-0.217	-0.158	0.034	0.452	0.503
R.Length	-0.149	-0.631	-0.205	-0.37	-0.065	0.301	-0.364	-0.417
R.Diameter	0.573	-0.045	0.126	-0.064	-0.015	-0.433	-0.667	0.13
RMF	-0.166	0.456	-0.621	-0.231	-0.073	0.242	-0.351	0.372
SLA	-0.218	0.108	0.228	-0.293	-0.865	-0.23	0.044	-0.008
SRL	-0.496	-0.327	0.39	0.055	0.14	0.037	-0.238	0.644
R.tips.length	-0.055	-0.241	-0.343	0.808	-0.381	-0.026	-0.147	0.004

Legend: Principal component analysis trait loadings. Values indicate the strength (greater absolute value) and direction (positive or negative) that a single trait aligns with a PC axis. See Table 3.1 for trait abbreviations.

Table S3.4

Method	Variable	Degrees of Freedom	F and p-value
ANOVA of PC1	**Site** **Maternal treatment** Interaction Residual	2 2 4 145	**107.1, < 0.001** **5.2, < 0.01** 0.91, 0.46
Tukey multiple comparison of means post-hoc comparison of ANOVA for PC1	Low – Mid site Low – High site Mid – High site Drought – Control Control – Water Drought – Water		<0.001 <0.001 0.97 0.37 0.12 0.004
ANOVA of PC2	**Site** Maternal treatment *Interaction* Residual	2 2 4 145	**7.2, < 0.001** 1.9, 0.16 *2.3, 0.059*
Tukey multiple comparison of means post-hoc comparison of ANOVA for PC2	Low – Mid site Low – High site Mid – High site Drought – Control Control – Water Drought – Water *Low Drought – High Water *Low Control – High Water *Low Drought – Mid Control *Low Control – Mid Control		0.03 < 0.001 0.60 0.18 0.98 0.28 0.04 0.02 *0.056* 0.03
ANOVA of PC3	*Site* Maternal treatment Interaction Residual	2 2 4 145	3.0, *0.054* 0.24, 0.79 1.5, 0.20
Tukey multiple comparison of means post-hoc comparison of ANOVA for PC3	Low – Mid site *Low – High site* Mid – High site		0.71 *0.06* 0.26
ANOVA of PC4	Site Maternal environment Interaction Residual	2 2 4 145	0.95, 0.39 0.27, 0.76 0.78, 0.54

Legend: Differences in seedling trait PC axes between sites and maternal environment treatments compared using ANOVA statistical comparisons and Tukey post-hoc tests. Significant comparison groups (p-value < 0.05) are bolded and marginally significant (p<0.10) are italicized. Pairwise comparisons are calculated using a Tukey multiple comparisons of means test with a 95% family-wise confidence level. *See table below for full pairwise interaction comparisons.

Pairwise comparison groups		PC1 p-value	PC2 p-value
high:water	high:control	0.871	0.973
high:water	high:drought	0.039	0.68
high:drought	high:control	0.574	0.997
mid:water	mid:control	0.999	0.283
mid:water	mid:drought	1	1
mid:drought	mid:control	1	0.346
low:water	low:control	0.946	0.971
low:water	low:drought	0.762	0.997
low:drought	low:control	1	1
low:drought	mid:control	0	*0.056*
low:drought	mid:drought	0	1
low:drought	mid:water	0	0.999
low:control	mid:control	0	0.035
low:control	mid:drought	0	0.999
low:control	mid:water	0	0.984
low:water	mid:control	0	0.508
low:water	mid:drought	0	1
low:water	mid:water	0	1
high:drought	low:control	0	0.535
high:drought	low:drought	0	0.724
high:drought	low:water	0	0.998
high:control	low:control	0	0.168
high:control	low:drought	0	0.265
high:control	low:water	0	0.9
high:water	low:control	0	0.025
high:water	low:drought	0	0.04
high:water	low:water	0	0.427
high:drought	mid:water	0.604	0.985
high:drought	mid:control	0.862	0.774
high:drought	mid:drought	0.819	0.968
high:control	mid:drought	1	0.736
high:control	mid:water	1	0.737
high:control	mid:control	1	0.99
high:water	mid:control	0.716	1
high:water	mid:drought	0.998	0.285
high:water	mid:water	0.992	0.221

Legend: Pairwise significant differences of PC values for maternal environment treatments and populations from Table S3.4. P-values indicate that pairwise comparisons between groups are significant and that these groups are significantly (p<0.05) or marginally (p<0.10) different from each other. Table is shown in color where teal indicates low-elevation, pink indicates mid-elevation, and purple indicates high elevation maternal treatment groups.

Table S3.5

Model	**Method**	**Variable**	**D.F.**	**F and p-value**
Population, maternal treatment, and population by maternal treatment interaction	PERMANOVA Bray Curtis, 999 permutations	Maternal environment **Population** **Interaction** Residual	2 2 4 145	1.98, 0.069 14.71, **0.001** 2.09**, 0.015**
High elevation site Mid-elevation site Low elevation site	PERMANOVA Bray Curtis, 999 permutations, run separately for each site	**Maternal environment** Maternal environment *Maternal environment*	2,72 2,42 2,31	2.87, **0.015** 1.50, 0.16 1.91, *0.078*
Drought maternal treatment Control maternal treatment Water maternal treatment	PERMANOVA Bray Curtis, 999 permutations, run separately for each maternal treatment	**Population** **Population** **Population**	2,42 2,59 2,44	4.59, **< 0.001** 10.25, **< 0.001** 3.20, **< 0.01**

Legend: PERMANOVA comparison of seedling trait values across populations, maternal environment treatment, and their interaction. Seedling traits included in the model are the same as those included in the PCA analysis (see Table 3.1). Significant parameters (p-value < 0.05) are bolded, marginally significant (p<0.10) are italicized and indicate that at least one level of this parameter is different from another level.

Table S3.6

Performance metric models for individual plant trait values: Survival and Flowering

Response	Predictive variable	Intercept (standard error)	Estimate (std.error)	logLik	Chi sq	p-value	R2 marg.	R2 cond.
Survival	Intercept only	57.75 (1.30)	NA	- 727.17	NA	NA	0	0.10
	Height day 5	51.75 (3.18)	0.11 (0.05)	- 725.04	4.26	**0.04**	0.02	0.11
	Days to emergence	55.33 (3.08)	0.31 (0.35)	- 726.82	0.70	0.40	0.00	0.11
	Seed mass	60.69 (3.22)	- 668.44 (662.47)	- 726.79	0.76	0.38	0.01	0.13
Flowering	Intercept only	-0.54 (0.70)	NA	- 87.5	NA	NA	0	0.18
	Height day 5	-0.28 (1.23)	- 0.005 (0.02)	- 87.52	0.06	0.80	0.03	0.18
	Days to emergence	-1.43 (1.08)	0.12 (0.11)	- 86.92	1.25	0.26	0.00	0.19
	Seed mass	1.50 (1.15)	- 488.07 (252.91)	- 85.94	3.22	*0.07*	0.17	0.20

Legend: Dry-down survival time and the probability of flowering were modeled separately for each trait that was collected for every individual plant (height at day 5, days to emergence, seed mass). All models included the population by treatment as a random effect. The log-likelihood, chi-square statistic, and p-value were determined by comparing the model with the trait to an intercept only model that included the random effect of population by maternal treatment. The marginal r-squared indicates the variation explained by the trait value while the conditional R-squared value indicates the variation explained by the trait and the random effect. Dry-down survival (days alive without watering) was modeled using a linear model (lmer, package lme4) and R-squared values were calculated from the package MuMin. Flowering was modeled as a binomial model with a logit link (glmmTMB package); R-squared values for the probability of flowering were calculated using McFaddens r-squared. Significant parameters (p-value < 0.05) are bolded and marginally significant (p<0.10) are italicized.

Performance metric models for individual plant trait values: Seed Production

	Predictive variable	Intercept (standard error)	Estimate (standard error)	LR statistic	p-value (Chi-square)	DF	R squared value	Residuals meet assump-tions
Low elevation population	Intercept only	4.80 (0.10)						
	Height day 5	4.79 (0.44)	0.00 (0.01)	0	0.99	21	0	yes
	Days to emergence	4.86 (0.42)	- 0.01 (0.06)	0.02	0.88	21	0	yes
	Seed mass	4.90 (0.44)	- 47.06 (198.66)	0.05	0.83	21	0	no
Mid-elevation population	Intercept only	3.5 (0.09)				28		
	Height day 5	3.17 (0.51)	0.01 (0.01)	10.47	**0.005**	26	0.03	yes
	Days to emergence	3.07 (0.42)	0.06 (0.05)	11.58	**0.003**	26	0.06	no
	Seed mass	2.41 (0.61)	239.62 (127.09)	3.10	*0.078*	27	0.11	yes

Legend: LR= likelihood ratio. DF = degrees of freedom. Due to the low sample size (see Table S3.2) and high variance between means and standard deviations across populations and maternal environment treatments (Figure 3.3c), we modeled seed production using a negative binomial linear model separately for the low and mid-elevation populations (MASS package, function glm.nb); the high elevation population did not have enough data points to model (n=6). As seed production was modeled separately for each population, there is no random effect in these models. The comparison model here includes only the intercept, e.g., the population mean. Not all models were able to satisfy assumptions for the distribution of residuals. R-squared values are delta values from the MuMIn package. Significant parameters (p-value < 0.05) are bolded and marginally significant (p<0.10) are italicized.

Table S3.7

Comparison	Statistical Test	group 1	group 2	n	p-value	adjusted p-value
Is flowering different between sites?	Chi-square	Chi-squared value = 48.97, df = 2, p-value = **0**				
	Pairwise fisher test	**AR**	**BC**	101	0	**0.0001**
		AR	**CC**	141	0	**0**
		BC	**CC**	96	0	**0**
Is flowering different between maternal treatments at low elevation (BC)?	Fisher's test					*0.062*
	Pairwise fisher test	Drought	Control	22	0.494	0.524
		Water	Control	19	0.262	0.524
		Water	Drought	15	0.044	0.132
Is flowering different between maternal treatments at mid-elevation (AR)?	Fisher's test					0.157
Is flowering different between maternal treatments at high elevation (CC)?	Fisher's test					*0.084*
	Pairwise fisher test	Drought	Control	48	0.045	0.136
		Water	Control	43	0.669	0.669
		Water	Drought	45	0.192	0.384
Is flowering different between each population by maternal family category?	Fisher's test			169		**0.0005**
	Pairwise fisher test	ARD	ARW	53	0.151	1
		ARC	ARD	45	0.117	1
		ARC	ARW	48	1	1
		ARC	BCC	33	0.067	0.941
		ARC	BCD	29	0.011	0.262
		ARC	BCW	26	1	1
		ARD	**BCC**	38	0.001	**0.015**
		ARD	**BCD**	34	0	**0.003**
		ARD	BCW	31	0.32	1
		ARW	BCC	41	0.039	0.774
		ARW	BCD	37	0.005	0.13
		ARW	BCW	34	1	1
		ARD	CCC	48	0.727	1
		ARD	CCD	50	0.022	0.468
		ARD	CCW	45	0.269	1
		ARC	CCC	43	0.048	0.792
		ARC	**CCD**	45	0	**0.002**
		ARC	CCW	40	0.014	0.304
		ARW	CCC	51	0.039	0.774
		ARW	**CCD**	53	0	**0.002**
		ARW	CCW	48	0.011	0.259
		BCC	BCD	22	0.494	1
		BCC	BCW	19	0.262	1
		BCD	BCW	15	0.044	0.792
		BCC	**CCC**	36	0	**0.006**
		BCC	**CCD**	38	0	**0**
		BCC	**CCW**	33	0	**0.001**
		BCD	**CCC**	32	0	**0.001**

Is flowering different between each population by maternal family category?	Pairwise fisher test	**BCD**	**CCD**	34	0	**0**
		BCD	**CCW**	29	0	**0**
		BCW	CCC	29	0.131	1
		BCW	CCD	31	0.004	0.116
		BCW	CCW	26	0.062	0.936
		CCC	CCD	48	0.046	0.792
		CCC	CCW	43	0.669	1
		CCD	CCW	45	0.192	1

Legend: Fisher's exact test for count data; comparing flowering between maternal environment treatments and populations. For each comparison the fisher, which allows unbalanced sample sizes, or chi-square test indicates if there is a difference among any of the groups, while a pairwise test compares each pair of subgroups. n indicates the sample size for the specific group comparison. Populations are abbreviated and indicated by color: AR = Arboretum, mid-elevation population (pink); BC= Blue Chute, low-elevation population (teal); CC= Camp Colton, high elevation population (purple). Population by maternal environment treatments are abbreviated as populations followed by C = control maternal environment, D = drought maternal environment, W= well-watered maternal environment, i.e., ARD = mid-elevation, drought maternal treatment. The p-value indicates the difference between the two groups, while the adjusted p-value accounts for error from calculating a high number of pairwise comparisons. Significant parameters (adjusted p-value < 0.05) are bolded and marginally significant (adjusted p-value <0.10) are italicized.