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Implications of Within-Species Root and Shoot Trait Variation and Plasticity for Species Coexistence

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Abstract

Understanding the outcomes of interactions from assessing shoot traits has practical applications and has elucidated major ecological patterns. Roots and shoots differ in their functions and can differ in responses to abiotic and biotic stimuli. And while roots are more difficult to characterize because they tangle and are embedded in soil, they are likely key drivers of interaction outcomes and influence survival. Generally, drawing conclusions only on one part of the plant masks crucial ecological information. In this dissertation, I use four studies to assess four factors that influence plant traits and interaction outcomes. The factors are 1) water availability, 2) population variation due to seed source, 3) plasticity, and 4) neighbor identity and traits. First, I use a meta-analysis on competition studies with varying water availability that partition roots or shoots to assess the contribution of plant parts to competition. I found that root-root interactions and shoot-shoot interactions had opposing responses to water availability: root-root interactions were intense under low water availability whereas shoot-shoot interactions were more intense under high water availability. The competitive relationships denote that root interactions in resource limited environments have a greater impact and should be considered in understanding the dynamics of interacting plants. Second, using a native perennial grass *Pseudoroegneria spicata*, I assessed the traits and variability in root and shoot traits of three wild and three cultivar accessions. Wild collections had higher survival, and six of eight measured traits showed greater variability in wild compared to cultivar collections. One key root trait, root tip count, was 51% greater on average in wild compared cultivar collections. While cultivars are important to meet seed need for restoration, cultivation practices should limit trait variation loss that could hinder survival and long-term persistence. Next, I assess how plasticity and neighbor identity affect interactions important for species coexistence. The Modern Coexistence Theory denotes that species coexistence is hinged upon stabilizing mechanisms, where intraspecific competition is more intense than interspecific competition, and equalizing mechanisms should function to keep traits important to competition equal between interacting species to avoid competitive exclusion. In the third study, I used a transplant study to show that induced plasticity affected subsequent intraspecific interactions important to stabilizing niche mechanisms, and thus coexistence. I induced plasticity through either intraspecific or interspecific interactions, then moved these induced plants to new intraspecific neighbors to assess if induced plasticity affected subsequent intraspecific interactions. Plasticity induced from interspecific competition lead to subsequent intraspecific interactions that were more negative than plants induced by intraspecific competition. Roots showed stronger responses than shoots to the outcomes of induced plasticity and the interaction outcomes show that interspecific interactions may have a role in population dynamics important to species coexistence. Lastly, I tested if population variation affected stabilizing niche mechanisms and found that populations varied in whether they competed more intensely with intraspecific compared to interspecific neighbors, and this was influenced by neighbor identity. This indicates that certain intraspecific and interspecific population combinations may promote or hinder stabilizing mechanisms and species coexistence. I also tested whether neighbors demonstrated equalizing mechanisms in which competition becomes more intense with increasing trait distance. The interaction outcomes show variation in whether interacting pairs showed equalizing mechanisms by population identity of both neighbors, whether the interactions were intraspecific or interspecific, and whether traits were root- or shoot-derived. Restoration practitioners should consider interactions of populations and species selected to be mixed in restorations which may influence long-term coexistence. In all, the outcomes of these four studies have implications for ecological theory in which the impact of

root traits, population trait variation, and plasticity have been understudied. The study outcomes also inform restoration practices in systems where belowground interactions strongly influence community dynamics.

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Dedication

I dedicate this dissertation to every Black woman who came before and after me, who built a world that was not built for her.

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CHAPTER ONE

Root and shoot competition lead to contrasting competitive outcomes under water stress: a systematic review and meta-analysis

Chapter one is published in PLOS One with coauthor Dr. F. Fort, under Foxx AJ, Fort F (2019) Root and shoot competition lead to contrasting competitive outcomes under water stress: A systematic review and meta-analysis. PLoS ONE 14(12): e0220674. I conceptualized the study, curated the data, analyzed the data, performed the literature search, created the visualizations and wrote the original draft. F. Fort reviewed and edited the manuscript.

Abstract

Background: Competition is a critical process that shapes plant communities and interacts with environmental constraints. There are surprising knowledge gaps related to mechanisms that underlie competitive processes, though important to natural communities and agricultural systems. One being the contribution of different plant parts on competitive outcomes and the effect of environmental constraints on these outcomes. Objective: Studies that partition competition into root-only and shoot-only interactions assess whether plant parts impose different competitive intensities using physical partitions and serve as an important way to fill knowledge gaps. Given predicted drought escalation due to climate change, we focused a systematic review – including a meta-analysis on the effects of water supply and competitive outcomes. Methods: We searched ISI Web of Science for peer-reviewed studies and found 2042 results. From which eleven suitable studies, five of which had extractable information of 80 effect sizes on 10 species to test these effects. We used a meta-analysis to compare the log response ratios (lnRR) on biomass for responses to competition between roots, shoots, and full plants at two water levels. Results: Water availability treatment and competition treatment (root-only, shoot-only, and full plant competition) significantly interacted to affect plant growth responses (p < 0.0001). Root-only and full plant competition are more intense in low water availability (-1.2 and -0.9 mean lnRR, respectively) conditions than shoot-only competition (-0.2 mean lnRR). However, shoot-only competition in high water availability was the most intense (-0.78 mean lnRR) compared to root-only and full competition (-0.5 and 0.61 mean lnRR, respectively) showing the opposite pattern to low water availability. These results also show that the intensity of full competition is similar to root-only competition and that low water availability intensifies root competition while weakening shoot competition. Conclusions: The outcome that competition is most intense between roots at low water availability emphasizes the importance of root competition and these patterns of competition may shift in a changing climate, creating further urgency for further studies to fil knowledge gaps addressing issues of drought on plant interactions and communities.

Introduction

A major question among plant ecologists is to understand plant competition mechanisms and their outcomes from different perspectives. Many contemporary ecological endeavors seek to elucidate the role of competition in community structure, processes, and species coexistence (Chesson, 2000, 2008; Mayfield & Levine, 2010; HilleRisLambers *et al.*, 2012; Godoy O, Kraft N, 2014; Kraft *et al.*, 2014). Evidence shows that competition impacts survival, and higher level processes such as community diversity and spatial structure (Schamp & Aarssen, 2009; Kunstler *et al.*, 2012). Past work dived deeply into understanding the role of pair-wise species competition on outcomes observed in communities and in field settings (Aerts, 1999; Acciaresi & Guiamet, 2010; Owen *et al.*, 2013; Parkinson *et al.*, 2013). But, only a small section of the literature describes the competitive contributions of roots and shoots separately (Fig. 1.1) and their interaction with environmental constraints - which is critical considering the contribution of roots and shoots to ecosystem processes and responses to environmental changes(Diaz, S., Hodgson, J.G.; Thompson, K.; Cabido, M.; Cornelissen, J.H.C.; Jalili, A; Montserrat-Marti, G.; Grime *et al.*, 2004; Bardgett *et al.*, 2014; Bu *et al.*, 2019).

Most competition studies focus on competitive outcomes on shoots. But competitive behaviors resulting from shoot competition, may not influence competitive root responses in the same plant (Murphy & Dudley, 2007), thus the influence and outcome of roots interaction needs specific consideration. Traits can predict competitive ability and performance in environments (Violle *et al.*, 2007; Funk *et al.*, 2008), and Kembel & Cahill (Kembel & Cahill, 2011) showed that roots face different environments than shoots leading to variable correlation of above- and belowground traits in response to the environment. A meta-analysis on studies that physically partitioned roots and shoots during competition under nutrient stress found that roots imposed more intense competition than shoots reporting a 42% biomass reduction – indicating intense competition. (Kiaer *et al.*, 2013). An important remaining question is on the role of water in competition.

Water is a critical resource that allows plant growth, and related physiological processes such as cell growth and nutrient transport to shoots (Hsiao, 1973; Hsiao & Xu, 2000). In cases of low water availability plants can close stomata to limit water loss and CO_2 capture (Taiz & Zeigler, 2002). Plants can also respond to low water availability by allocating more mass to roots to acquire the limited resource (Wang & Taub, 2010; Poorter *et al.*, 2012). Generally, while water stress reduces plant size, root allocation, branching, length, and uptake increase to maintain soil water capture capacities (Sharp & Davies, 1979; Jupp & Newman, 1987; Berendse & Móller, 2009; Silva *et al.*, 2012) (Fig. 1.2). Conversely, water stress reduces shoot growth, leaf area, new leaf production, and photosynthetic light conversion (Sharp & Davies, 1979, 1985; Jentsch *et al.*, 2011; Silva *et al.*, 2012) (Fig. 1.2). Resulting diminished light interception and metabolic activity aboveground (Gargallo-Garriga *et al.*, 2014), coupled with increased absorptive root area under water stress should intensify competition between roots more than between shoots (e.g. (Casper & Jackson, 1997)), but the literature presents mixed evidence related to their outcomes.

Despite established patterns of the effects of water stress, water stress intensifies, decreases or produces no measured outcomes on root-only or shoot-only competition (e.g. (Dauro & Mohamed-Saleem, 1995; Weigelt *et al.*, 2005; Lamb *et al.*, 2007). The different physiological processes of roots and shoots to drought, may reduce resource need. These differing activity levels during drought may also have strong effects on above- compared to belowground performance that may affect the intensity of root and shoot competition in water limited environments. This is critical due to the predicted variable global precipitation patterns and increased regional aridity due to climate change (Seager *et al.*, 2007). Environmental constraints such as resource stress, change the intensity of the competition among species (Rajaniemi *et al.*, 2003; Liancourt & Lavorel, 2013; Fort *et al.*, 2014a; Silvertown *et al.*, 2015) and low water availability can intensify (Weigelt *et al.*, 2000; Hanke *et al.*, 2015) or weaken competition (McCluney *et al.*, 2012).For example, water loss of a nurse shrub due to dry soil reduced mortality in a protégé shrub (Prieto *et al.*, 2011). Despite the substantial impacts water limitation imposes on competition and survival compared to nutrient stress (Coomes & Grubb, 2000), the literature pool on water and competition is comparatively small so syntheses would advance our knowledge by elucidating patterns.

We conducted a systematic review and meta-analysis to provide resolution on the intensity of root and shoot competition under water stress. We assessed whether roots and shoots impose different competitive intensities in studies that physically partitioning roots and shoots during competition experiments under different water availabilities (Fig. 1.1). We hypothesize that: 1) competitive intensity of root-only, shoot-only, and full competition will differ under varying water availabilities; 2) competitive intensity will differ between low and high water availability treatments; and 3) root competition will differ from shoot competition at varying water availabilities.

Methods

Literature Search

We sought peer reviewed literature using the ISI Web of Science searching platform. A search was performed on 2 May 2019 of the following title and topic with Boolean terms and wildcard symbols to broaden the search: [(shoot* AND root*) OR (above AND below)] AND [(competit* OR interact*)], topic: "water stress." Search results were refined by research areas of plant sciences, agriculture, genetics, heredity, forestry, and environmental sciences, and ecology (See SI table 1.2 and SI table 1.3 for study checklists (Koricheva & Gurevitch, 2014)). Citations within relevant articles were searched as well. Abstracts were then evaluated for relevance and kept if they met the following experimental criteria: experimental designs that contained root-only, shoot-only, and or full competition, and a control group (Fig. 1.1), all under a high and low water availability treatments. Authors were contacted for data sharing when essential data were not imputable or extractable.

Data Collection

Studies were included in the analyses if we acquired response variables, standard deviation, and sample sizes, either from the study, the study authors, or from figures. When data were only available in graphics, those data were extracted using the free web-based application WebPlotDigitizer v4.1 (Rohatgi, 2015). We extracted data from figures from three studies (Salinger & Bornkamm, 1982; Lamb *et al.*, 2007; Bartelheimer *et al.*, 2010). Two studies implemented multiple water treatments (Wilkinson & Gross, 1964; Bornkamm *et al.*, 1975), so data from the two extreme treatments were used (highest and lowest water availability). Nutrient treatments were used in some studies, but this was not replicated in all studies nor a target hypothesis so, only data from the lowest nutrient level were utilized. Fixed effects from each study included water treatment (low and high-water availability treatments), competition treatment (control, root-only, shoot-only, or full competition) (Fig. 1.2), and focal species nested within study as a random effect.

Analyses

All analyses were performed in R v3.6.1 (R Core Team, 2019). We constructed mixed effects meta-regression models to compare the log response ratio values (lnRR). Models were constructed using the “rma.mv” function in the “metafor” package (Viechtbauer, 2010) in R (R Core Team, 2019). Models were compared using logliklihood ratio test that used the “anova” function. To test whether water treatments modulated outcomes of the competition treatments, the full model assessed the interaction between water availability levels (low and high availability) and competition treatments (root-only, shoot-only, and full). The reduced models were compared to the full model to determine which explained more variation in plant growth. The reduced models assessed plant growth response to water availability, or plant growth responses to competition treatment, and plant growth responses to the additive effects of competition and water treatments.

The effect sizes lnRR [53]. Log response ratios are the proportional change in treatment groups compared to the control group (Hedges *et al.*, 1999). They are symmetric around zero and taking the log linearizes the ratio and leads to a generally normal distribution when the treatment mean is not zero (Hedges *et al.*, 1999). Log response ratios measure the intensity of interactions; negative values denote competition and positive values denote facilitation, while a lnRR of zero denotes no effect of treatment (Valentine *et al.*, 2010). The lnRR values were calculated in R using the "ROM" measure in the "escalc" function in the "metafor" package (Viechtbauer, 2010). The "ROM" measure underlies the equation:

$$\text{lnRR} = \ln \frac{X_E}{X_c} \qquad (1)$$

Where X_E is the biomass mean of treatment group plants compared to the mean of the control group X_C. Here, the lnRR values were calculated over study and species and compared between root-only, shoot-only, and full competition, as well as water availability levels. The calculated lnRR is the most likely effect size but confidence intervals are important in interpreting meta-analyses outcomes (Valentine *et al.*, 2010). They indicate how confident one is in the directionality of an effect size and tell the full range of effect size for the treatment (Valentine *et al.*, 2010). If the lower bound confidence interval overlaps with zero, the results are not statistically significant (Valentine *et al.*, 2010). The sampling variances of the lnRR were calculated in R using the "escalc" function, and the equation follows Hedges et al. [53]:

$$\frac{SD_E^2}{n_E * X_E^2} + \frac{SD_C^2}{n_C * X_C^2} \qquad (2)$$

Where n_E and n_C and SD_E and SD_C are the sample sizes and standard deviations for the experimental and control groups respectively. Standard deviation was not reported in two suitable studies (Wilkinson & Gross, 1964; Bornkamm *et al.*, 1975), but were imputed to reduce publication bias and improve variance estimates compared to when data from an incomplete study are excluded (Lajeunesse, 2013). So, the standard deviation was calculated using F-statistics reported in the original study using equation 3 (L. Hedges, *Personal communication*):

$$s = \sqrt{(Y_C - Y_E)^{-2} * \left(\frac{F}{\frac{n_C + n_E}{n_C * n_E}}\right)^{-1}} \qquad (3)$$

where n_C and n_E are the sample sizes of the control group and treatment group respectively. Additionally, Y_C and Y_E are the mean values of the control group and treatment group respectively, and so is the imputed standard deviation. Standard deviations were also imputed for one study (Wilkinson & Gross, 1964) using a linear regression between sample sizes and pooled standard deviation values of studies with known standard deviation values using the following equation (Lajeunesse, 2013):

$$SD_j = avgX_j * (\sum_i^K SD_i \,/\, \sum_i^K avg\ X_i) \qquad (4)$$

where SD_j is the standard deviation of the study with missing information and SD_i is the standard deviation of samples with full information, X_i is the mean of the lnRR of full studies and X_j is the mean of the lnRR of the study with missing information. We performed contrasts to test the hypotheses that root competition differed from shoot competition at differing water levels, and the hypothesis that competitive intensity differed between water availability levels. Contrasts were specified in the “anova” function from the “car” package (Fox & Weisberg, 2019). Finally, we tested for publication bias by performing a Rank Correlation Test for Funnel Plot Asymmetry using the “ranktest” function in the “metafor” package. This helps determine if the observed outcomes and variances are correlated, indicating publication biases (Viechtbauer, 2010).

Results

Literature search

The search results yielded 2042 studies (Fig. 1.3).The broad search terms led to many studies that were usual competition experiments that lacked partitions or had suitable methods but manipulated nutrients (see Kiær *et al.*, 2013) and not water levels, or manipulated no resource. Eleven studies with applicable methods were found. One researcher provided data from her study (Weigelt *et al.*, 2005). Five studies with extractable information were included in the meta-analysis on ten species, containing 106 data points, and 80 lnRR outcome measures (Data for calculations; SI Table 1.2). Data useful for calculating effect sizes and variance were unavailable in figures or through authors in other studies and were excluded from analysis. One excluded study used trees as focal plants (Putz & Canham, 1992) while all others utilized herbaceous or shrub species. Furthermore, this study (Putz & Canham, 1992) and another [Dauro & Mohamed-Saleem, 1995– also with missing data] used spatial (site differences) and temporal (drought year and rainy season) proxies for water treatments likely introducing heterogeneity and doubts on whether the effect sizes are drawn from the same population – an assumption of fixed effects meta-analytic models (Cohn & Decker, 2003). Another study (Salinger & Bornkamm, 1982) was excluded due to the response measure being shoot to root ratio while all other studies used direct biomass measures. In total, 16 species were represented in all twelve studies published across a 46-year period from 1961-2007 (Table 1.1).

Interaction outcomes from meta-analysis

The model that best fit the data included an interaction between competition treatment and water treatments ($Q_{df = 5}$ = 395.5, $p < 0.001$) (Table 1.2), whereby competition and water treatments interacted to significantly affect plant growth. Root-only, shoot-only and full competition exhibited different responses to water treatments while opposing competitive outcomes are recorded at low water availability (Fig. 1.4). Shoot-only competition in high water availability resulted in a lnRR of -0.78, while, root-only and full competition are -0.5 and -0.61 respectively, meaning shoot-only competition was on average more intense (Fig. 1.4). Conversely, at low water availability, root-only and full competition treatments resulted in more intense competition (lnRR = -0.9, and lnRR = -1.2, respectively) than shoot-only competition (lnRR = -0.2) (Fig. 1.4).

Root only-competition significantly differed from shoot-only competition at low water availability ($p < 0.0001$) and under high water availability ($p = 0.04$), where root-only competition was more intense under low water availability compared to high water availability. Though there are large confidence intervals for shoot-only competition at high water availability reduces our certainty of the true effect size.

The heterogeneity between studies (Q_m on 5 df) is 395.5 indicating that heterogeneity between studies is high and given a $Q > 100$ we reject the null hypothesis that the variance component is 0 (Hedges *et al.*, 1999). Large heterogeneity indicates that here are differences between studies and unexplored sources of variation we did not capture in the analyses. This is reinforced by the high I^2 values (Table 1.2) denoting that a large part of the variation remains unexplained. Root-only and shoot-only competition had significantly different responses to water treatments ($p < 0.001$) where root-only competition was more intense than shoot-only competition under low water availability and the opposite pattern at high water availability treatments (Fig. 1.4).The overall plant response was only slightly impacted by water availability (p = 0.1). Low water availability caused weaker competition compared to high water availability when aggregated over effect sizes of all treatments. The rank correlation test for funnel plot asymmetry to test for publication bias revealed some correlation between studies (Kendall's tau = 0.153, p = 0.05) indicating publication bias.

Study Assessments: Competitive outcomes

Welbank (Welbank, 1961) is the earliest experiment considered and assessed competition between *Impatiens parviflora* and *Agropyron repens* in pots and only included full competition and shoot-only competition which provides indicative rather than direct impacts of root competition. Full competition under low water availability had a slower growth rate (biomass) than in high water availability and full competition suppressed growth rate more than shoot-only competition, indicating that the inclusion of root intensified competition. In another study, Wilkinson & Gross (Wilkinson & Gross, 1964) aimed to understand the role of competition in *Trifolium repens* in stands of *Dactylis glomerata* and introduced *T. repens* into stands of *D. glomerata* in a greenhouse study where roots and shoots were separated by clear plastic. The biomass of *T. repens* at low water availability was highest in full competition followed by root-only competition. Outcomes in low water availability showed that full competition had the greatest mass followed by root-only competition, then by shoot-only competition, indicating that competition was least intense for shoot-only competition.

Bornkamm et al. (Bornkamm *et al.*, 1975) explored the role of water availability on competition that could pattern distribution of the co-occurring grasses *Arrhenatherum elatus* and *Bromus erectus*. *A. elatus* had smaller root mass at low water availability compared to high water availability indicating suppression, and larger roots in shoot-only and full competition treatments under low water availability. *B. erectus* had smaller roots in high water availability for root- and shoot-only competition but had larger roots in low water availability showing the opposite pattern. The follow-up study (Salinger & Bornkamm, 1982) used the same experimental design (Bornkamm *et al.*, 1975) and found that *B. erectus* allocated more mass to shoots under root-only competition and low water availability compared to high water availability. *B. erectus* also allocated less to shoots in low water compared to high water availability in both shoot-only and full competition treatments pointing to increased competition. *A. elatus* showed a differing response and had lower S:R ratio in low water availability in root-only and full competition, while it had equal S:R in the shoot-only competition for both water levels. Another study (Putz & Canham, 1992) assessed methods to curtail tree encroachment into shrub areas and compared the interactions of *Cornus racemosa* on *Acer rubrum* seedlings in a field study using site differences as a proxy for water treatment and trenches with weed cloth and wire to tie shoots. Measuring basal area daily growth rate, the authors found that the growth of *A. rubrum* was most suppressed by shoot-only competition - being two times smaller than under root-only competition - meanwhile full competition most suppressed the basal area at the driest site and shoot-only plants had two times the basal area than root-only competition indicating weaker competition in the shoot-only treatment. These site differences also introduce soil property and site history differences that could affect plant growth in addition to treatments imposed.

Two studies utilized partitioning experiments in agricultural systems to understand competition in intercropped systems. Dauro & Mohamed-Saleem (Dauro & Mohamed-Saleem, 1995) evaluated the impacts of competition between intercropped *Triticum durum* var. Boolai and *Trifolium quartinianum* in field plots using wet and dry seasons as a proxy for water treatment and reflective foil and plywood to separate shoots and roots, respectively. In both the dry and wet season shoot-only competition did not significantly affect either species' biomass, meanwhile root-only competition in the dry season suppressed *T. quartinianum* significantly leading to increases in biomass for *T. durum* competitors. Semere & Froud-Williams (Semere & Froud-Williams, 2001) explored ways that intercropping interactions improved yield of *Zea mays* and two pea cultivars with leafy and less-leafy phenotypes in a greenhouse. The authors found that pea cultivar identity and low water availability impacted root-only competition on *Zea Mays*. Both pea cultivar's growth were not significantly affected by shoot-only competition, while root-only competition and low water availability reduced mass by 43%. These results indicate that root-only competition impacted growth while shoot-only competition had smaller effects, and that water stress and root-only competition suppressed the growth of *Z. mays* more than shoot-only competition. Interestingly, pea cultivar competitive intensity in shoot-only treatment did not differ given the differences in leaf phenotype.

Haugland & Froud-Williams (Haugland & Froud-Williams, 1999) explored the role of competition in grassland establishment of established *Lolium perenne* and *Phleum pretense* seedlings in boxes in the greenhouse. The outcomes are not clearly reported likely due to lack of statistically significant findings in competition treatments with water treatments. However, the authors found that low water availability reduced growth of both species and that shoot-only competition from *L. perenne* reduced the biomass of *P. pratenses* more than root-only competition. Some studies utilized this approach on outdoor settings and mesocosms. Lamb et al. (Lamb *et al.*, 2007) were interested in identifying the role of root-only and shoot-only competition and productivity gradients in Canadian grassland in the field with PVC pipes for root exclusion and plastic netting for shoot exclusion. Focal species were *Artemisia frigida* and *Chenopodium leptophyllum* and neighbors were a mixture of grass and tree species in the natural vegetation. Shoot biomass for *A. frigida* under root-only and full competition was similar and smaller than shoot-only competition under both water treatments indicating more intense competition and suppression in these treatments. Shoot biomass in shoot-only competition was smaller with higher water - compared to lower water availability. *C. leptophyllum* under low water availability for full and root-only competition had similar shoot mass outcomes, while shoot mass in shoot-only competition treatment was higher. At higher water availability, full competition had the lowest shoot mass mean followed by root-only then shoot-only competition. These results show that competition intensifies when roots interact and under low water availability. The natural vegetation could have potential diversity effects that could influence interaction outcomes though provides a robust comparison of field performance.

Weigelt et al. (Weigelt *et al.*, 2005) assessed root allocation in response to competition and resource stress in dune species *Carex arenaria*, *Corynephorus canescens*, and *Hieracium pilosella* in an outdoor sandbox mesocosm calculating competitive intensity from total plant biomass. This study assessed root-only and full competition treatments only and did not report on competition by water treatments responses likely due to the lack of statistical significance. The authors found that competition for all species was generally more intense under low - compared to high water availability. Lastly, one study explored the role root-only or shoot-only competition played in niche segregation of co-occurring species *Senecio aquaticus* and *Senecio jacobea* using *Phleum pratense* competitors in mesocosms under drought and water-logged conditions (Bartelheimer *et al.*, 2010). *S. aquaticus* is adapted to wet soil (e.g. marshes) and had the largest shoot mass in shoot-only competition followed by full, then root-only competition at low water availability. It performed better in high water availability for all treatments, and the competitive hierarchy of low water availably was maintained. *S jacobea* had similar mass in shoot-only and root-only competition treatments and full competition had the smallest mass at low water availability. This species also had the largest shoot mass in shoot-only followed by, full, then root-only competition at low water availability indicating less intense competition with shoot competitors at low water availability.

Discussion

The impact of increasing drought in a changing climate (IPCC, 2014) and ever-present competition have large ramifications for natural plant communities and agricultural systems. Specifically, competition and water stress impacts community membership (Verwijmeren *et al.*, 2014; Kraft *et al.*, 2014) and crop yield (Acciaresi & Guiamet, 2010; Leguizamón *et al.*, 2011) and has global importance for plant conservation and food security. We demonstrate that water availability significantly modulates competitive outcomes where high water availability intensified shoot-only competition while weakening root-only competition respective to competitive outcomes of low water availability. These study results are important as short-term effects of competition were a top predictor of species' abundance in the field (Howard & Goldberg, 2001). This systematic review combines study assessments and a meta-analysis on empirical evidence to reveal competitive patterns and influence future work to advance our knowledge.

Shoot competition responses to water availability

We show in meta-analysis and in study evaluations that shoot-only competition was more intense under high water availability than in low water availability treatments. Higher aboveground biomass in high water availability treatments may have resulted from plentiful soil resources available for biomass production(Sharp & Davies, 1979, 1985; Jentsch *et al.*, 2011; Silva *et al.*, 2012). Furthermore, greater aboveground mass could be in response to light competition for shade avoidance responses denoting intensified competition through imposing shade (Gundel *et al.*, 2014; van Gelderen *et al.*, 2017). From a community perspective, research suggests that light competition is important in ecosystems with high aboveground productivity (Twolan-Strutt & Keddy, 1996) and thus aboveground competition can impact patterns of community diversity and dynamics (Kraft *et al.*, 2015).

To the contrary, the weakest competitive treatment was shoot-only competition in low water availability. Water stress is known to limit plant growth leading to a reduction in leaf area which limits shading and light competition that an individual can impose on its neighbor (Semere & Froud-Williams, 2001). Results of the meta-analysis showed that competition weakens at low water availability when shoot competition is included, and seem to agree with the stress gradient hypothesis which notes that facilitation and weak competitive interactions may dominate at high-stress levels compared to low-stress (Bertness & Callaway, 1994; McCluney *et al.*, 2012). Weak competitive interactions could be a result of plants allocating less mass aboveground or slowing metabolic activity aboveground for survival and defense under stressful conditions (Gargallo-Garriga *et al.*, 2014). This is interesting given that competition in dry environments is high, though thought to be concentrated belowground (Fowler, 1986), however, we clearly demonstrate that when shoot competition is considered alone water availability is a key factor modulating its intensity and this needs exploration in different biomes.

Root responses to water availability

Root-only competition was weaker at high water availability than low water availability but was the most intense competition group of this study at low water availability. This suggests that higher water supply weakens belowground competition and shows different patterns to shoot-only competition. These results are in line with Lamb et al. (Lamb *et al.*, 2007), but counter Bartelheimer et al. (Bartelheimer *et al.*, 2010) who showed competitive suppression in root-only treatments under high water supply. On the other hand, root-only competition was the most intense competition treatment under low water availability. Intense root competition may be driven by roots responding to water stress by increasing root allocation and intensity of soil exploration resulting in increased nutrients and water uptake (Sharma, RB and Ghidyal, 1977; Gedroc *et al.*, 1996; Wang & Taub, 2010; Poorter *et al.*, 2012). High root biomass and root length production are known to induce intense competition between plants (Mommer *et al.*, 2012) and these morphological changes in response to water stress likely also increase competition due to reduced resources (Acciaresi & Guiamet, 2010; Treder *et al.*, 2016). Research suggests that root competition is more intense in dry environments where productivity is concentrated belowground (Fowler, 1986; Schenk & Jackson, 2002) and root-only competition was more intense than shoot-only competition under low water availability. These results along with meta-analytic findings of Kiær et al. (2013) on nutrients indicate that when soil resources are limited, root competition is more intense than shoot competition. Despite this strong evidence of a positive effect of water shortage on the root competition we may expect conflicting responses when species evolve in differing environments, though more studies are needed to better assess this hypothesis.

Whole plant outcomes and implications

The results of studies reviewed highlight the variability in species response to low water availability but generally are in line with the findings of this meta-analysis that root-only competition differs from shoot-only competition. But the contrasting results between shoot-only, root-only, and full competition suggest that the contributions of root and shoot competition are not additive. Rajaniemi et al. (Rajaniemi *et al.*, 2003) showed that root-only competition experimental assemblages resulted in lower species diversity compared to shoot-only competition assemblages. Also, Lamb et al. (Lamb *et al.*, 2009) showed shoot competition negatively impacted community evenness but was through indirect increases in competitive root responses. While aboveground competition has documented impacts on community structure (Fortunel *et al.*, 2016), root competition also has strong and apparent consequences for plant communities. Because we see contrasting outcomes in root-only and shoot-only competition, researchers should increase the assessment of belowground ecology to draw more accurate conclusions about competition particularly if environmental constraints would lead to a shift in biomass allocation (Cahill, 2002).

Study limitations

These results show important interactions between plant competition and water availability. The fixed effects used in these models significantly explained variation in effect sizes but including other effects such as target species life history, non-target life-history, and experimental setting may reduce residual heterogeneity. Given the small number of studies, these factors could not be reliably tested without replication. Other sources of variation were in the differences in materials used to partition plants (e.g. mesh vs. solid aboveground dividers) and implementation of water stress where amounts that were considered “high” and “low” differed by study. Additionally, the adaptations of target species could have influenced competitive outcomes and responses to water stress. For example, Bartelheimer et al. (Bartelheimer *et al.*, 2010) used *Senecio aquaticus* – a wetland adapted species – which performed poorer than the terrestrial congener in low water availability.

Five studies ignored the role of intraspecific competition in the set-up and had focal plants interact with conspecifics both above and belowground. Given that many species compete more intensely with conspecifics than heterospecifics (Adler *et al.*, 2018) this could impact the outcomes of competitive intensity recorded. Additionally, considering proper comparison groups is important for quantifying the effect of a treatment. Monoculture groups with root-only, shoot-only, full treatments under all applied water treatments serve as appropriate controls for partition studies.

Finally, we excluded several known suitable studies from the meta-analysis due to missing information introducing publication bias (BMJ, 2015). More studies in this area are needed particularly to provide resolution for whether plants alter allocation in response to the source of below or aboveground competition, shedding light on long-posited hypotheses (Gedroc *et al.*, 1996). The results of relevant treatments in suitable studies were likely not reported due to lack of significance, introducing selective reporting bias (BMJ, 2015). Authors should publish full study results related to original hypotheses presented and parameters (e.g. sample size, responses, measures of variability) for future synthesis and knowledge advancement.

Conclusions

The intensity of root-only and shoot-only competition showed opposing trends under differing water availability. Our results show that roots have major implications in competitive outcomes for plants when soil resource are limited. Importantly, if we only record aboveground responses to water stress or competition, we may conclude weak competition or facilitation when belowground responses may reveal contrasting evidence. Future research should tie in the role that root and shoot competition have on species coexistence in plant communities.

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CHAPTER TWO

Hidden variation: cultivars and wild plants differ in root and shoot trait variation

Abstract

Restoration practitioners have many seed material choices when restoring plant communities, and for some species, cultivars may be the most affordable and accessible material available. However, the process of plant selection and commercial seed production usually limits genetic and trait variability in cultivars. This variation can be critical to survival and persistence in heterogeneous environments, which are common restoration goals. Several studies have shown negative impacts of plant selection and commercial production on trait values and variation, particularly in aboveground traits, but impacts on root traits in wild-collected material relative to cultivars have rarely been assessed. This is a critical gap, especially in arid environments where root traits play a key role in plant survival. To compare root and shoot trait values and variability between wild and cultivar accessions, we grew seedlings of three wild-collected accessions and three cultivars of *Pseudoroegneria spicata* – including ‘Whitmar’ and ‘Goldar’ and a “selected germplasm”, ‘P-7’, with high neutral genetic diversity, developed following selection on progeny produced after open pollination among 25 populations in a nursery. We grew the plants in sand in a common environment experiment in a growth chamber for four weeks, then harvested the plants and compared trait variation and average trait values among collection type (wild-collected versus cultivar) for four aboveground (shoot) and four belowground (root) traits. We found that wild-collected accessions had significantly greater variation in two root traits and one shoot trait, while trait values differed significantly between collection type for three root traits and one shoot trait. Specifically, wild-collected plants had 51% more root tips on average,

although there was significant variation among accessions for this trait, and higher survival compared to cultivars. These results show the importance of including root trait values and variation when assessing accessions for restoration use, and warrants reexamining of cultivation and production practices that may reduce trait variation and limit restoration success.

Introduction

Restoration practitioners have several plant material choices available when restoring native plant communities, ranging from wild-collected material to highly selected and commercially-produced cultivars. Wild-collected materials often have higher neutral genetic diversity than commercially-available material, particularly cultivars (Burton and Burton 2002, Tang and Knapp 2003; although this is not always the case (Chivers et al. 2016)). Genetic variation is important for population establishment and persistence in heterogeneous environments (Hughes & Stachowicz, 2004; Hughes *et al.*, 2008). However, the major limitations of using wild-collected materials are quantity available given the need, and cost and labor required to collect the material (Haidet & Olwell, 2015). Additionally, the amount of material needed to restore landscapes can easily decimate natural populations (Dorner, 2002; Broadhurst *et al.*, 2008).

A native seed industry arose to address the need for large volumes of affordable seeds of native species (Dyer *et al.*, 2016). Growers across the world, including in the United States, now sell a wide range of plant materials for restoration purposes (Ladouceur *et al.*, 2018; White *et al.*, 2018). In many cases, growers collect seeds from wild populations and grow them in seed farms to increase the seed available, which can lead to unintentional selection (Montalvo *et al.*, 1997; Dyer *et al.*, 2016). Furthermore, for some species and regions, important restoration species exist as cultivars produced through intentional selection and breeding programs. Cultivars usually ensure trait consistency and are bred for traits such as forage value, high aboveground biomass, and seed yield, which improve amenability to commercial production (Waters & Shaw, 2003; Aubry *et al.*, 2005; Leger & Baughman, 2015). Selection on traits and variation can happen during the production of cultivars – partly due to the use of agronomic techniques that select for uniform plants that accommodate machinery (Roundy, 1999; Dyer *et al.*, 2016) and propagation in high resource environments with weed removal (Patel, 2013). Selection can cause plants to shift phenology in response to harvest time and harvest equipment (Dyer *et al.*, 2016), lose genetic variation (Dyer *et al.*, 2016; Nagel *et al.*, 2019), lose seed dormancy (Ensslin *et al.*, 2018), and change flowering effort (Nagel *et al.*, 2019) within a short time frame. These activities can impact both traits and variation relative to wild populations, but to-date studies of the impacts of plant selection and commercial production have largely focused on changes in aboveground traits, leaving knowledge gaps of potential impacts of selection on root traits in native plants.

Of the few studies that have compared root traits in the context of sourced plant material, Solomon (2019) found little evidence for trait differences between cultivar and wild-collected accessions for root mass, volume, length, diameter, and root to shoot ratio in western grass species *Poa secunda* and *Elymus elymoides*. The author did find that one of three *P. secunda* wild collections had six times greater root allocation than all other collections, and cultivars were twice the size aboveground relative to wild collections (Solomon 2019). Conversely, Klopf and Baer (2011) found that cultivars had larger root systems and used more nitrogen than wild-collected accessions, and identified no differences in shoot traits between collection types. Crops have similar propagation methods to cultivars and commercially-available material, and have shown differences in root performance, whereby some domesticated crop species exhibit reduced root competitiveness (Kiaer *et al.*, 2013) and reduced root plasticity and root diameter compared to wild relatives (Grossman & Rice, 2012). Researchers also often assess wild progenitors to cross with crops to increase variation in root traits (Kumar *et al.*, 2012). This indicates that selection during crop growth and development can have large effects on root traits, supporting further inquiry into the impacts of plant selection and production practices on root traits.

Belowground performance is a critical determinant of restoration success in arid environments. Here, plants must cope with severe drought (e.g. Comstock and Ehleringer 1992) and capture limited resources while competing with other species. Other species include *Bromus tectorum*, a successful invasive plant of the arid Western United States. *B. tectorum* has thin and highly branched roots that proliferate at very low temperatures, unlike many native plants, aiding its dominance (Evans, 1961; Harris, 1967). These factors point to the critical role of root traits in these environments, specifically, root tips and root allocation. Root tips take up an average of 80% of the water and nitrogen used by plants (Sharma and Ghidyal 1977, Lazof et al. 1992, Varney and Canny 1993, Sorgonà et al. 2005, Sorgonà et al. 2007). Greater number of root tips and greater root allocation improve survival of plants out-planted in arid environments, by improving performance in drought and conferring competitive ability (Wang and Taub 2010, Acciaresi and Guiamet 2010, Stevanato et al. 2011, Atwater et al. 2015; Foxx and Kramer *in review*). Furthermore, research has shown that plants with greater root allocation (Rowe & Leger, 2011) and greater number of root tips (Atwater *et al.*, 2015) are more likely to survive in restoration in the arid Great Basin in the western US, providing more evidence that root traits are critical in restoration contexts in arid systems.

To investigate above- and belowground traits and variation, we conducted a common garden experiment in a growth chamber using three wild-collected and three cultivar accessions of *Pseudoroegneria spicata* (Pursh) A. Love. Numerous cultivars have been developed for revegetation use for this species, and wild-collected material is also commonly used in semi-arid regions of Western North America (Larson et al. 2000, Bradley St. Clair et al. 2013). Practitioners value *P. spicata* for its forage value and its drought resistance due to its extensive root system (Ogle *et al.*, 2010). Therefore, this species is ideal for this study assessing above- and belowground trait variation due to the importance of its root system for performance in restorations and the multiple cultivars developed and currently used widely in the western United States. We compared root traits; root tip count, root mass, root mass fraction, and root length to comparable shoot traits; leaf count, shoot mass fraction, and plant height; and survival, between wild collections and cultivars. We hypothesized that: 1) variation in trait values differ by collection type, with variation greater in wild-collected compared to cultivars, 2) trait values differ between cultivars and wild collections, and 3) survival differs by accession and collection type, being greater in wild collections.

Materials and Methods

Species and accessions

Pseudoroegneria spicata, or bluebunch wheatgrass, is a cool season, long-lived perennial bunchgrass native to the western US (Ogle et al. 2010). We used six accessions of *P. spicata* obtained from the U.S. Germplasm Research Information Network (GRIN; USDA, Agricultural Research Service, National Plant Germplasm System, 2020) or donated (‘Whitmar’; Rainier Seeds, Davenport, WA, USA). The three wild-collected accessions were from three different locations in Utah, USA, collected following the Seeds of Success protocol (Haidet & Olwell, 2015) in 2009 (Table 2.1). We also used three commercially available cultivars and one selected germplasm: ‘Goldar’, ‘Whitmar’, and ‘P-7’ (Table 2.1). ‘Goldar’ was released in 1989 and was selected from a single source for its large aboveground size, seed production, and root system to stabilize the soil (Ogle *et al.*, 2010). ‘Whitmar’ is an awnless cultivar selected from a single source first released in 1946. The selected germplasm ‘P-7’ resulted from selection on a 25-accession “polycross”, which was achieved through open pollination of 23 geographically diffuse wild-collected sources and two cultivars ‘Whitmar’ and ‘Goldar’, resulting in higher neutral genetic variation than ‘Goldar’ and ‘Whitmar’ (Larson et al. 2000). Whether this neutral diversity translates to trait variation is unknown. While ‘P-7’ is not officially a cultivar, it is treated as one in our analyses because its selection history is closer to the other two cultivars compared to the wild collections.

Seed germination

In February 2018 we surface sterilized 200 seeds per accession with 8% bleach solution for 30 seconds followed by a DI water rinse for one minute. Next, we placed fifty seeds per accession on four - 90 mm diameter petri dishes filled with 1.5% solidified agar for cold moist stratification at 3°C in a refrigerator at the Chicago Botanic Garden (Glencoe, IL, USA) until germination (emergence of the radicle) was observed. We checked germination three times weekly and moved germinants to watered, randomized cone-tainers in a growth chamber at 25°C/20°C day/night with a 14hr/10hr photoperiod.

Plant growth conditions

The plants were grown in 6.4 cm diameter x 30.5 cm height Ray Leach cone-tainers (Stuewe and Sons, Tangent, OR, USA) containing commercial sand with a 5 mm layer of loam topsoil on top to encourage establishment. Accessions were randomized in 13 cone-tainer racks (blocks) and were rotated three times weekly to reduce position effects. The initial sample size for each accession was 30 plants. Germinants were watered every other day for seven days to encourage establishment, then three times weekly thereafter. Each cone-tainer was watered with the same amount of water - 10 mL. We applied 10 mL of half-strength Murashige-Skoog (4.43 g/1L of DI water) (Sigma-Aldrich, St. Louis, MO) immediately following watering at week three. Plants were harvested in random order between 23 to 28 days after being planted, with an equal subsample of all accessions harvested on each harvest day. This plant age coincided with the time at which most roots reached the bottom of the cone-tainer in a pilot study under the same conditions and planting materials (Foxx, *unpublished data*).

Sample processing and data collection

Plants were washed gently of sand at harvest and imaged with a five-megapixel camera. Plants were placed in a 30.5 cm x 25.4 cm x 10.2 cm rectangular container with a black sheet of paper at the bottom and filled with water to spread the roots for more accurate assessments and provide greater contrast to the roots. Photos were taken from 30 cm above the plant, and we used these images to visually count the number of root tips. We counted the number of leaves and used a ruler to measure length of the longest root and the longest leaf. Plants were then stored in coin envelopes and dried in an herbarium drier for one week, then placed at room temperature for three months prior to weighing. Following weighing with a laboratory balance, mass data (mg) were used to calculate root mass fraction (RMF: root mass/total mass) and shoot mass fraction (SMF: shoot mass/total mass). Root mass, shoot mass, and plant height were log transformed to meet assumptions of normality. We also assessed plant survival with the initial sample size and number of plants that survived to the end of the experiment.

Analyses

Variation homogeneity tests comparisons between collection type

All analyses were performed in R (R Core Team 2019, v3.6.0). To test whether collection type impacted trait variation, we used Bartlett's test for homogeneity of variances to compare variances of six continuous variables (root mass, RMF, root length, shoot mass, SMF, and plant height) using the "bartlet.test" function in the "stats" package (R Core Team, 2019). We also tested whether collection type impacted trait variation in two other traits (leaf count and root tip count) using the similar Levene's test of equality of variance for data that are not normally distributed. We used the "leveneTest" function in the "car" package with "center = mean" argument for this test (Fox & Weisberg, 2019).

Trait comparisons between collection type and accessions

To assess if accession source or collection type impacted the root and shoot traits of *P. spicata*, we used linear mixed effects models to test collection type and source accession and their interaction on root mass, root length, shoot mass, and plant height on models specified using the "lmer" function in the "lme4" package (Bates *et al.*, 2015). Block and age at harvest were included as random effects. We used stepwise backwards elimination of non-significant variables ($p \leq 0.05$) to select the minimally adequate model (Crawley, 2005). Due to unequal variances of RMF and SMF, we used the non-parametric Mann-Whitney U test on ranks to compare collection type using the "wmwTest" function in the "asht" package (Fay, 2018) and the Kruskal-Wallis test to compare RMF and SMF by accession identity using the "kruskal.test" test in the "stats" package (R Core Team, 2019). We used generalized linear mixed effects models to test collection type and source accession and their interaction on root tip count and leaf count on models specified using the "glmer" function in the "lme4" package (Bates *et al.*, 2015). Block and age at harvest were included as random effects. We used stepwise backwards elimination of non-significant variables ($p \leq 0.05$) to select the minimally adequate model

(Crawley 2005). To perform a Tukey post hoc test to determine which accessions differed in root tip count, we used the "glht" function in the "multcomp" package (Hothorn *et al.*, 2008). Lastly, we compared survival proportion between accessions and by collection types using the "prop.test" function in base R.

Results

Trait variability between collection types

The variation between collection types for RMF, SMF, and root tips markedly differed ($p = 0.02$, $p = 0.02$, $p = 0.07$, respectively). RMF and SMF for wild-collected plants had 46% more variability than cultivars, and wild-collected plants had 37% greater variability in root tip count than cultivars (Table 2.2). There were no significant differences in variability between collection types for all other traits (SI, Table 2.1). However, the differences in variance for wild-collected accessions was higher compared to cultivars for all traits except root length, which was 4% lower in wild-collected accessions and equal between collection types for leaf count (Table 2.2).

Trait comparisons

Survival and SMF were greater in wild accessions, whereas RMF and root mass were greater in cultivated accessions. Survival, RMF, and SMF varied by accession as well (SI Table 2.1). The interaction between accession and collection type explained differences in root tip count ($p < 0.0001$; random effects variance, age = 0.06, block = 0.01) (Fig. 1.1, SI Table 2.1). Wild-collected accessions produced 51% more root tips on average (median = 68, $\overline{x} = 69.4 \pm 3.2$ se; SI Table 2.1) than the cultivars (median = 38, $\overline{x} = 45.6 \pm 3.2$ se). The 'Whitmar' cultivar had a similar root tip count to all wild collections according to the Tukey post hoc test ($p = 0.95$). Leaf count did not vary by accession or collection type ($p = 0.9$). Root mass differed by collection type where cultivated accessions had slightly larger root mass than wild collections (*p*

= 0.05; random effects variance, age = 0.04, block = 0.01), as well as greater RMF ($p = 0.0006$; Fig. 2.1, SI Table 2.1). Shoot mass did not differ by collection type or accession, while SMF differed by accession ($p = 0.0006$) and collection type ($p = 0.08$; Fig. 2.1, SI Table 2.1). Root length and plant height traits were not explained by accession or collection type ($p = 0.9$ and $p = 0.1$).

Survival comparisons by collection type

Survival proportion differed by collection type ($p = 0.05$; SI Table 2.1) and by accession ($p = 0.08$, SI Table 2.1). The wild-collected accessions had 38% higher survival compared to the cultivars. ‘Whitmar’ had the lowest survival and ‘Goldar’ had the highest survival for the cultivars, while accession Wild 3 had the lowest survival and Wild 1 had the highest survival of the wild collections.

Discussion

Large scale restoration in the United States annually requires millions of tons of seeds for millions of hectares (Oldfield & Olwell, 2015). Collecting native seeds only from wild populations would decimate source populations to meet these restoration demands (Dorner, 2002). An agronomic approach of developing cultivars or selected germplasm suited to commercial production is one way restoration demand is being addressed (Montalvo *et al.*, 1997; Dyer *et al.*, 2016). But concerns about how well these materials represent wild populations have been raised, with evidence for change through the selection and production process assessed mostly on aboveground traits (*e.g.* Montalvo et al. 1997, Roundy 1999, Espeland et al. 2017). We found that plants grown from wild-collected accessions of *Pseudoroegneria spicata* had greater trait variation than cultivars in six of the eight root and shoot traits studied. Notably, the ‘P-7’ selected germplasm produced specifically to encompass greater genetic variation than wild populations (Larson et al. 2000) did not have greater variation than either wild-collected population. Root tip count, RMF, and SMF had significantly greater variation in wild-collected accessions compared to cultivars, with 51% more root tips on average than cultivars as well as higher seedling survival. These findings indicate that choice of accession and collection type matters and may influence whether or not restoration outcomes meet desired goals.

Greater trait variation in wild compared to cultivar accessions

Results that wild-collected accessions have more trait variability than cultivars suggest that the wild populations may be more likely to establish and persist in heterogeneous conditions at restoration sites. However, because source conditions of wild-collected plants are likely more variable than the selection and commercial production environments used to develop and produce cultivar accessions in our study, these results may be driven either by genetic or environmental factors, or a combination of the two (Falconer & Mackay 1996). If driven by genetic factors, wild-collected populations will contain more heritable genetic variation in measured traits than cultivars, which are predicted to lose heritable variation due to intentional or unintentional selection in the selection and production process (Espeland et al. 2017). Root traits are moderately to highly heritable [*e.g.*, lateral root count in alfalfa (Johnson et al. 1996), root biomass in wheat (Mathew *et al.*, 2018), and in certain tree species (Kormanik *et al.*, 1997b)], which may suggest that variation seen here is due to heritable genetic variation. This would provide strong evidence that cultivars may not be appropriate sources for restoration because they lack important adaptive variation that will allow them to survive under heterogeneous conditions in the short-term, and to adapt to changing conditions over the long-term.

At the same time, the conditions at the source site for each accession may explain some of the differences in trait values observed in this study through plasticity. Plasticity may occur as a result of transgenerational maternal effects that persist over multiple generations (Herman *et al.*, 2012). However, if differences in variation among collection type are driven by environmental factors, for example, variation in the growing conditions at wild-collection sites relative to the conditions at the field used to produce cultivar seeds may lead to transgenerational plasticity (Herman et al. 2012), then the implications for restoration outcomes are less clear. In the short-term, this variation may still confer important benefits to the restored population. For example, plants from maternal and grandmaternal plants grown in drought had significantly longer roots and larger biomass than plants from maternal plants grown in well-watered conditions (Herman *et al.*, 2012). This would still favor selection of wild-collected material over cultivars. However, these differences by collection type would not be predicted to persist beyond the first few generations and would not directly provide a greater ability to adapt to environmental changes over time. In order to determine which factor(s) may be driving the patterns identified here, a common garden experiment using a new generation of seeds produced in the same environment is necessary (Bischoff & Muller-Scharer 2010). It is worth noting that plasticity can be driven by the current environmental conditions a plant is exposed to (*e.g.* Padilla et al. 2007, Drenovsky et al. 2012, Roscher et al. 2015), and if plasticity improves fitness, plasticity can be adaptive (Bradshaw, 1965; Miner *et al.*, 2005). However, we do not expect our results to be influenced by the environment plants were grown in for the study, as plasticity was controlled as much as possible with a common environment experimental design in a growth chamber with controlled watering and nutrient applications.

Trait values in cultivar and wild-collected accessions

We found wild collections of *P. spicata* produced 51% more root tips on average than the cultivars. Number of root tips strongly influences competition (*e.g.* Kormanik et al. 1997b, 1997a, Stevanato et al. 2011; Foxx and Kramer, *in review*), resource uptake (*e.g.* Varney and Canny 1993, Sorgonà et al. 2005), and survival (Atwater *et al.*, 2015). Therefore, any plant selection or production process that leads to fewer root tips in cultivated material could prove detrimental to meeting goals of long-term persistence in restorations, particularly in arid regions. In the western US, the competitive, fibrously rooted invasive grass *Bromus tectorum* has dramatic negative impacts on the native plant community (Reichenberger & Pyke, 1990; Melgoza & Nowak, 1991; Chambers *et al.*, 2007) and appears to be selecting for root traits in co-occurring native plants that increase competitive ability belowground. For example, Rowe and Leger (2011) found that the native grass *Elymus multisetus* from *B. tectorum*-invaded sites had greater allocation to fine roots and tolerance to competition from *B. tectorum* than at uninvaded sites (Rowe & Leger, 2011). Atwater et al. (2015) showed that plants with greater root trait values had nearly ten times higher predicted field survival than individuals with inferior traits. Additionally, root tip count had greater positive impacts on plant aboveground growth when grown in a drier field compared to a site with greater moisture. Furthermore, field establishment from seed often fails in arid regions (Knutson *et al.*, 2014), and drying is a major factor leading to seedling mortality (Fenner, 1987), pointing to the importance of root system traits for performance. Thus, assessing population root traits and planting collections with high root tip count may improve survival and establishment.

The wild collections of this study had lower root mass fraction (RMF) values than cultivars. This was driven by differences in shoot mass between collection type, as wild collections had greater shoot masses, while root mass averages of both collection types were similar. Lower RMF in wild collections is surprising at first glance, and we expected the opposite of higher RMF in wild collections. Larger RMF values indicate larger belowground capacity for resource absorption and can confer competitive benefit (Acciaresi & Guiamet, 2010) and drought tolerance (Wang & Taub, 2010). Ferguson et al. (2015) found that plants with larger root allocation were more likely to persist in restoration in the arid Great Basin, pointing to the importance of root system and allocation. Generally, roots of herbaceous plants are larger than shoots in environments with lower mean annual precipitation (Schenk & Jackson, 2002). Water is likely more limited at natural sites compared to seed production fields; indeed, the wild collected sites received 203 mm on average less precipitation (Sevier County, UT, National Oceanic and Atmospheric Administration) than the cultivar commercial production locations (AgWeatherNet, Washington State University).

Furthermore, the most common traits sought in cultivars and native seed material release in an arid ecosystem are forage value, aboveground yield, and seed yield (Leger & Baughman, 2015). For all these reasons, we expected cultivars to be larger aboveground and RMF higher in wild collections. However, when larger shoot mass is considered along with root tip count, evidence shows that greater lateral root count, a major component of root tip count, results in greater shoot mass (Lamb *et al.*, 2000) due to greater access to soil resources. Additionally, shoot growth and metabolic activity are suppressed more than roots under water stress (Sharp & Davies, 1979; Silva *et al.*, 2012; Foxx & Fort, 2019), and because populations have differential stress-allocation responses (Leguizamón *et al.*, 2011), the wild collected plants may have better supported shoot growth through root activity. Taking the RMF and root tip count findings together, although cultivars had greater root allocation, there were pronounced differences in root architecture compared to wild collections. These trait results indicate that measuring more traits than plant mass and allocation will improve our characterizations of accessions and performance as more native plants are assessed in future studies aimed at informing plant material use in restorations.

Cultivar performance and cultivation recommendations

Fewer root tips in cultivars has been found in at least one other study. A commercially available accession of *Elymus elymoides* had fewer root tips than seven wild accessions (Foxx and Kramer *in review*). Of note, one cultivar in this study ('Whitmar') had more root tips than the two other cultivars and was similar to the wild collections. This outcome was driven by a small number of individuals (five of 18) with root tip counts greater than 70. This accession had the lowest survivorship of all accessions – indicating that more traits are involved in survival probability, potentially including coleoptile thickness (Larson *et al.*, 2015) and root length (Lloret *et al.*, 1999; Atwater *et al.*, 2015). We were not able to obtain more biotic or abiotic information on the original source of 'Whitmar' that could explain these root trait outcomes. The 'P-7' selected germplasm had low variation in root tip count, though we expected this accession to have high trait variation because it was developed through a 25-accession "polycross" aimed at increasing genetic diversity (Jones et al. 2002). While this accession has been shown to have high neutral genetic variation (Larson et al. 2000), these markers are not correlated with adaptive traits under selection (Hughes et al. 2008; Espeland et al. 2017). Future research should assess adaptive trait variation and aim to link traits to plant performance.

The seed production industry often uses agricultural approaches to produce native seeds (Roundy, 1999). Agricultural approaches may include: harvest once per year, favor plant uniformity that accommodates machinery (Dyer *et al.*, 2016), employ high water and nutrient resource inputs, remove weeds (Patel, 2013), and grow plants in monocultures that exacerbate intraspecific competition (Nagel *et al.*, 2019). These conditions lead to both intentional and unintentional selection (Dyer et al. 2016), potentially leading to declines in trait variation and/or loss of traits that may not be favored in the restoration (Dyer *et al.*, 2016; Nagel *et al.*, 2019). For example, loss of genetic and phenotypic variation in aboveground traits has been documented in cultivation (García *et al.*, 1997; Nissim *et al.*, 2004; Flint-Garcia, 2015; Hernández-Terán *et al.*, 2017). Other impacts of cultivation include selection for greater aboveground mass leading to increased competitiveness relative to wild sources (e.g., *Panicum virgatum* L. cultivars (Eckberg *et al.*, 2018) and in cultivar-wild hybrids (Schröder & Prasse, 2013)). Recommendations to maintain genetic variation through production could, in turn, maintain trait variation. Recommendations include harvesting seeds multiple times, promoting gene flow, limiting the number of generations in production, limiting plant maintenance, tracking sources separately, and keeping as many plants alive and contributing to the seedlot as possible (Basey *et al.*, 2015; Dyer *et al.*, 2016; Espeland *et al.*, 2017).

Future work on root traits in restoration materials

The results that wild collections had greater trait variability and different trait values than cultivar accessions indicate that the process of plant selection and cultivation may impact root traits as well as shoot traits, translating to lower survival. Repeating this study with more species and populations will help clarify trait variability trends. Additionally, assessing plasticity through drought experiments would directly elucidate the extent to which wild populations or cultivars have differential plastic responses – differences that have been found in plastic root responses of cultivars compared to wild progenitors (Grossman & Rice 2012). Assessing the extent of trait plasticity will also provide more evidence of the plant’s performance related to observed traits.

Likewise, while trait assessments in controlled settings have direct applications to performance in the field (Schroeder-Georgi et al. 2016), trait assessments in spatially and temporally heterogeneous environments will help quantify more aspects of a population's performance. Finally, differences in root tip count by collection type highlight the importance of quantifying root traits, particularly because the similar root mass between wild accessions and cultivars belied differences in root architecture where number of root tips in wild accessions outnumbered those in cultivars.

Conclusions

Wild collections and cultivars of *P. spicata* have different levels of trait variation in the key root trait, root tip count, as well as differing mean values in other traits. Differing trait variation in wild compared to cultivar accessions has major implications for plant performance as seen here in differences in seedling survival. While many studies have shown truncated trait and genetic variation in cultivars compared to wild populations, more studies on root traits will further our understanding of plant selection and cultivation impacts. Plant selection and cultivation practices should work to maintain high trait variability in material produced for restoration to provide ecological benefits and contribute to long-term restoration success.

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CHAPTER THREE

Induced plasticity impacts the strength of conspecific interactions

Abstract

Plant interactions play key roles in species coexistence in The Modern Coexistence Theory. This theory emphasizes stabilizing mechanisms in which conspecifics must compete more intensely than heterospecifics to promote species coexistence. Plastic responses can alter traits and interaction intensity, which may, in turn alter the strength of intraspecific interactions that can hinder or promote species coexistence. But whether plasticity induced by different types of competitors can impact mechanisms of coexistence remains unknown, and more generally, we lack an empirical understanding of the impact of plastic responses on plant-plant interactions. Here, I induced plastic responses via intraspecific and interspecific interactions, then studied the effects of these induced responses on subsequent intraspecific interactions using the grass *Pascopyrum smithii,* native to the western United States. Using a two-part greenhouse study, I first induced plastic responses in *P. smithii* by growing it with conspecific or with heterospecific neighbors of the invasive grass *Bromus tectorum*. Second, I used a transplant experiment to test whether these induced plants interacted with new conspecific neighbors differently based on whether plastic responses were induced by conspecifics or heterospecifics. First, heterospecific root and shoot mass showed plastic responses to neighbors, but not in the expected direction; both root and shoot mass were significantly greater with heterospecific neighbors compared to the control, showing that the invasive promoted facilitation. Second, heterospecific-induced individuals exhibited lower rhizome production than conspecific-induced individuals (p = 0.002). This indicates that heterospecific-induced individuals lead to more intense intraspecific

competition than did conspecific-induced individuals, even though the induced effect was positive. These results have implications for plants that encounter new neighbors through disturbance, a perennial life cycle, and transplantation from seed farm to restoration. All of these scenarios have implications for long-term species coexistence through plastic responses to a previous environment that could indirectly affect stabilizing mechanisms. This study suggests that heterospecific interactions may play an indirect role in stabilizing niche mechanisms via induced plasticity, thereby furthering our understanding of how plastic responses impact interactions and species coexistence.

Introduction

Competition is a critical process that structures plant communities and affects which species assemble and coexist (Mayfield & Levine, 2010; HilleRisLambers *et al.*, 2012; Kraft *et al.*, 2014). Strong intraspecific competition is a major factor that promotes species diversity and coexistence; when individuals limit conspecific growth more than heterospecific growth, populations can rebound from low densities, which promotes species diversity through comparatively weaker interspecific interactions (*i.e.*, a stabilizing mechanism; Chesson, 2000, 2008; Mayfield & Levine, 2010; HilleRisLambers *et al.*, 2012; Turcotte & Levine, 2016, Adler et al. 2018). However, phenotypic plasticity can alter the intensity of interactions (Miner *et al.*, 2005; Ashton *et al.*, 2010; Schiffers *et al.*, 2011; Brandt *et al.*, 2015). If plasticity affects the outcome of competitive interactions, particularly the relative strength of intraspecific vs. interspecific competition, predicted coexistence may be altered too (*reviewed in* Miner *et al.*, 2005; Turcotte & Levine, 2016).

Plastic responses that weaken intraspecific relative to interspecific competition should decrease diversity through competitive exclusion, as competitive species will be favored and dominate (*reviewed in* Miner et al. 2005, Berg and Ellers 2010, Turcotte and Levine 2016). One study showed that plasticity led to decreased competitive intensity when *Bromus hordeaceus* was grown with conspecifics, as plants avoided competitive root growth and nutrient uptake in zones near roots of conspecific neighbors (Schiffers *et al.*, 2011). Species coexistence would be hindered if *B. hordeaceus* then competed more intensely with heterospecifics, but this was not tested. Furthermore, plastic responses that increase resource uptake between heterospecifics can intensify competition (*e.g.* Acciaresi & Guiamet, 2010), but in-turn may hinder species diversity and coexistence. We can advance our understanding of how plasticity affects species coexistence through studies that directly manipulate plasticity and quantify the effects on competition (Turcotte & Levine 2016).

To-date, researchers have predominantly used predator-prey and plant-herbivore interactions to clarify the effects of plastic responses on biotic interactions. Such studies induce plastic responses, then introduce new interactions with the induced individual to characterize the impact of induced plastic responses on interaction outcomes (Relyea, 2002; Cipollini *et al.*, 2003; War *et al.*, 2011). Karban et al. (2000) showed that tomatoes near clipped sagebrush plants detected leaf damage in the neighbor, which induced volatile production that reduced herbivory relative to tomatoes near unclipped sagebrush plants. Relyea (2002) provided an example of plasticity that affected competition. Tadpoles exposed to differing competitor densities were more active and had faster growth rates, and these altered traits improved their competitive ability for food capture with subsequent conspecific competitors. However, empirical research on plant-plant induced responses impacting competitive interactions is currently lacking, despite the fact that interactions form the foundation for The Modern Coexistence Theory. Available observational data suggests these interactions are important: Taylor & Aarssen (1990) noted that the identity of associates growing with three species (*Medicago sativa, Phleum pratense,* and *Trifolium pratense*) influenced the competitive performance of tillers transplanted from a field to a competition experiment.

Evidence that prior plant-plant interactions affect subsequent interactions also comes from transgenerational plasticity and transplant studies. In transgenerational plasticity studies, researchers induce responses in the maternal plant, then quantify effects in the offspring (e.g. Bell & Sultan, 1999). These studies have shown that exposure to competition in maternal plants (Heger *et al.*, 2014; Eilers & Heger, 2019), including competition from invasive species (Mealor & Hild, 2006; Goergen *et al.*, 2011; Ferrero-Serrano *et al.*, 2011; Oduor, 2013), impacts the competitive ability and fitness of offspring compared to offspring from uninduced maternal plants. Transgenerational plasticity studies help to elucidate effects of plasticity on interactions, but the evidence is potentially less direct due to factors like recombination and outcrossing that lead to genetic differences between maternal plants and offspring. Transplant experiments are another tool that can provide strong inference related to plasticity, and researchers use this method to assess changes in interactions of organisms moved to novel climates or with novel competitors (Alexander *et al.*, 2015; Chang & Marshall, 2017; Nooten & Hughes, 2017; Cui *et al.* 2018). These studies largely show that transplanted plant communities change their interaction intensity under new climates and novel competitors. The use of a transplant approach allows the impacts of induced plastic responses on subsequent interactions to be more directly quantified.

Understanding the effects of induced plasticity on future competitive interactions has real-world implications that impact species coexistence. For instance, perennial plants exposed to different forms of disturbance or changes in native and invasive species cover, as well as nursery-grown seedlings transplanted for restoration, may all interact with different associates over time, and these different interactions may affect the outcome of subsequent interactions. To elucidate whether plant-plant interactions with conspecific or heterospecific neighbors induce plastic responses, and to determine whether these induced responses impact subsequent intraspecific interactions, I performed two interaction experiments in a greenhouse, growing a native grass *Pascopyrum smithii* (Rydb) A. Love as the focal plant with a co-occurring invasive grass, *Bromus tectorum* L. as the non-focal plant. Specifically, I first used intraspecific and interspecific interactions to induce focal plants of *P. smithii,* and then assessed impacts of induced plasticity on aboveground and belowground traits. I then tested whether conspecific-induced and heterospecific-induced plants differed in subsequent interactions with new conspecifics, thereby impacting an important component of stabilizing niche mechanisms. I hypothesized that in experiment one: Above- and belowground traits in *P. smithii* differ when grown with conspecific or heterospecific neighbors, where interactions will be more competitive when *P. smithii* is grown with a conspecific compared to a heterospecific neighbor. In experiment two I hypothesized that when grown with new conspecifics, traits differ for plants induced by conspecific neighbors compared to heterospecific neighbors.

Materials and Methods

I conducted three greenhouse studies between the summer of 2017 and in spring of 2018 at the Chicago Botanic Garden (Glencoe, IL, USA) to assess 1) whether plant-plant interactions led to plastic responses, 2) if those plastic responses affected future conspecific interactions, and 3) assess the impacts of disturbance from transplantation. The focal plant is *Pascopyrum smithii,* (‘Arriba’ cultivar (CRB, 2013)) which is a native perennial rhizomatous bunchgrass, widely used in restoration in the western United States. *Pascopyrum smithii* was grown in this study with the invasive annual grass *Bromus tectorum*, which is a conservation concern because it displaces native species and alters wildfire regimes (Chambers et al. 2007). Seeds of *P. smithii* were purchased in July 2014 (Central Milling Wheatland, UT, USA), and *B. tectorum* seeds were wild collected from Grand County, UT in June 2013. Seeds were stored in a seed drier at 13°C at 15% relative humidity in the Dixon National Tallgrass Prairie Seed Bank (Glencoe, IL, USA).

Seed germination

I germinated seeds on agar-filled 95 mm diameter petri dishes at 11°C/1°C day/night with a 12 hr/12 hr photoperiod in an incubator (Percival-Scientific, IA, USA) beginning 16 May 2017. Previous tests showed 75% germination at this temperature for seed dormancy break (Kramer & Foxx 2016), so I germinated 600 seeds at 50 per dish for a total of 12 dishes for both *P. smithii* and *B. tectorum.* I checked germination every two days and moved germinants to pots in the greenhouse after radicle emergence. Germination occurred over multiple days, but all interacting germinants in each pot were added simultaneously to minimize size differences.

Planting and experiment conditions

Experiments were sequential, and experiment one lasted for four weeks and experiment two lasted for four weeks. Experiment one and two ran from 6 June – 18 September 2017 under 21°C conditions in the daytime. I used Twenty 7.6 cm × 7.6 cm × 15.2 cm rectangular pots (Stuewe & Sons, OR, USA) per treatment for each experiment. Thus, experiment one had three treatments with 60 pots total, and experiment two had two treatments with 40 pots total. I organized pots into trays, or blocks, with 18 pots each. I filled pots with fine commercial sand. The blocks for experiment one and two were organized into separate blocks on the same greenhouse bench. I placed mesh squares at the pot bottoms to keep sand from leaking through irrigation holes. I added three plants per pot for the interaction treatments in the first experiment. I planted the focal plant in the center to interact with two neighbors, then tagged plants after emerging. I added one plant in the control group pots. I watered plants twice weekly, rotated blocks weekly, and added Murashige-Skoog (Sigma Aldrich, MO, USA) half strength nutrients (4g/L) at week two of experiment one and two. The greenhouse was sprayed weekly with MainSpring GNL insecticides (MainSpring, MD, USA) for aphids, mites, and thrips.

Experiment one: Induced plasticity

This experiment used interactions to induce plastic responses. I used three treatments with *P. smithii* as the focal plant: the control group with one plant; *P. smithii* with two conspecifics; and *P. smithii* with two heterospecific *B. tectorum* individuals (Box 3.1A). After four weeks of growth, half of the plants were randomly designated to either be harvested for data collection, or excavated and moved to new pots with new *P. smithii* neighbors.

At harvest, I gently separated the plants and washed the roots of sand. I placed each focal seedling individually on a sheet of paper and scanned them using an Epson expression 10000XL scanner (Epson, CA, USA). I counted the number of root tips from the scanned images. I recorded root mass and shoot mass after drying in an herbarium drier at 95°C for five days. Additionally, I calculated the functional trait root mass fraction (RMF: root mass/ plant mass) because greater root allocation in response to intraspecific compared to interspecific interactions has been argued to be a be a signal of strong intraspecific competition (Bennett *et al.*, 2016).

For plants selected for transplant to experiment two, I first watered the pots to saturation. Then I removed the focal plant using two spatulas, taking care to avoid disturbing the focal plant's roots, while keeping the soil column around the roots intact as much as possible. The focal plant was transferred to pots with two same-age *P. smithii* plants then watered to saturation once more (‘consequences of induced plasticity’; (Box 3.1B).

Experiment two: Consequences of plasticity experiment

This experiment tested the outcome of induced plastic responses in focal *P. smithii* plants using plants from experiment one. Using the same propagation methods and materials as experiment one, I created two treatments: (i) conspecific-induced *P. smithii* paired with new conspecific neighbors and (ii) heterospecific-induced *P. smithii* paired with new conspecific neighbors (Box 3.1, B). I started the conspecifics in experiment two concurrently with experiment one: four weeks before I added the same age induced focal plants. I placed a Ray Leach- cone-tainer (Stuewe & Sons, Inc., OR, USA) in the middle as a place holder for the induced plant, and two *P. smithii* germinants were planted around the cone-tainer. The transplanted plants grew for an additional four weeks with the new conspecifics and were eight weeks of age at harvest. I washed the plants of sand at harvest, but roots of neighbors and the focal plant were highly tangled and not separated. Therefore, my two response variables for this experiment were shoot mass for each focal plant and cumulative rhizome count in each pot. I counted the total number of rhizomes for all three plants per pot. I placed plants in paper coin envelopes in an herbarium drier prior to weighing aboveground biomass.

Supplemental experiment: Testing the impact of transplantation

I performed a supplemental experiment to assess the impact of focal plant excavation on growth of *P. smithii*. I propagated seedlings as in experiments one and two, though here, single plants were grown in commercial sand in the same size pots for four weeks. Plants were watered twice weekly and fertilized weekly with a 237 ppm of nitrogen, phosphorous, and potassium containing solution. I excavated half of the plants at week four to simulate disturbance from transplantation, then returned the plant to the same pot. The other half served as the control group where I did not excavate the plants. Plants grew for eight weeks, and the experiment ran from 7 March – 14 May 2019 at 16˚C/21˚C day night temperatures and 56% relative humidity.

Analyses

All analyses were carried out in R (R Core Team 2019 v3.6.0). I applied a square root transformation to shoot mass data to meet assumptions of normality for experiments one and two. For experiment one, I determined if interactions induced plastic responses by testing whether traits of plants in the conspecific and heterospecific treatments differed from the control treatment. I used linear models with treatment (control, conspecific and heterospecific interactions) as a categorical predictor variable, and shoot mass, root mass, and RMF as response variables in separate models. I used a generalized linear model with a Poisson error distribution to analyze the effects of treatment on root tip count. For experiment two, I determined whether heterospecific-induced treatments differed from conspecific-induced treatments. I tested if shoot mass and rhizome count responses diffed by induced treatments using separate linear and generalized linear models with a Poisson error distribution, respectively. Lastly, to determine the impact of focal plant excavation on plant growth, I used linear models to analyze the effect of excavation treatment on shoot mass and root mass.

Results

Experiment one: Induced plasticity

Interaction treatments lead to differences in plant traits. Shoot mass of focal plants differed across the interaction treatments ($R^2 = 0.37$, $F_{2,34} = 11.5$, $p = 0.0002$). Shoot mass in the heterospecific treatment was higher than the control ($p < 0.0001$), indicating facilitation, while shoot mass in the conspecific treatment was marginally higher than the control (p = 0.06, Fig. 3.1A). Belowground, root mass and RMF also differed across the treatments (root mass: R^2 = 0.14, $F_{2,36}$ = 3.2, p = 0.05; RMF: $F_{2,34}$ = 19.4, R^2 = 0.53, p = 0.01). Root mass followed a similar pattern as shoot mass, where root mass in the heterospecific treatment was higher than the control (p = 0.02), but root mass in the conspecific treatment did not differ from the control (p = 0.4, Fig. 3.1B). Conversely, RMF was lowest in the heterospecific treatment (vs. control, p = 0.003) and slightly higher in the conspecific treatment (vs. control, p = 0.2; Fig. 3.1C). Root tip count did not differ across the three treatments ($F_{1,35}$ = 49.3, p = 0.9, Fig. 3.1D).

Experiment two: Consequences of Induced plasticity

The outcomes of conspecific interactions differed depending on whether plasticity was induced by conspecific or heterospecific individuals for cumulative rhizome count, but not for shoot mass (Fig. 3.2). The cumulative rhizome count was higher when induced by conspecific plants compared to heterospecific induced plants ($F_{1,28}$ = 10.8, R^2 = 0.25, p = 0.003; Fig. 3.2B). Shoot mass did not differ between treatments (R^2 = 0, $F_{1,28}$ = 0.42, p = 0.40; Fig. 3.2A), though the effect was in the direction of larger shoot mass in the conspecific-induced compared to the heterospecific induced treatment.

Additional experiment: Testing the impact of transplantation

Mean shoot mass was affected by excavation (R^2 = 0.30, $F_{1,29}$ = 11.5, p = 0.002), where plants in the excavated treatment were smaller aboveground than plants in the control group (Fig. 3.3A). Conversely, mean root mass of plants in the excavated treatment did not significantly differ from the control group (p = 0.30; Fig. 3.3B).

Discussion

Whether induced plastic responses impact mechanisms of coexistence remains unknown, and more generally, we lack a thorough empirical understanding of the impact of induced plasticity on future plant-plant interactions (*reviewed in* Turcotte & Levine, 2016). The goal of this study was to induce plastic responses in the native grass *P. smithii* through intraspecific and interspecific interactions with the invasive grass *B. tectorum,* and then test whether those induced responses impact subsequent intraspecific interactions. I found evidence that *B. tectorum* induced plastic, facilitative responses in *P. smithii*. Importantly, I also found that induced plastic responses altered the strength of conspecific interactions, where heterospecific-induced plants were more competitive with new conspecifics than conspecific-induced plants, even though the induced interactions were positive. This is the first study to empirically demonstrate different plastic responses to conspecific and heterospecific competitors that influences subsequent interactions with conspecifics and, therefore, an important aspect of species coexistence.

Heterospecific interactions induced plastic responses

Trait differences between the control group and the treatment groups provide evidence that interactions induced plastic responses. Heterospecific neighbors exerted greater impacts on focal *P. smithii* plants than conspecific neighbors. For example, shoot mass and root mass were greatest in the heterospecific treatment, intermediate in the conspecific treatment, and lowest in the control. If stabilizing niche mechanisms are at play in a population, conspecific interactions should be more competitive than heterospecific interactions (Chesson 2000; Adler et al. 2018), which *P. smithii* demonstrates here.

The facilitative effects of *B. tectorum* on *P. smithii* biomass are surprising due to *B. tectorum's* invasive status and documented suppressive impacts on competitor biomass (Phillips & Leger, 2015). The competitive ability of this invasive species likely stems from a fibrous, resource-acquisitive root system (Evans, 1961), as well as mediated reductions in arbuscular mycorrhizal fungi colonization, observed in a native grass, *Elymus elymoides* (Owen *et al.*, 2013). However, this is not the first evidence that the *P. smithii* 'Arriba' cultivar collection used in this study competes less intensely with heterospecifics than with conspecifics and have done so with native heterospecific neighbors (Foxx *unpublished data*). Therefore, *P. smithii's* interaction dynamics with heterospecifics may be a characteristic of this cultivar. Facilitation can have important outcomes for plant interactions (Callaway & Walker, 1997; Callaway & Al., 2002) and recent research showed facilitation to explain 40% of interactions for in Tibetan alpine meadow species (Lyu *et al.*, 2017). Overall, the signals of facilitation in this and other studies call for further exploration, particularly as it relates to The Modern Coexistence theory, as facilitation is not currently integrated into this theory (Chesson 2000).

We found that for RMF, intraspecific interactions were likely more competitive than interspecific interactions, and this pattern may be indicative of intense conspecific competition important for stabilizing mechanisms (Bennet et al. 2016). We found RMF was greatest in the conspecific treatment, which was not statistically different from the control, while RMF in the heterospecific treatment was significantly lower than both the conspecific and control treatments. Generally, greater root mass allocation provides increased absorptive area and resource uptake (Wang & Taub, 2010), and is an indicator of intense competition (Berendse and Möller 2009, Acciaresi and Guiamet 2010, Leguizamón et al. 2011). It is noteworthy that measures of root and shoot traits may illustrate different contributions to species coexistence, and exploring multiple traits related to plastic responses is important to further our understanding of the role of plasticity in competitive and coexistence contexts. In particular, these results support previous findings that belowground traits and interactions are particularly important when assessing potential mechanisms of species coexistence (Silvertown *et al.*, 2015; Abbott & Stachowicz, 2016).

However, not all root traits measured here were equally informative or responsive, as root tip number surprisingly lacked plastic responses to neighbors. This contradicts evidence that plant roots tend to show dramatic plastic responses (Osmont *et al.*, 2007) of greater root tip proliferation under competition (Stevanato *et al.*, 2011; Phillips & Leger, 2015) and resource stress (Jupp & Newman, 1987; Sorgonà *et al.*, 2005, 2007). The colonization of arbuscular mycorrhizal fungi (AMF) may explain the lack of plastic responses in root tip count in which the associate AMF hyphae function to capture resources (Hodge et al. 2009), reducing the need for greater root tip proliferation. I observed emerging evidence that AMF colonization differed by treatment, but further work is needed. Generally, microbiota strongly mediate species coexistence (Hart *et al.*, 2003; Casper & Castelli, 2007; Bever *et al.*, 2010, 2015; Mangan *et al.*, 2010). A promising area of future work is to couple AMF assessments and plastic responses to fully typify the belowground contribution to species coexistence.

Heterospecific- and conspecific-induced plasticity differentially impact subsequent interactions

I found evidence that the outcome of intraspecific interactions depended on whether the focal plant was induced by conspecific or heterospecific neighbors. Rhizome production in the conspecific-induced treatment was significantly higher than in the heterospecific-induced treatment. Rhizome production is known to decline under water stress and competition (Qi *et al.*, 2012), including for *P. smithii* (Dong *et al.*, 2012; Bam, 2018; Zhang *et al.*, 2018). However, because the rhizome count in this study was for all individuals in a pot, it is unclear whether lower rhizome counts in the heterospecific-induced treatment are a result of: i) the focal plant producing fewer rhizomes due to previous interspecific interactions, ii) the non-focal individuals producing fewer rhizomes in the presence of the heterospecific-induced focal plant, or iii) a combination of both of these responses. In any case, the individuals should not differ in their rhizome production between treatments other than in response to differences in induction treatment in experiment one. Therefore, either interpretation provides evidence of a below-ground effect of induction by heterospecific or conspecific neighbors. Furthermore, the aboveground results followed the same pattern, with a non-significant trend toward lower shoot mass of the focal plant in the heterospecific induced treatment.

This study suggests that interspecific interactions may play a role in stabilizing niche mechanisms. Heterospecific-induced plasticity promoted stronger intraspecific competition in the subsequent experiment, thereby acting as an indirect stabilizing mechanism. The mechanism for how previous interspecific interactions could lead to indirect stabilizing mechanisms could lie in trait hierarchies of induced plants and new conspecific neighbors. Trait hierarchies are trait differences between a superior and inferior competitor that impact interactions – often termed fitness inequalities (Chesson 2000; Mayfield & Levine 2010). In this study, heterospecific-induced *P. smithii* plants were larger than conspecific-induced plants. Thus, heterospecific-induced plants were likely able to impose more intense competition on new conspecifics than conspecific-induced plants. Fitness inequalities are usually assessed between species (Mayfield and Levine 2010, Kunstler et al. 2012, Fort et al. 2014; Fort et al. 2015; Kraft et al. 2014), and not between conspecific neighbors, though trait differences between conspecific neighbors should also influence interaction outcomes critical to stabilizing mechanisms (*reviewed in* Ehlers et al. 2016). Future work should assess fitness inequalities between interacting conspecifics and the impact of trait variation on outcomes to expand our understanding of how traits influence interaction-based stabilizing niche mechanisms.

Restoration implications

The finding that plasticity induced by different competitors can impact subsequent conspecific interactions is of concern to practitioners interested in restoring and growing native plants, because this can affect long-term persistence of restored populations. In particular, it provides further support to previous research showing that the identity of associates growing with plants before out-planting can impact that plant's performance and interactions when transplanted to a new environment (Taylor & Aarssen 1990). Plants grown for commercial purposes are usually grown with conspecifics (Espeland *et al.*, 2017), and plastic responses to conspecifics compared to heterospecifics can result not only in the same plant, as found here, but also over multiple generations such as in transgenerational plastic responses (Rottstock *et al.*, 2017).

Future work and study limitations

For a perennial species, immigration and asynchronous emergence of neighbors can bring about new competitors within the lifetime of a plant and may affect its traits and offspring's traits. Similar methods could be used to understand the consequences of varying neighbors on a single perennial plant to understand the role iterative interactions play in subsequent interactions and coexistence. MORE research is needed to elucidate the relationship between maternal effects and potential impacts on species coexistence such as through stabilizing mechanisms.

The transplant methodology used here is the first of its kind to induce plastic responses in plants to assess impacts on subsequent interactions, a concept first review by Turcotte and

Levine (2016). The transplant approach helps to elucidate impacts of multiple biotic and abiotic environments on individuals (*see* Alexander et al. 2015). It also has advantages over transgenerational plasticity experiments when it comes to drawing inferences about changes to interaction outcomes. Transplant experiments have the issue of distinguishing between natural changes in growth and ontogeny and experimental effects. Plants in the subsequent experiment are older than plants in the prior experiment, and traits in the subsequent experiment are due in part to growth and development. Furthermore, if the lag time between an induced response and treatment application is great, the induced response may not affect subsequent interactions (Miner *et al.*, 2005). Additionally, plant life stage can influence the outcomes of species interactions (Callaway & Walker 1997). For instance, the intensity of conspecific competition for the cycad *Dioon sonorense* decreased with age, whereas heterospecific competition increased with age (Álvarez-Yépiz *et al.*, 2014). Seedlings, like the ones used here, are thought to be more sensitive to competition (Foster, 1999; Young *et al.*, 2005; James *et al.*, 2011), suggesting that the current study which focused on seedling responses, is likely most appropriate. However, future studies should explicitly incorporate plant interactions at different life stages in transplant studies and incorporate multiple interactions to determine consequences of plasticity in subsequent interactions.

In my additional experiment to test the impact of transplantation, excavation impacted shoot mass, whereas root mass was not affected. This means that interaction assessments in experiment two based on shoot mass likely underestimate the intensity of interactions. But because both treatments were excavated in the same manner, impacts on both treatments should be equal, and allows conclusions to be drawn based on the interaction treatments imposed. The excavation study showed that transplanting is less likely to impact interactions between roots in this study, so I am confident in the rhizome production outcome as a signal for plant interactions. Generally, competition is often more intense belowground (Kiaer *et al.*, 2013; Foxx & Fort, 2019), especially in arid environments (Fowler, 1986; Schenk & Jackson, 2002), such as where these seeds were sourced. As transplant experiments such as this are increasingly used, future research should investigate ways to mitigate transplant impacts.

Conclusions

Using a two-part interaction study, I show that interactions induced plastic responses in *P. smithii,* and that heterospecific-induced plants had more competitive interactions with conspecifics than conspecific-induced plants. This study provides some mechanistic evidence of how plasticity induced by interactions may affect species coexistence, via alterations to the strength of stabilizing mechanisms in the form of effects on intraspecific interactions. Furthermore, I show that previous interspecific interactions may function as an indirect stabilizing niche mechanism, which has implications for better understanding community dynamics of plants who encounter new neighbors in their lifetime. Future research should assess induced plasticity in different abiotic and biotic contexts to provide more empirical evidence on the ecological consequences of plasticity.

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CHAPTER FOUR

Source population and neighbor trait distance modulate plant interaction intensity

Abstract

Intraspecific trait variability affects plant competitive ability, but intraspecific variability effects on interaction outcomes has not been explored at length empirically in mechanisms of The Modern Coexistence Theory. The Modern Coexistence theory is predicated on two principles: 1) stabilizing niche mechanisms, in which stable species coexistence is achieved when intraspecific competition is greater than interspecific competition, and 2) equalizing mechanisms, in which the traits that influence competitive outcomes are similar among interacting species to limit competitive exclusion. Stabilizing mechanisms have been documented extensively among species, but evidence is lacking whether it varies by population. Additionally, research on the outcomes of equalizing mechanisms is often assessed through comparing interactions between one population of each species under study, though populations can vary in how the interact with one another. The contribution of root and shoot traits to competition raise questions on whether they exhibit differential outcomes with trait distances. In this study I used an interaction experiment in the greenhouse with three populations of two native western US forb species to test if stabilizing niche mechanisms vary by population. By also assessing how trait hierarchical distance and the type of interaction (inter- or intraspecific) affected interaction intensity I investigate equalizing mechanisms. And I determined whether these results vary depending on whether root or shoot traits are measured. I found evidence that population vary in whether they compete more intensely with intraspecific neighbors compared to interspecific neighbors. I also found varied evidence for whether trait hierarchical distance increased or decreased

competitiveness, with notably variable outcomes based on population identity, whether traits were root- or shoot-derived, and whether the pairings were interspecific mixtures, intraspecific mixtures, or monocultures. This is the first study to compare population responses and to compare the outcomes of root and shoots in a species coexistence context.

Introduction

There is clear evidence that plant traits vary among species as well as within and among populations of the same species (e.g. Zhang et al. 2003; Barney et al. 2009; Siefert et al. 2015; Owen et al. 2019; Zeldin et al. 2020). Some of the variation within species may be driven by adaptation to different abiotic and biotic factors, including water availability (Leguizamón et al. 2011) and invasive species (Mealor & Hild, 2006; Goergen *et al.*, 2011; Ferrero-Serrano *et al.*, 2011; Oduor, 2013). Intraspecific trait variation likely leads to intraspecific variation in responses to plant interactions (e.g. Bossdorf et al., 2009; Kulpa & Leger, 2013; Leguizamón et al., 2011; Phillips & Leger, 2015). The outcomes of interactions are important for species coexistence, in particular under the Modern Coexistence Theory (Chesson 2000). However, we lack empirical evidence of how intraspecific variation affects interactions in relation to species coexistence.

Aspects of both intraspecific and interspecific interactions are important for promoting species coexistence. One key component of the Modern Coexistence Theory is stabilizing niche mechanisms, which are density-dependent processes that cause intraspecific individuals to compete more intensely than interspecific individuals (Adler et al., 2018; *c.f.* Aguiar et al., 2001; Chesson, 2000; Mayfield & Levine, 2010). Stabilizing niche mechanisms allow populations to return from low densities as the presence of fewer intraspecific individuals lessens competitive intensity (Harpole & Suding, 2007) and allows for increased species diversity as a result of comparatively weaker interspecific competition (Chesson, 2000; HilleRisLambers et al., 2012). Several studies have demonstrated that stabilizing niche mechanisms improve species coexistence (e.g. HilleRisLambers et al. 2002; Adler et al. 2006; LaManna et al. 2017; Bever et al. 2015; Levine & HilleRisLambers 2009; Adler et al. 2018). While there is a dearth of empirical data on the subject, previous studies have suggested that stabilizing niche mechanisms are not necessarily observed in all populations (Kunin 1992) or species (Comita et al. 2010). No study to my knowledge explicitly addresses population variation in stabilizing niche mechanisms.

The second component of the Modern Coexistence theory is equalizing mechanisms, which are density-independent mechanisms that help achieve species coexistence via limited differences between traits important for competition and fitness (Chesson, 2000; HilleRisLambers et al., 2012; Turcotte & Levine, 2016). Otherwise, trait distance would lead to competitive hierarchies and promote exclusion (Adler et al., 2018; Ågren & Fagerström, 1984). To-date, equalizing mechanisms have exclusively been assessed using only one population of a species (Kunstler *et al.*, 2012; Fort *et al.*, 2014b, 2015; Kraft *et al.*, 2014). However, because different populations of different species can vary in their interaction outcomes, it is likely that the competitive outcome of different interspecific pairings will vary based upon which populations are used. This prediction has yet to be explicitly tested, despite its importance to understanding which populations of species can coexist.

The trait distances related to equalizing mechanisms are hierarchical, meaning the direction and magnitude of distance matter to interaction outcomes. Hierarchical trait distances are usually calculated by subtracting the trait of the focal plant from the trait of the non-focal neighbor plant (Kunstler *et al.*, 2012; Fort *et al.*, 2014b; Kraft *et al.*, 2014). Under The Modern Coexistence Theory, a positive relationship between hierarchical trait distance and increasing negative interactions between two neighbors indicates fitness inequalities, and the conditions for equalizing mechanisms are not met (i.e., trait hierarchy hypothesis; Box 4.1B) (Kunstler *et al.*, 2012; Fort *et al.*, 2014b, 2015; Kraft *et al.*, 2014; Gross *et al.*, 2015; Carmona *et al.*, 2019). Alternatively, the trait distance hypothesis predicts that greater distance between neighbors lead to more positive interactions (Box 4.1C) (Fargione & Tilman 2005; Bennett et al. 2016). There is mixed evidence of whether greater trait distance increases or decreases negative interactions, but to-date the majority of studies that explicitly test the relationship between trait distance and interaction intensity support the trait hierarchy hypothesis (Kunstler *et al.*, 2012; Fort *et al.*, 2014b, 2015; Kraft *et al.*, 2014; Gross *et al.*, 2015; Carmona *et al.*, 2019).

Presently, nearly all research investigating the impact of trait hierarchies on coexistence has focused at the interspecific level (Carmona et al., 2019; Fort et al., 2015, 2014; Gross et al., 2015; Kraft et al., 2014; Kunstler et al., 2012, but see Abbott & Stachowicz, 2016). Yet both theoretical and empirical evidence suggests that trait hierarchies at the intraspecific level also play a role in driving mechanisms of coexistence (Barabas & D’Andrea, 2016; Hart et al., 2016; Lichstein et al., 2007; Ehlers et al. 2016). One empirical example of intraspecific trait distance affecting competition comes from Abbott & Stachowicz (2016) which showed that trait distance between interacting genotypes of eel grass led to competitive exclusion (i.e., the trait hierarchy hypothesis, Box 4.1, B). This study shows that equalizing mechanisms are manifest at the intraspecific level, but further research is needed to investigate the extent to which intraspecific trait variation impacts competition intensity and related impacts on coexistence.

While many empirical studies investigating the impacts of trait distance on interaction intensity focus on above-ground traits (Kraft et al. 2014; Gross et al. 2015), it is increasingly clear that roots are strong drivers of competition, with recent research showing that root-root competition is more intense than shoot-shoot competition, with different outcomes in response to resource stress (Foxx & Fort, 2019; Kiaer et al., 2013). This is supported by Abbott & Stachowicz (2016), where increasing trait distance in root mass values between a focal plant and a neighbor increased competition and influenced coexistence, whereas distance in aboveground biomass had little effect. Furthermore, Bennett et al. (2016) quantified the intensity of intraspecific and interspecific interactions and showed that plants that had stronger intraspecific competition (one component of stabilizing mechanisms) allocated more biomass to their root systems. These studies implicate the importance of root responses in competition for both stabilizing and equalizing mechanisms, and thus species coexistence. Further research is needed to quantity the influence of roots relative to shoots to uncover patterns that underpin mechanisms of species coexistence.

In this study I tested stabilizing niche mechanisms, whereby intraspecific individuals compete more intensely than with interspecific individuals, and whether this varies among populations of two different species competed in inter- and intraspecific pairs. Stabilizing niche mechanisms were measured by calculating interaction intensity based on full plant mass for all possible interspecific and intraspecific interactions. I also tested fitness inequalities in two ways: 1) to explicitly compare interspecific mixtures, intraspecific mixtures, and monoculture, I characterized the relationship between neighbor root and shoot mass hierarchical trait distance and root allocation patterns, and 2) I compared intraspecific and interspecific mixtures to characterize the relationship between neighbor root and shoot length hierarchical trait distance and interaction intensity. To do this, I used three populations of two co-occurring forb species native to the US. Using a greenhouse study, I interacted two individuals in each of three interaction types: monoculture, intraspecific mixture, and interspecific mixture. After seven weeks of growth, I harvested plants and assessed full plant mass, root and shoot mass, root and shoot length, and root and shoot mass fraction. First (H1), I hypothesize that focal populations vary in whether they compete more intensely with intra- compared to interspecific neighbors. Second (H2), I hypothesize that neighbor mass distance influences allocation, and these relationships are affected by source population, interaction type, and whether traits are root- or shoot derived. And third (H3), I hypothesize that neighbor root and shoot length distance affect interaction intensity, and focal population source and interaction type affect these relationships.

Materials and Methods

Species and population information

I used two co-occurring forbs native to the western United States from the Asteraceae: *Machaeranthera canescens* (Pursh) A. Gray and *Heterotheca villosa* (Pursh) Shinners. These are priority species for use in large-scale restoration efforts in the Colorado Plateau (Wood *et al.*, 2015). *Machaeranthera canescens* is a common, short-lived perennial forb growing between 15 and 75 cm tall that flowers in late summer (Tilley *et al.*, 2014), and *H. villosa* is a perennial forb growing between 5 and 70 cm (Semple, 1996). I used three populations of *M. canescens* and three populations *H. villosa* from the Colorado Plateau (Table 4.1). Seeds of both species were requested through the Seeds of Success program, which uses standardized seed collection protocols for seeds collected in the wild (Haidet & Olwell, 2015).

Propagation and set-up

I propagated and established plants in the greenhouses at the Chicago Botanic Garden (Glencoe, IL, USA). I planted seeds in 200-cell trays with loam soil. I planted one seed into each cell then watered. Planting was carried out over 10 days in August 2018. Immediately after planting, I placed all trays in refrigerators at 3°C in the dark for two - three weeks to break dormancy, then moved to a fog house simultaneously at 18.3°C under ambient light to keep the soil moist and encourage establishment for four weeks. Following establishment, I moved plants to experimental treatments in a greenhouse at 20°C/17.2°C day/night temperatures and 60% relative humidity. I moved seedlings from the 200-cell tray after watering to keep soil intact as much as possible. I planted the seedlings between 8 October 2018 and 16 October 2018 into 5.1 cm x 10.2 cm rectangular pots (Stuewe & Sons, Tangent, OR) with a 30% sand and 70% loam soil mixture then I immediately watered the seedlings. I added two seedlings to each pot, and 18 pots per tray for a total of 358 pots. There were 22 trays total, representing blocks. I counted the number of true leaves for each seedling upon planting because seedlings varied in initial size at planting. Plants interacted for seven weeks and I watered once every four days and fertilized the plants at week one and week four with a solution containing 237 ppm nitrogen, phosphorous, and potassium. The greenhouse was sprayed weekly with MainSpring GNL insecticides (MainSpring, MD, USA) for aphids, mites, and thrips.

Experimental treatments

Machaeranthera canescens acted as the focal plant in all pairwise competition treatments. There were 18 interaction treatments: nine intraspecific and nine interspecific treatments (Table 4.2). For the intraspecific treatments, three were single-population monocultures for each of three *M. canescens* focal populations. The six intraspecific mixture interaction treatments each had one of three focal *M. canescens* populations paired with each of the other two *M. canescens* populations for the following population combinations: AB, AC, BA, BC, CA, CB (Table 4.2). For the nine interspecific mixtures, each focal *M. canescens* population interacted with each of the three *H. villosa* populations. Thus, 'interaction type' represents three levels: monoculture, intraspecific mixtures, and interspecific mixtures.

Data collection

At harvest, some pots had roots grow through drainage holes, which I cut at the base of the pot and did not consider because the growth was likely in response to soil spilled below the pots in the trays. Roots of the two interacting plants were separated using hair conditioner (Alberto Vo5, Melrose Park, IL, USA) diluted in tap water, then rinsed with water. Next, I measured plant height and length of the longest root. I then separated plants into aboveground and belowground parts before I placed each part in separate in coin envelopes. Plants were then dried in an herbarium drier at 95˚C for one week. Plant samples were then kept at room temperature until processing two months later. Dried roots and shoots were then weighed.

Calculations

I used full plant mass, root mass, shoot mass, root length, and shoot length to make seven calculations (Table 4.3). I calculated root mass fraction (RMF: root mass divided by total mass), and shoot mass fraction (SMF: shoot mass divided by total mass). To address hypothesis one, I calculated the log response ratio (lnRR) as a metric of interaction intensity. The lnRR is calculated for all treatments as the log of the treatment mean divided by the monoculture mean (Hedges et al., 1999) and represents the proportional size difference between plants in intraspecific and interspecific mixtures relative to the monoculture. Negative values denote competition, zero values denote neutral interactions, and positive values denote facilitation (Hedges et al., 1999; Suding et al., 2003). I calculated lnRR on full plant mass.

I calculated hierarchical trait distances (hereafter, trait distance) to address hypotheses on equalizing mechanisms using the equation $t_a - t_b$, where t_a is the trait of the focal plant and t_b is the trait of non-focal neighbor plant (Kunstler *et al.*, 2012; Fort *et al.*, 2014b; Kraft *et al.*, 2014). I made one calculation for each pot between plant pairings for four trait distances: 1) root length difference, 2) plant height difference, 3) root mass difference, and 4) shoot mass difference (Table 4.3). Trait distances close to zero indicate the neighbor traits were similar, whereas negative values indicate that the focal plant was smaller than the non-focal plant, and positive values indicate that the focal plant was larger than the non-focal plant. I calculated lnRR on root mass and shoot mass to test H2 and utilized lnRR on full plant mass to address H3. The following variables were square root transformed to meet assumptions of normality: full mass, shoot length, root length distance between neighbors; and non-focal root mass, and plant height distance between neighbors were log transformed. I removed one focal SMF, one non-focal SMF, and one focal RMF that were outliers.

Analyses

All analyses were carried out in R (R Core Team, 2019, v3.6.1). I first determined if initial leaf count affected the relationships tested by comparing mixed effects models with initial leaf count difference between neighbors as a random effect in all models to fixed effects models without the random effect. Models without the random effect had higher loglikelihood values, therefore, I did not include the random effect in the models and proceeded with fixed effects-only models.

I tested whether interaction intensity varied with interaction type by focal population (H1). I compared two linear models for each of the three focal populations: the maximal model containing lnRR response and interaction type predictor, and the null model. I used a likelihood ratio test to compare the models and interaction type predictor was retained if the $p \leq 0.05$.

To determine the relationships between root and shoot mass distance and root and shoot mass allocation (H2), I tested if neighbor trait distance in root mass and shoot mass influences RMF and SMF, respectively, and whether these relationships were moderated by interaction type, neighbor identity, focal plant source population, and whether traits are derived from roots or shoots. I used a linear model with a continuous predictor of root and shoot mass distance and continuous responses of SMF and RMF. The maximal models contained a five-way interaction between mass difference, population source, neighbor identity nested within interaction type, interaction type, and whether traits are derived from roots or shoots. I selected the best model using the "stepAIC" function in the MASS package (Venables & Ripley, 2002) which selects models with the lowest Akaike Information Criterion (AIC) value.

To assess the relationships between root and shoot length distance and interaction intensity (H3), I tested if neighbor trait distance in root length and shoot length influences interaction intensity based on root mass and shoot mass, respectively, and whether these relationships were moderated by interaction type and focal plant source population. I used linear models with a continuous predictor root and shoot length distance and interaction intensity based on root and shoot mass. The maximal models contained a three-way interaction between length difference, population source, and interaction type. I selected the best model using the "stepAIC" function in the MASS package (Venables & Ripley, 2002). I acquired summary statistics for best models selected using the AIC procedure using the "anova" and "summary" functions.

Results

Populations vary in whether they compete more intensely with intra- compared to interspecific neighbors (H1)

Interaction intensity varied among populations in intraspecific mixtures and interspecific mixtures (Fig. 4.1). The MACA-A population interacted with all interspecific neighbors and with MACA-B positively, whereas interactions with MACA-C were negative. Interaction type did not explain differences in interaction intensity (R^2 = 0.42, $F_{1,3}$ = 2.2, p = 0.2) (Fig. 4.1). The MACA-B population interacted with all interspecific neighbors positively and negatively with all intraspecific mixtures. The MACA-B population when interacting with MACA-A had a similar interaction outcome as the monoculture (lnRR near 0) (Fig. 4.2). Interaction type did not explain differences in interaction intensity (R^2 = 0.36, $F_{1,3}$ = 1.6, p = 0.3) (Table 4.4). The MACA-C population interacted positively with all intraspecific mixture and interspecific mixture neighbors. Interaction type did not explain differences in interaction intensity for MACA-C (R^2 = 0.36, $F_{2,3}$ = 1.6, p = 0.3) (Fig. 4.1).

Neighbor mass distance influences allocation, and these relationships are affected by source population, interaction type, and whether traits are root- or shoot derived (H2)

Variation in RMF and SMF is predicted by a five-way interaction between mass difference, focal population source, non-focal population source nested within interaction type, interaction type, and whether traits are derived from roots or shoots, (R^2 = 0.95, $F_{45,498}$ = 195.4, p < 0.0001) (Table 4.4). Root mass and shoot mass allocation (RMF and SMF, respectively) responded similarly to neighbor trait for root and shoot mass. As distance between neighbor masses increased, this meant the focal plant was larger than the non-focal plant, creating a hierarchy and the focal plant responded by increasing allocation to roots and shoots. This relationship led to positive slopes to distance in neighbor mass for interspecific mixtures for all three *M. canescens* focal populations (Fig. 4.2). Monoculture and intraspecific mixtures had the same pattern for allocation responses to mass distance. Neighbor's RMF and SMF responded in different ways to distance in neighbor root and shoot mass. For root mass, as the distance between neighbor masses increased, the focal plant was larger than the non-focal plant, the focal plant responded by increasing RMF. This relationship led to positive slopes in distance in neighbor mass for intraspecific mixtures and monocultures for all three *M. canescens* focal populations. Conversely for shoots, as the distance between neighbor masses increased, i.e. the focal plant was larger than the non-focal plant, the focal plant responded by decreasing SMF (Fig. 4.2). This relationship led to negative slopes to distance in neighbor mass for intraspecific mixtures and monocultures for all three *M. canescens* focal populations. The MACA-A monoculture treatment had positive slope, in other words, the distance between neighbor shoot mass increased as did the focal plant SMF.

Neighbor root and shoot length distance affect interaction intensity, and focal population source and interaction type affect these relationships (H3)

The MACA-A and MACA-B populations had similar relationships between distance in neighbor root and shoot length and interaction intensity where intraspecific mixtures and interspecific mixtures had positive and negative relationships, respectively (Fig. 4.3). For interspecific mixtures, as the focal plant became larger than the non-focal plant, interactions were more positive, denoting increasing facilitation with increasing trait distance. Whereas for intraspecific mixtures, as the focal plant became larger than the non-focal plant, interactions were more negative, denoting increasing competition with increasing trait distance. The MACA-C population showed a differing response to MACA-A and MACA-B, where both the intraspecific mixtures and interspecific mixtures had positive relationships for how distance in neighbor root and shoot length affected interaction intensity (Fig. 4.3). As the focal plant became larger than the non-focal plant, interactions were more positive, denoting increasing facilitation with increasing trait distance. The relationship between interaction intensity and distance in neighbor root and shoot length was explained by the interaction between focal population source and interaction type (Linear model: $R^2 = 0.78$, $F_{11,18} = 5.7$, $p = 0.0006$) (Table 4.5).

Discussion

This study provides empirical evidence that intraspecific variation at the population level influences mechanisms of coexistence, as support for stabilizing niche mechanisms and equalizing mechanisms differed among populations of *Machaeranthera canescens,* and were influenced by interaction type (interspecific mixtures, intraspecific mixtures, and monocultures), trait hierarchy, and whether belowground and aboveground trait distance were investigated. These results illustrate why intraspecific variation warrants further explicit consideration in The Modern Coexistence Theory. Specifically, I found that not all populations displayed evidence of stabilizing niche mechanisms, as they vary in whether they compete more intensely with intraspecific neighbors compared to interspecific neighbors. I also found that not all populations exhibit equalizing mechanisms, and this varied with neighbor identity and whether traits were root- or shoot-derived. In this study, I demonstrate that populations varied in how they responded to neighbor trait distance, and whether the neighbor was of the same or a different species. This is the first study to compare population responses and to compare the outcomes of root and shoots in a species coexistence context.

Stabilizing mechanisms vary by population

Populations had different responses to intraspecific and interspecific interactions. This denotes variation in stabilizing mechanisms by population (Fig 4.4) and the need to consider the performance of individual populations, rather than making broad generalizations about a species from one population. One study assessed density dependence in two populations of *Diplotaxis erucoides* but did not identify or hypothesize about intraspecific variation in density dependence (Kunin 1992). The variability in intensity of interactions with interspecific neighbors of *M. canescens* also underscores the need to assess performance of multiple populations and species, though theory and most empirical assessments focus on interspecific relationships (Chesson, 2000; Kunstler et al., 2012; Carmona et al., 2019). This also highlights the impact that different species and populations have on plant growth and interaction outcomes.

Plants from different populations or genotypes respond differently to competition (Ehlers et al., 2016; Leguizamón et al., 2011; Stevanato et al., 2011), and intraspecific variation plays a dynamic role in species coexistence (Ehlers et al. 2016). Results of this study indicate that mixing populations of the same species may lead to different competitive outcomes based on population identity. For example, MACA-C had positive interactions with all interacting populations and species. If this population were mixed with either MACA-A or MACA-B, their interaction dynamics could lead to positive population growth for both populations but may in turn have negative outcomes for another interacting species. However, if MACA-A and B were mixed, my results suggest that they would not behave differently than if they were grown separately. Because intraspecific variation affects community structure and composition (Booth & Grime, 2003; Crawford & Rudgers, 2012; Crutsinger et al., 2008; Hughes et al., 2006), assessing variation in competitive outcomes requires more attention.

Root and shoot traits respond variably to mass distance and its influence on allocation

The root and shoot competitive responses to trait distance provided evidence in support of trait hierarchy hypothesis and trait distance hypothesis, which varied by population and interaction type. For allocation patterns, the focal plant imposed more intense root competition with greater neighbor distance (i.e. the focal plant had larger mass than the non-focal plant). This indicates a competitive hierarchy between neighbors for RMF and competitive exclusion is predicted when root traits differ greatly, hindering coexistence. This study is in line with Abbott & Stachowicz (2016) which showed that for eelgrass genotypes, root mass increased with increases in multivariate trait distance between neighbors. Greater allocation to roots or shoots signals the ability of roots or shoots to impose intense competition belowground and aboveground, respectively (Acciaresi & Guiamet 2009; Poorter et al. 2012), where plants tend to allocate less to roots when grown alone and under less intense resource competition (Acciaresi & Guiamet 2009; Berendese & Möller 2009).

Aboveground responses for interspecific mixtures had similar patterns for all three focal *M. canescens* populations. As neighbor shoot mass distance increased, so did focal plant shoot mass fraction. The positive relationship results in similar interaction outcomes as root mass fraction and mass distance in which the focal plant imposed more intense shoot competition when there were shoot mass distance in which the focal plant was larger than the non-focal plant. This indicates a competitive hierarchy between interspecific neighbors for SMF and competitive exclusion is predicted when shoot traits differ greatly hindering coexistence.

Intraspecific mixtures and monoculture treatments had varied root and shoot relationship directions, whereby population differences and interaction type affected responses to neighbor mass. For MACA-C and MACA-B, roots and shoots had opposing responses in which relationships belowground were positive and negative aboveground. Response patters indicate that intraspecific interactions for these populations follow trait hierarchy belowground and trait distance outcomes aboveground. This outcome is important as it indicates differing rules for interactions above- and belowground. The trait distance hypothesis denotes that large trait distance between interacting individuals weakens competition (Macarthur & Levins, 1967; Mayfield & Levine, 2010; Kraft *et al.*, 2014). Furthermore, these results also support hypotheses that shoot competition is symmetrical and proportionate to shoot size (Casper & Castelli, 2007; Del Río et al., 2014; Freckleton & Watkinson, 2001).

Lastly, MACA-A showed differing aboveground relationships compared to MACA-B and C, in which intraspecific mixtures differed from the monoculture with negative and positive relationships, respectively. The relationship differences demonstrate that for MACA-A responses in intraspecific mixtures cannot be equated to responses or performance in single population monocultures. The response of MACA-A monoculture treatment follows outcomes of trait distance hypothesis and agree with other findings that demonstrate the importance of light availability on interaction outcomes (Kunstler *et al.*, 2012). The other populations and intraspecific treatments may fail to exhibit this relationship because the plants under study are from dry, light unlimited environments, grown in a light-unlimited greenhouse, and may be exhibiting opposing responses because light is not a limiting resource. In general, the relationship between neighbor mass distance and allocation provides some evidence that root traits and shoot traits are explained by different mechanisms for intraspecific neighbors – adding potential caveats of expectations for fitness inequalities important to species coexistence.

Trait distance influence interactions along interaction types and populations

Results of this study show for the first time that equalizing mechanisms can play out at the intraspecific level and not at the interspecific level. The intraspecific and interspecific treatments had differing relationships with neighbor root and shoot length distance and interaction intensity for MACA-A and B. Intraspecific mixtures followed a trait hierarchy: as neighbor root and shoot length distance increased, so did negative competitive interactions. These relationships are in line with other studies conducted at the species level (Fort et al., 2015, 2014; Kraft et al., 2014; Kunstler et al., 2012; Gross et al. 2015; Carmona et al. 2019).

Conversely, interspecific mixtures followed a trait distance hypothesis relationship: as neighbor root and shoot length distance increased, so did positive facilitative interactions. The MACA-C population exhibited a trait distance hypothesis pattern for both intraspecific and interspecific mixtures. The trait distance hypothesis relationship between interspecific neighbors is thought to hinder stable species coexistence because variability in interacting species' trait values is an expectation (Chesson 2000). Thus, if any of the *M. canescens* A and B and *H. villosa* populations co-occurred, the stabilizing niche mechanisms of MACA-A and B would need to act to overcome the negative impacts from trait distance. The results on stabilizing mechanisms of this study (Fig. 4.1) suggest they may.

Restoration implications

Restoration practitioners seek long-term persistence of planted material introduced to revegetate habitats (SER, 2002). Some restoration practitioners have recently begun introducing multiple populations of a species at a site ('admixture provenancing,' *e.g.* Breed et al., 2013; Bucharova et al., 2019). Mixing multiple populations has many ecological benefits, such as: greater variability, higher aboveground productivity (Crutsinger *et al.*, 2006; Hughes *et al.*, 2008), higher plant establishment from low initial densities (Cook-Patton et al., 2016), and increases in the abundance and diversity of higher trophic level organisms (Crawford & Rudgers, 2013; Crutsinger et al., 2006). However, these are outcomes evaluated with short-term studies and we do not know if in the long run certain genotypes outcompete others – resulting in lower genotypic richness and the over-representation of competitive genotypes (*see selection effect*

Hughes et al. 2008). Booth & Grime (2003) provided some evidence of the selection effect in which high initial genotypic diversity of a species mixture promoted higher species diversity over time, though there was a reduction in surviving genotypes at the study's conclusion. While high initial intraspecific variation had benefits towards species diversity, certain populations and combinations may fail to persist, and loss of planted material is detrimental to the resource intensive nature of seed-based restoration methods. In this study, population combinations that interacted with facilitation might have short-term restoration benefits if combined in admixtures but fails to exhibit density dependence that would benefit its own population at low densities leading to extinction (*e.g.* Population C, Fig 4.1). Forecasting the effects of combining populations and species should be a more informed process that considers both intraspecific and interspecific interactions and how processes change over time.

Future research

This study provided evidence that competitive responses to trait hierarchies vary when assessing different populations, on root or shoot traits, and among intraspecific and interspecific neighbors. To assess patterns in these outcomes, future research should assess how other species and populations interact with each other and whether their traits and interactions would hinder or promote species coexistence. Furthermore, the species of this study are from the arid western US, and competition is thought to be concentrated belowground in these biomes (Fowler, 1986) and may explain the differential above- and belowground responses to competition. Species from other biomes may respond differently and represents interesting future studies. These species may have grown smaller than if grown with more space, though growth in controlled settings provides important proxies to field conditions (Schroeder-Georgi *et al.*, 2016). Future work assessing coexistence mechanisms on multiple populations in outdoor field settings would curtail issues of pot size constraining root growth and introduce other biotic and abiotic factors that may influence coexistence. Furthermore, more work assessing traits other than mass is extremely important in identifying patterns in interaction outcomes with greater resolution such as root architectural traits and other functional traits (*e.g.* Fort et al., 2015, 2014).

Conclusions

This study shows that trait hierarchies that form equalizing mechanisms differ between root and shoot traits – even at the population level - and that populations can vary in these hierarchies. Furthermore, I show that stabilizing mechanisms are not observed in all populations of the same species. The results assessing equalizing mechanism provide further caveats to explore on how and why root and shoot traits differ in response to neighbor's traits. These outcomes also have important implications for restoration for population mixing whereby some populations may have better or poorer coexistence probabilities when mixed. This work should stimulate future studies on the mechanisms underlying stabilizing niche mechanisms and equalizing mechanisms related to traits, specifically how root and shoot traits influence species coexistence to deepen reasonings of the Modern Coexistence Theory.

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Tables & Figures

CHAPTER ONE

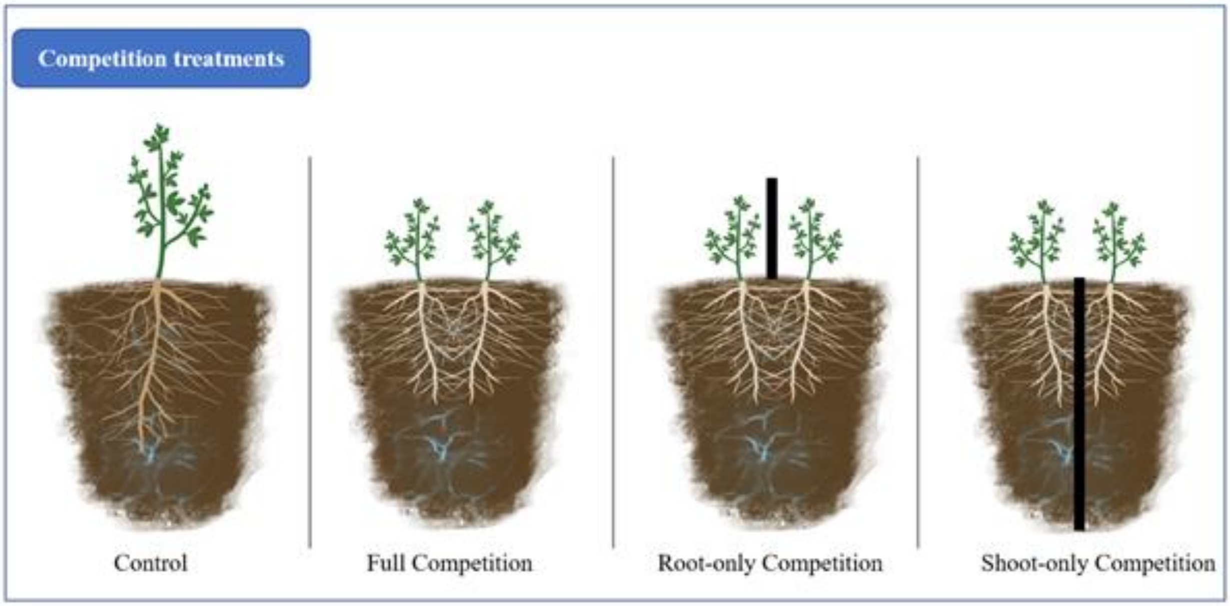


Figure 1.1. Study treatments. Competition treatments of root-only, shoot-only, full competition and, monoculture of partition studies.

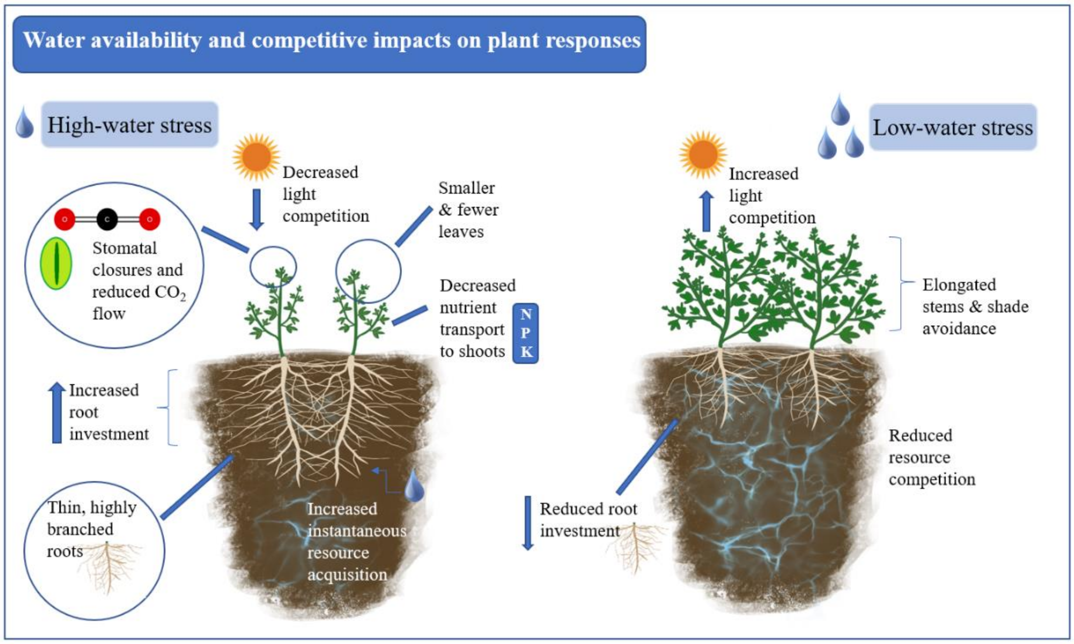


Figure 1.2. Competition and water stress impacts. Morphological and physiological above- and belowground competitive responses to water availability.

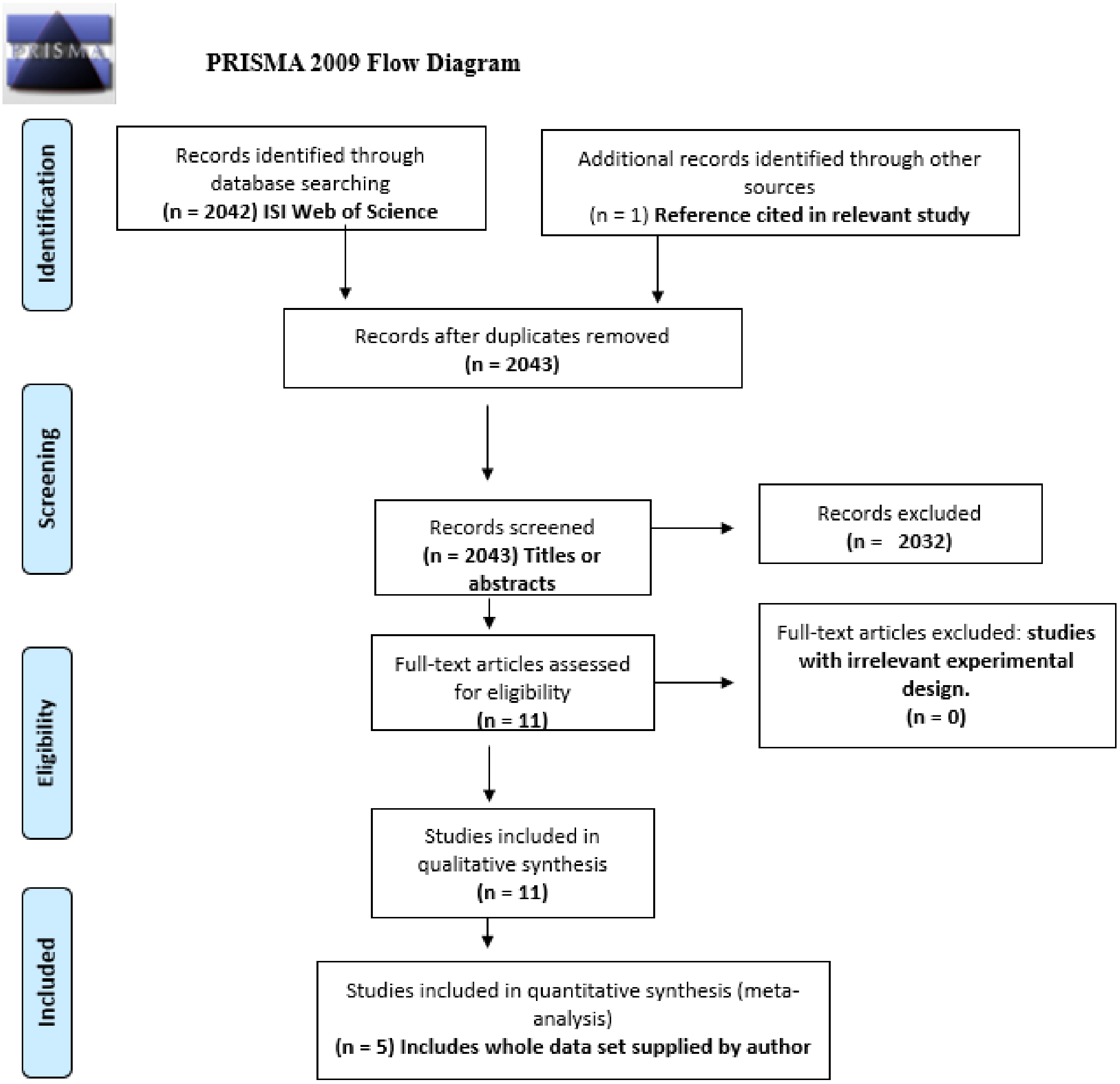


Figure 1.3. PRISMA flow diagram for study selection. *From:* Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097. **For more information, visit:** www.prisma-statement.org. (Moher D, Liberati A, Tetzlaff J, Altman DG, 2009)

Table 1.1. Characteristics of studies assessed in this systematic review.

Study	Experimental Setting	Target Species	Response measure	Used in Meta-analysis
Bartelheimer et al. 2010	Outdoor – mesocosm	*Senecio aquaticus; Senecio jacobaea*	Total biomass	Yes
Bornkamm et al. 1975	Setting unknown – pots	*Arrhenatherum elatius; Bromus erectus*	Root biomass	Yes
Lamb et al. 2007	Outdoor – plots	*Artemisia frigida; Chenopodium leptophyllum*	Shoot biomass	Yes
Weigelt et al. 2005	Outdoor – mesocosm	*Carex arenaria; Corynephorus canescens; Hieracium pilosella*	Total biomass	Yes
Wilkinson & Gross 1964	Greenhouse – pots	*Trifolium repens*	Total biomass	Yes
Salinger & Bornkamm 1982	Setting unknown – pots	*Arrhenatherum elatius; Bromus erectus*	Shoot:Root ratio	No
Putz and Canham 1992	Outdoor – plots	*Cornus racemosa*	Basal area daily growth rate	No
Dauro & Mohamed-Saleem 1995	Outdoor – mesocosm	*Triticum durum* var. Boolai; *Trifolium quartinianum*	Total biomass	No
Semere & Froud-Williams 2001	Greenhouse – pots	*Zea mays*	Shoot biomass	No
Haugland & Froud-Williams 1999	Greenhouse – pots	*Lolium perenne*	Total biomass	No
Welbank 1961	Outdoor – pots	*Impatiens parviflora*	Biomass growth rate	No

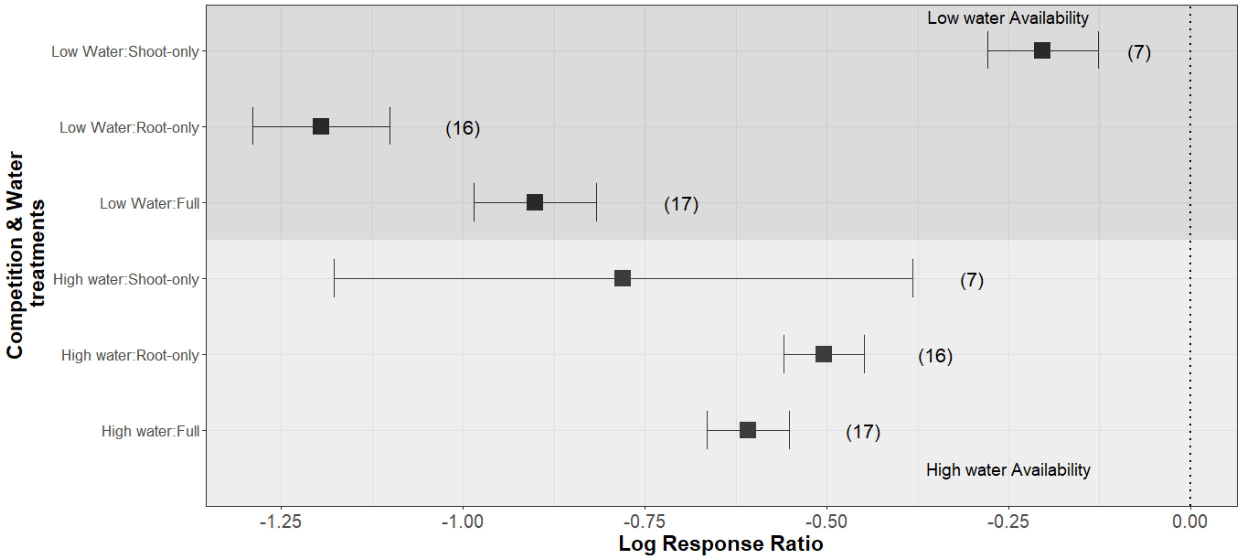


Figure 1.4. Effects of water availability and competition on plant growth**.** Meta-estimates (square points) and 95% confidence intervals. Smaller values indicate intense competition, while larger values indicate weaker competition. Sample sizes of lnRR values are in parentheses.

Table 1.2. Table of model outcomes. Q test statistic assess significance of between study variation (Hedges *et al.*, 1999); T^2 measures the between study variance; and I^2 measures variance explained by heterogeneity between studies (Viechtbauer, 2010).

Factor	Df	Q_m	T^2	I^2	P -value
Competition treatment	2	28.7	0.54	98.9%	<0.0001
Water treatment	1	218.1	0.57	99.4%	<0.0001
Competition + water treatment	3	312.6	0.53	98.7%	<0.0001
Competition * water treatment	5	395.5	0.53	98.1%	<0.0001

CHAPTER 2

Table 2.1. *Pseudoroegneria spicata* accession information for wild-collected and cultivar collections. Sample size surviving to harvest, as well as survival percentage are also provided.

Collection Name	Source: County, State	Collection Type	Sample size	Survival
‘Goldar’	Asotin County, WA	Cultivar	17	67%
‘P-7’	Multiple counties in six states* and one Canadian province	Cultivar**	18	60%
‘Whitmar’	Whitman County, WA	Cultivar	20	57%
Wild 1	Sevier county, UT	Wild-collected	27	90%
Wild 2	Sevier county, UT	Wild-collected	22	73%
Wild 3	Sevier county, UT	Wild-collected	21	70%

* Washington, Idaho, Oregon, Utah, Montana, and Nevada, and British Columbia (Canada).

** Counted as a cultivar in analyses, but officially released as “selected germplasm”

Table 2.2. Variation in traits of wild and cultivar assessments. Percent differences (% Difference) is the % variability of all three wild-collected accessions compared to all three cultivar accessions (100 – cultivar variance/wild-collected variance *100) and convey the wild accessions % variance compared to the cultivars.

Trait	Collection Type	Variance	% Difference	p-values	Trait type
Root tip count	Cultivar	43.5	+37%	0.07	Root
	Wild-collected	69.4			
Root length	Cultivar	0.75	-4%	0.9	
	Wild-collected	0.72			
Root mass	Cultivar	0.41	+25%	0.3	
	Wild-collected	0.55			
RMF	Cultivar	0.013	+46%	0.02	
	Wild-collected	0.024			
Leaf count	Cultivar	2.3	+0%	0.6	Shoot
	Wild-collected	2.3			
Plant height	Cultivar	0.05	+38%	0.1	
	Wild-collected	0.08			
Shoot mass	Cultivar	0.26	+13%	0.6	
	Wild-collected	0.30			
SMF	Cultivar	0.013	+46%	0.02	
	Wild-collected	0.024			

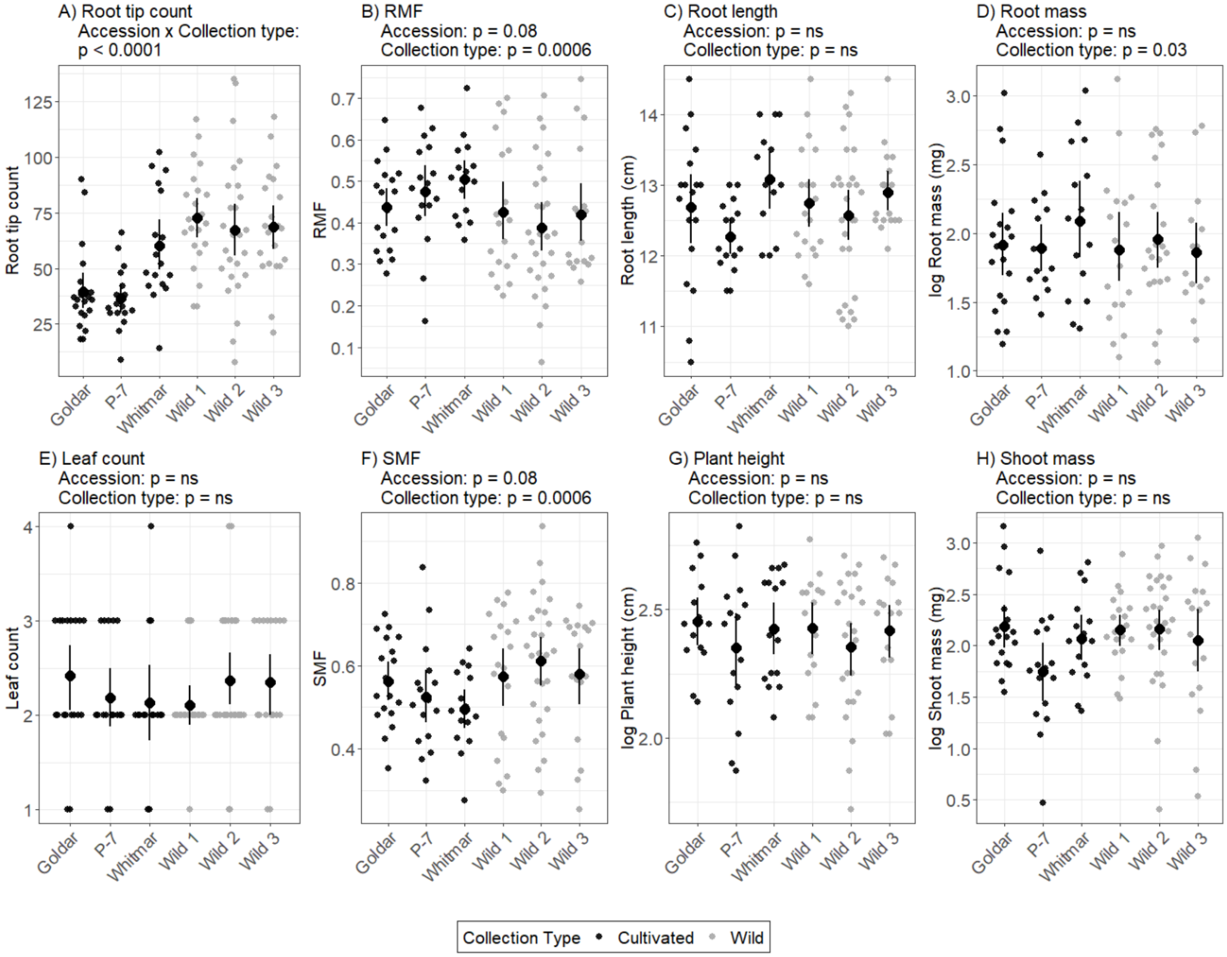
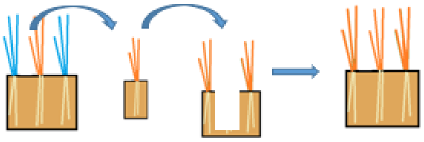
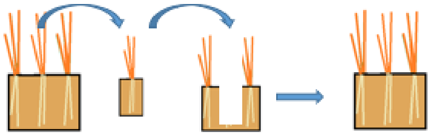


Figure 2.1. Mean and 95% confidence intervals of accessions of *Pseudoroegneria spicata* seedlings for root traits and shoot traits. A) root tip count, B) RMF, C) root length, D) root mass, E) leaf count, F) SMF, G) plant height, and H) shoot mass

CHAPTER THREE

Box 3.1. *Pascopyrum smithii* treatment groups and final sample size for the two main greenhouse experiments. Heterospecific interaction treatments are with *Bromus tectorum* neighbors. A) experiment one on induced plasticity, B) experiment two on the consequences of induced plasticity. Treatment sample sizes are in parentheses.

Experiment	**Treatment**	**Treatment diagram**
A) One: Induced plasticity	Control	 Harvested at 4 weeks (n = 14)
	Heterospecific competition	 Harvested at 4 weeks (n = 17) or excavated for experiment two
	Conspecific competition	 Harvested at 4 weeks (n = 13) or excavated for experiment two
B) Two: Consequences of Induced plasticity	Heterospecific induced	 Excavated at 4 weeks, moved with conspecifics, harvested at 8 weeks (n = 6)
	Conspecific induced	 Excavated at 4 weeks, moved with conspecifics, harvested at 8 weeks (n = 14)

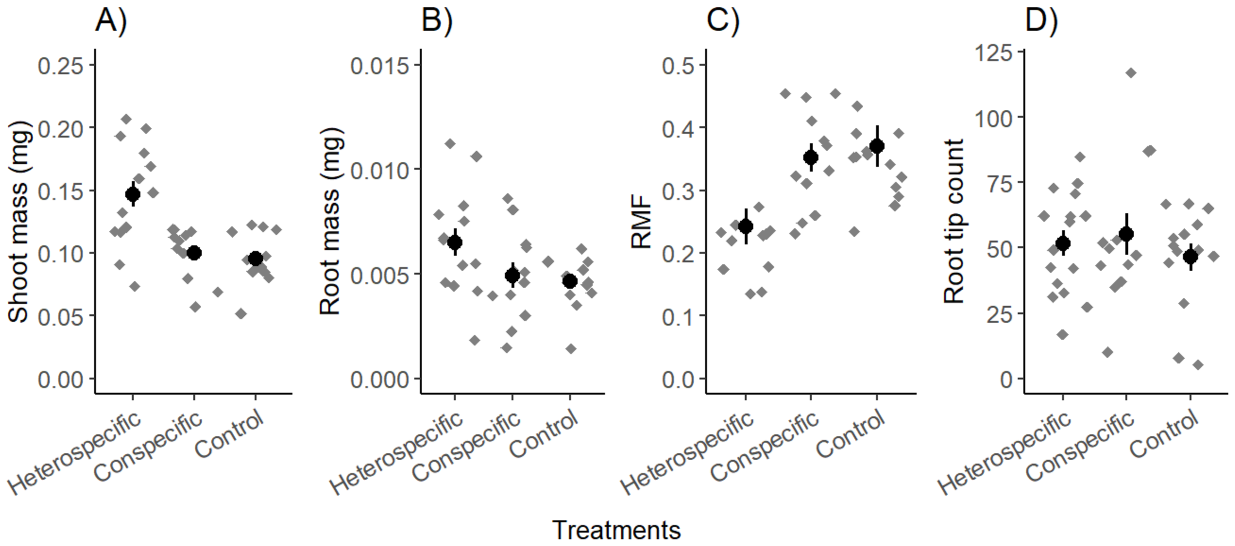


Figure 3.1. Mean ± standard error of the treatment group for *Pascopyrum smithii* plants growing alone and with conspecific and heterospecific neighbors for A) shoot mass, B) root mass, C) RMF, and D) root tip count. Black dots indicate treatment means, and grey diamonds indicate individual plant values.

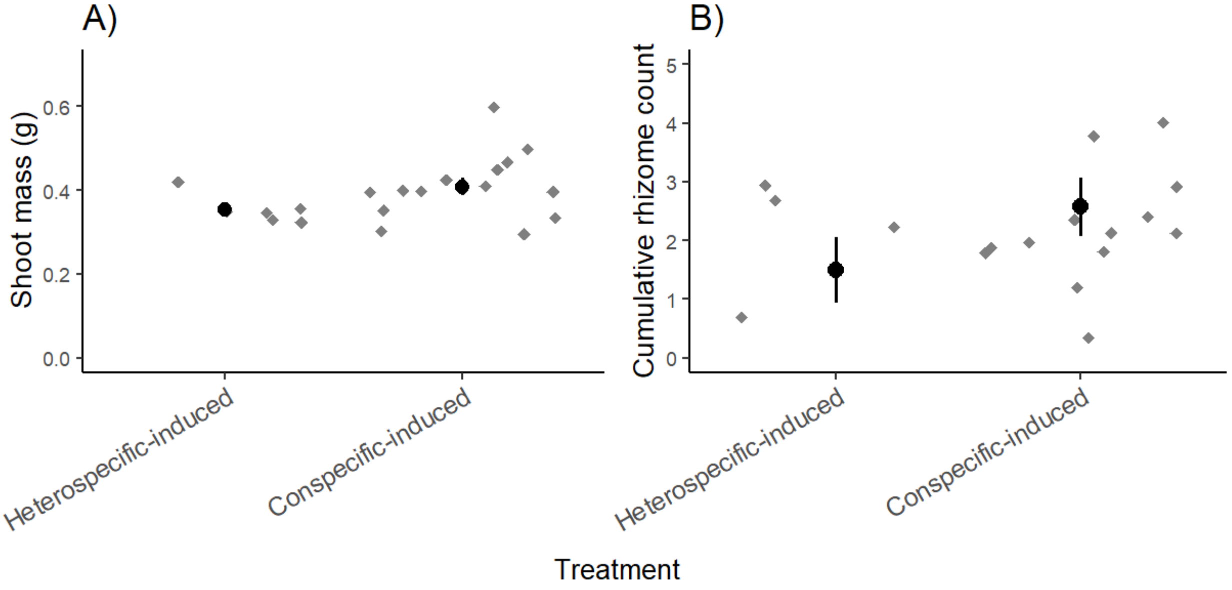


Figure 3.2. Mean ± standard error of *Pascopyrum smithii* plants induced by conspecific and heterospecific neighbors for A) shoot mass, and B) cumulative rhizomes per pot. Black dots indicate treatment means, and grey diamonds indicate individual plant values.

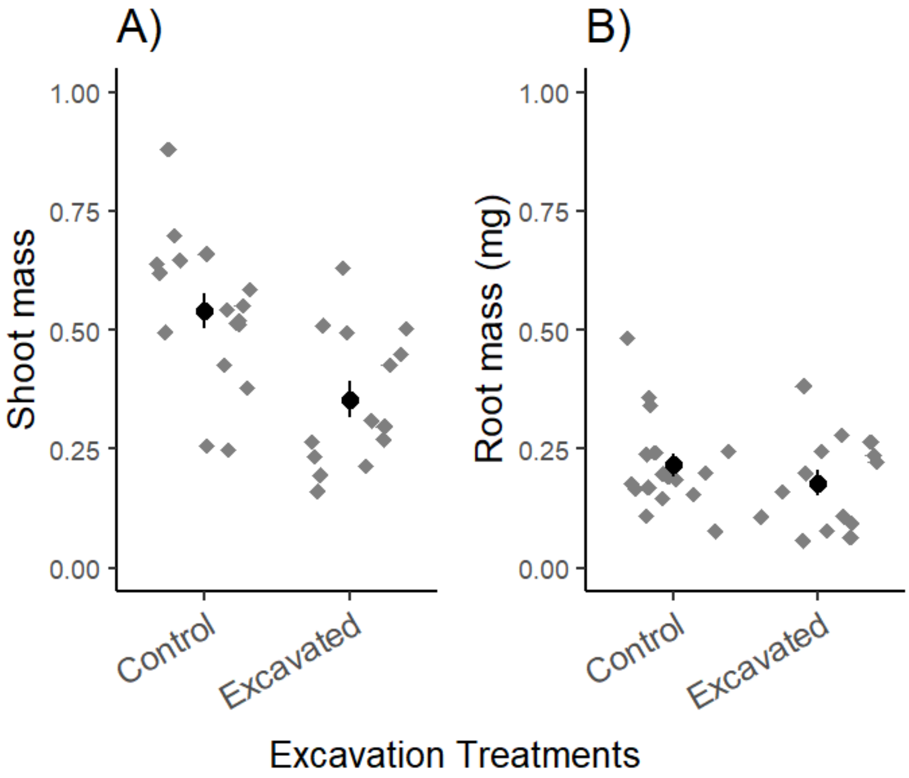
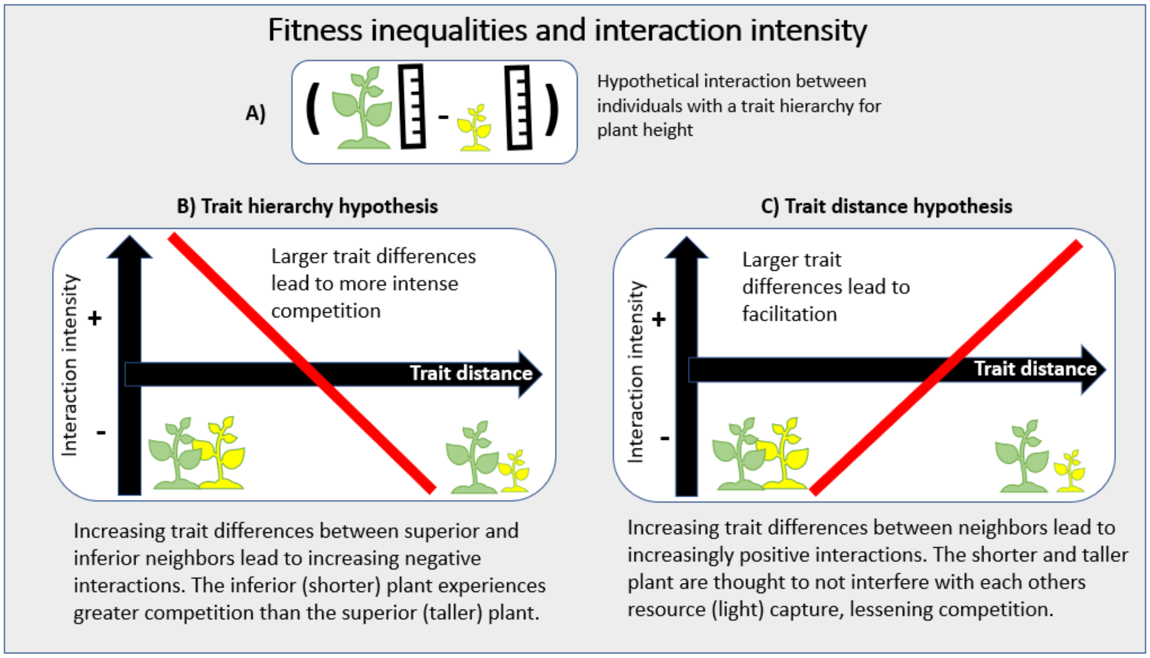


Figure 3.3. Mean ± standard error of *Pascopyrum smithii* plants excavated or undisturbed (control) for A) shoot mass, and B) root mass. Black dots indicate treatment means, and grey diamonds indicate individual plant values.



Box 4.1. Hypothesized relationships between trait distance and interaction intensity. Plant height hierarchical distance (A) are used here as an example. Under the trait hierarchy hypothesis, interaction intensity declines with trait distance (B), and conditions for equalizing mechanisms are not met. Under the Trait distance hypothesis, interaction intensity increases with trait distance (C), and conditions for equalizing mechanisms are met.

Table 4.1. *Machaeranthera canescens* and *Heterotheca villosa* population source information, and code names for the study.

Species	Population code	Source State	Latitude	Longitude
Machaeranthera canescens	MACA-A	Utah	39.0273	-109.2126
	MACA-B	Utah	40.0650	-109.2907
	MACA-C	New Mexico	36.2909	-107.8842
Heterotheca villosa	HEVI-A	New Mexico	35.9654	-107.0968
	HEVI-B	Utah	40.5149	-109.7113
	HEVI-C	Utah	39.1169	-111.2903

Table 4.2. Treatment descriptions for intraspecific and interspecific interactions, and replication within the study.

Interaction type	Populations or population mixtures	Number of replicates included in analyses
Monoculture 3 treatments	MACA-A + MACA-A	18
	MACA-B + MACA-B	17
	MACA-C + MACA-C	18
Intraspecific mixture 6 treatments	MACA-A + MACA-B	17
	MACA-A + MACA-C	18
	MACA-B + MACA-A	18
	MACA-B + MACA-C	14
	MACA-C + MACA-A	20
	MACA-C + MACA-B	20
Interspecific mixture 9 treatments	MACA-A + HEVI-A	19
	MACA-A + HEVI-B	17
	MACA-A + HEVI-C	15
	MACA-B + HEVI-A	19
	MACA-B + HEVI-B	20
	MACA-B + HEVI-C	18
	MACA-C + HEVI-A	15
	MACA-C + HEVI-B	16
	MACA-C + HEVI-C	19

Table 4.3. Responses calculated to assess hypotheses on stabilizing and equalizing mechanisms. Including calculation method, hypotheses tested, and notes on calculation and interpretation.

Responses and Predictors	Calculation made	Hypothesis tested	Sample size
Log Response Ratio (LRR)	log of the treatment mean divided by the monoculture mean for full mass	H1	15 treatments
Root Mass Fraction (RMF) and Shoot Mass Fraction (SMF)	root mass / total mass shoot mass / total mass	H2	358 plants
Root and Shoot mass	Focal plant root mass – non-focal root mass Focal plant shoot mass – non-focal shoot mass	H2	358 values on 716 plants
Root and Shoot Length	Focal plant root length – non-focal root length Focal plant shoot length – non-focal shoot length	H3	
Log Response Ratio (LRR)	log of the treatment mean divided by the monoculture mean for root and shoot mass	H3	10 treatments

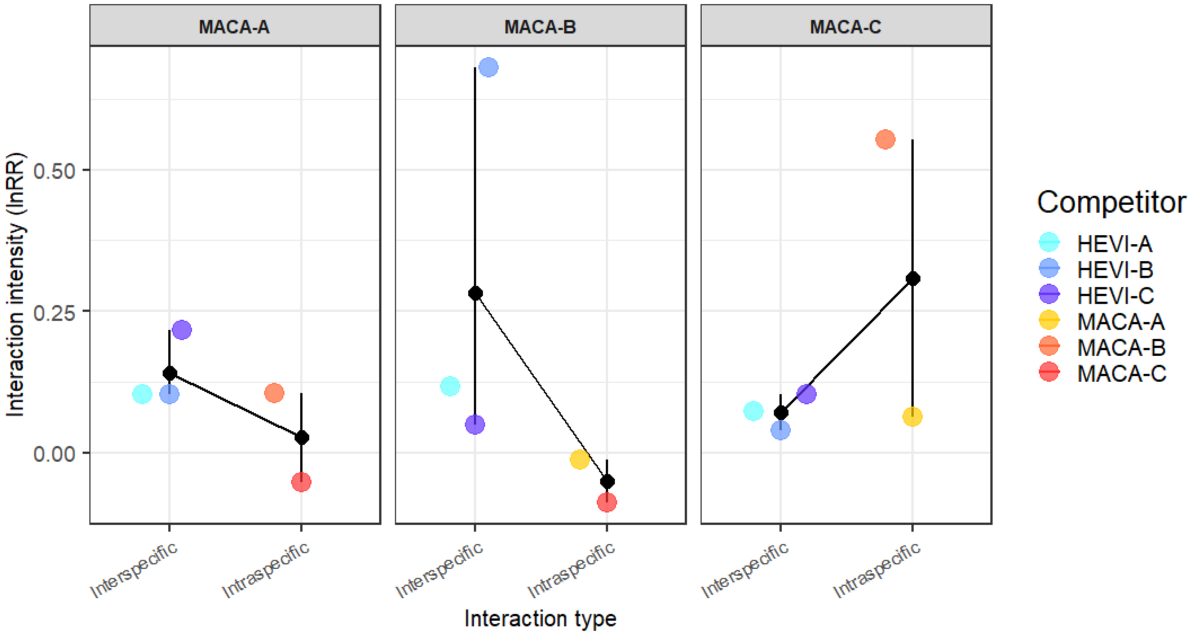


Figure 4.1. Relationships for interaction intensity and interaction type by population. Mean and 95% confidence intervals reported on interspecific mixed and intraspecific mixed outcomes by focal population. Colored dots indicate treatment means for pairings with each neighbor. Competitor indicates the three populations of both *M. canescens* (MACA) and three populations of *H. villosa*. (HEVI).

Table 4.4. Model AIC table assessing H2: Neighbor mass distance influence allocation, and these relationships are affected by source population, interaction type, and whether traits are root- or shoot derived. 'Population' indicates focal plant population identity. **Denotes the best model.

Model	AIC
lnRR ~ length distance * interaction type * population	-127.4**
lnRR ~ length distance + interaction type + population + length difference:interaction type + interaction type:population	-132.1
lnRR ~ length distance + interaction type + population + interaction type:population	-133.5

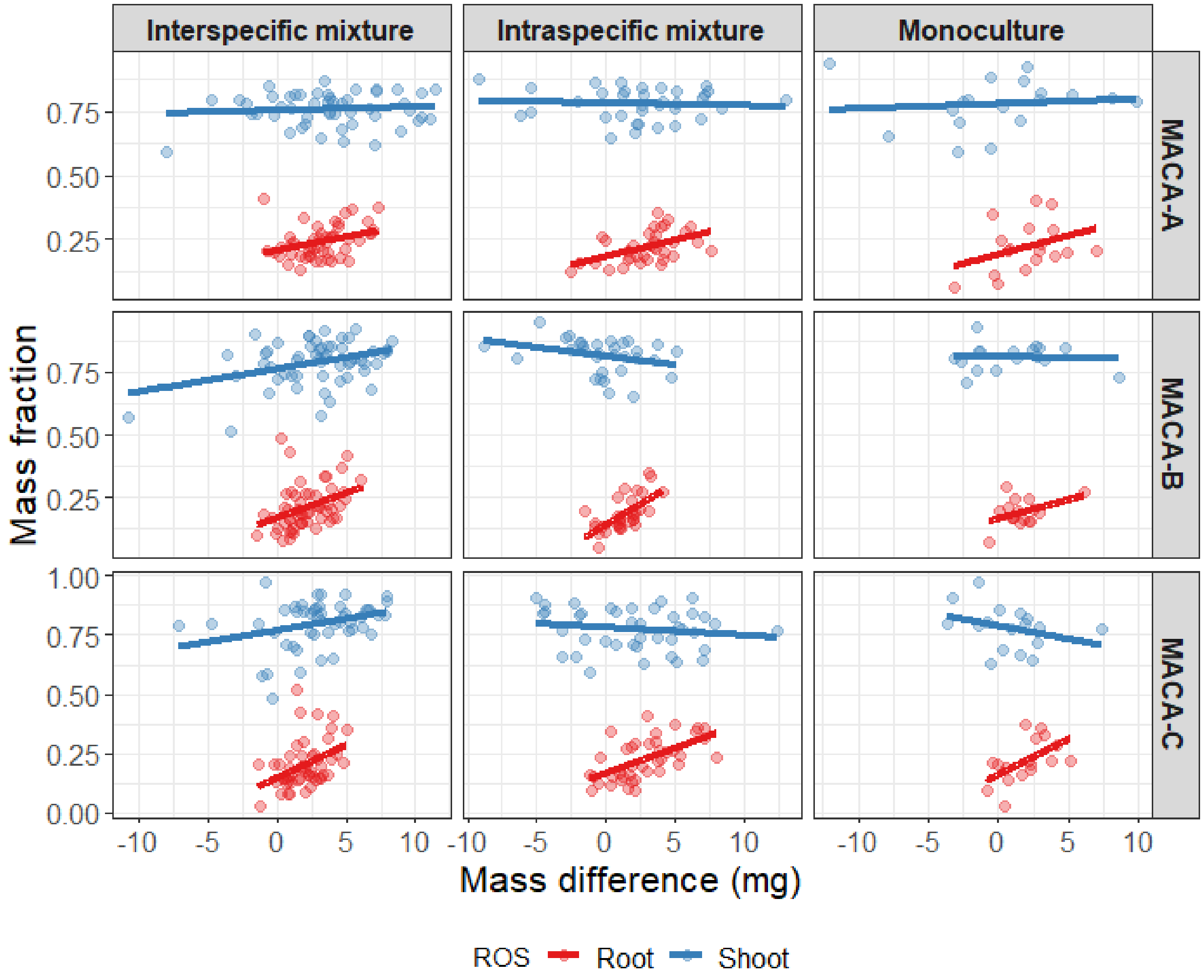


Figure 4.2. Relationships between mass fraction (RMF - red; SMF - blue) of the focal plant and hierarchical mass distance between focal and non-focal plants for the three focal populations and the interaction types. Data presented are raw values and lines represent the line of best-fit to the data. Lines of equations are in the supplemental materials (SI Table 4.1). Significant factors: Mass distance – $p < 0.0001$; Focal population – $p = 0.03$; Mass distance: Focal population – p = 0.08; Interaction type: Focal population – p = 0.008.

Table 4.5. Model AIC table assessing H3: Neighbor root and shoot length distance affect interaction intensity, and focal population source and interaction type affect these relationships. **Denotes the best model. The ROS variable is a factor with two levels denoting whether data are root- or shoot-derived.

Model	AIC
Mass fraction ~ mass distance * population * interaction type * ROS * competitor	-3199.1**
Mass fraction ~ mass distance + population + interaction type + ROS + interaction type:competitor + mass distance:population + mass distance:interaction type + population:interaction type + mass distance ROS + population:ROS + interaction type:ROS + mass distance:interaction type:competitor + population:interaction type:competitor + mass distance:population:interaction type + interaction type:ROS:competitor + mass distance:population:ROS + mass distance:interaction type:ROS + population:interaction type:ROS + mass distance:population:interaction type:competitor + population:interaction type:ROS:competitor + mass distance:population:interaction type:ROS	-3203.3
Mass fraction ~ mass distance + population + interaction type + ROS + interaction type:ROS + mass difference:interaction type:competitor + population:interaction type:competitor + mass distance:population:interaction type + interaction type:ROS:competitor + mass distance:population:ROS + mass distance:interaction type:ROS + population:interaction type:ROS + mass distance:population:interaction type:competitor + population:interaction type:ROS:competitor _	-3208.2

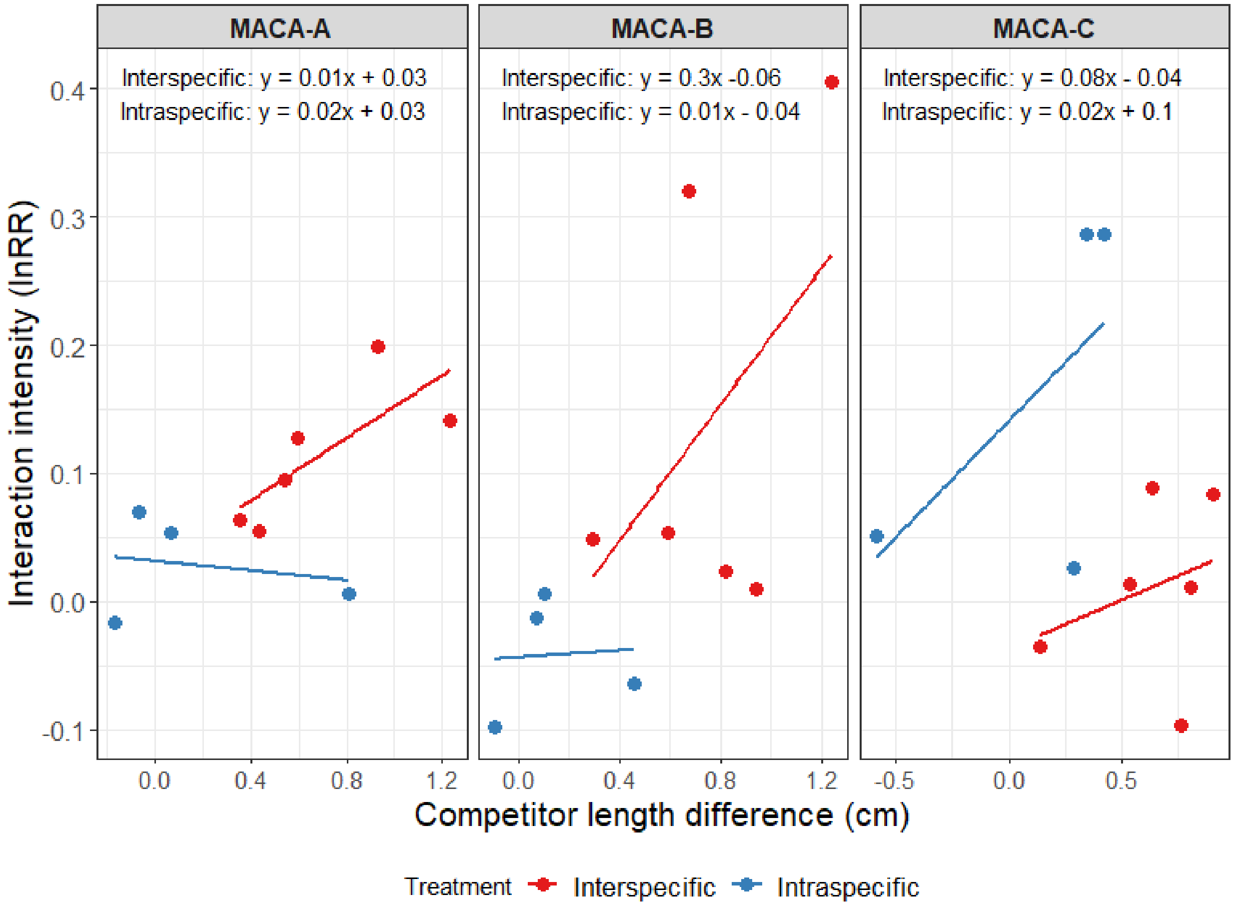


Figure 4.3. Relationships between interaction intensity and the hierarchical length (root and shoot) distance between focal and non-focal plants for each interaction type (the monoculture competition type is the reference group and equal to zero). Data presented are raw values and lines represent the line of best-fit to the data.

Literature Cited

Abbott JM, Stachowicz JJ. **2016**. The relative importance of trait vs. genetic differentiation for the outcome of interactions among plant genotypes. *Ecology* **97**: 84–94.
Acciaresi H, Guiamet J. **2010**. Below- and above-ground growth and biomass allocation in maize and Sorghum halepense in response to soil water competition. *Weed Research* **50**: 481–492.
Adler PB, Smull D, Beard KH, Choi RT, Furniss T, Kulmatiski A, Meiners JM, Tredennick AT, Veblen KE. **2018**. Competition and coexistence in plant communities: intraspecific competition is stronger than interspecific competition. *Ecology Letters* **21**: 1319–1329.
Aerts R. **1999**. Interspecific competition in natural plant communities: mechanisms, trade-offs and plant-soil feedbacks. *Journal of Experimental Botany* **50**: 29–37.
Ågren GI, Fagerström T. **1984**. Limiting Dissimilarity in Plants: Randomness Prevents Exclusion of Species with Similar Competitive Abilities. *Oikos* **43**: 369–375.
Aguiar MR, Lauenroth WK, Peters DP. **2001**. Intensity of intra- and interspecific competition coexisting shortgrass species. *Journal of Ecology* **89**: 40–47.
Alexander JM, Diez JM, Levine JM. **2015**. Novel competitors shape species' responses to climate change. *Nature* **525**: 515–518.
Álvarez-Yépiz JC, Búrquez A, Dovčiak M. **2014**. Ontogenetic shifts in plant-plant interactions in a rare cycad within angiosperm communities. *Oecologia* **175**: 725–735.
Ashton I, Miller A, Bowman W, Suding K. **2010**. Niche complementarity due to plasticity in resource use: plant partitioning of chemical N forms. *Ecology* **91**: 3252–3260.
Atwater DZ, James JJ, Leger EA. **2015**. Seedling root traits strongly influence field survival and performance of a common bunchgrass. *Basic and Applied Ecology*.
Aubry C, Shoal R, Erickson V. **2005**. Grass cultivars: Their origins, development, and use on national forests and grasslands in the Pacific Northwest. : 1–44.
Bam S. **2018**. Impacts of Altered Precipitation Frequency, Clipping, and Competition on Perennial Grasses Mediated Through Belowground Bud Bank in Northern Mixed-Grass Prairie. *Thesis: South Dakota State University*.
Barabas G, D'Andrea R. **2016**. The effect of intraspecific variation and heritability on community pattern and robustness. *Ecology Letters* **19**: 977–986.
Bardgett RD, Mommer L, De Vries FT. **2014**. Going underground: root traits as drivers of ecosystem processes. *Trends in Ecology & Evolution* **29**: 692–699.
Bartelheimer M, Gowing D, Silvertown J. **2010**. Explaining hydrological niches: The decisive role of below-ground competition in two closely related Senecio species. *Journal of Ecology* **98**: 126–136.
Basey AC, Fant JB, Kramer A. **2015**. Producing native plant materials for restoration : 10 rules to collect and maintain genetic diversity. *Native Plants Journal* **16**: 37–52.
Bates D, Maechler M, Bolker B, Walker S. **2015**. Fitting Linear Mixed-Effects Models Using lme4. ,. *Journal of Statistical Software* **67**: 1–48.
Bell D, Sultan S. **1999**. Dynamic Phenotypic plasticity for root growth in Polygonum: A comparative study. *American Journal of Botany* **86**: 807–819.
Bennett JA, Riibak K, Tamme R, Lewis RJ, Pärtel M. **2016**. The reciprocal relationship between competition and intraspecific trait variation. *Journal of Ecology* **104**: 1410–1420.

Berendse F, Móller F. **2009**. Effects of competition on root-shoot allocation in Plantago lanceolata L .: adaptive plasticity or ontogenetic drift? *Plant Ecology* **201**: 567–573.
Berg MP, Ellers J. **2010**. Trait plasticity in species interactions: A driving force of community dynamics. *Evolutionary Ecology* **24**: 617–629.
Bertness MD, Callaway RM. **1994**. Positive interactions in communities. *Trends in Ecology & Evolution*: 191–193.
Bever JD, Dickie IA, Facelli E, Facelli JM, Klironomos J, Moora M, Rillig MC, Stock WD, Tibbett M, Zobel M. **2010**. Rooting theories of plant community ecology in microbial interactions. *Trends in Ecology and Evolution* **25**: 468–478.
Bever JD, Mangan SA, Alexander HM. **2015**. Maintenance of Plant Species Diversity by Pathogens. *Annual Review of Ecology, Evolution, and Systematics* **46**: 305–325.
Bischoff A, Muller-Scharer H. 2010. Testing population differentiation in plant species - how important are environmental maternal effects. *OIKOS* **119**: 445-454.

BMJ. **2015**. What is publication bias in a meta-analysis? **351**.
Booth RE, Grime JP. **2003**. Effects of genetic impoverishment on plant community diversity. *Journal of Ecology* **91**: 721–730.
Bornkamm R, Salinger S, Strehlow H. **1975**. Productivity and Chemical Constituents of two Grasses under Pure and Mixed Cultivations. *Floral Biodiversity* **164**: 437–448.
Bossdorf O, Shuja Z, Banta JA. 2009. Genotype and maternal environment affect belowground interactions between Arabidopsis thaliana and its competitors. *Oikos* **118**: 1541–1551.
Bradley St. Clair J, Kilkenny FF, Johnson RC, Shaw NL, Weaver G. **2013**. Genetic variation in adaptive traits and seed transfer zones for Pseudoroegneria spicata (bluebunch wheatgrass) in the northwestern United States. *Evolutionary Applications* **6**: 933–948.
Bradshaw AD. **1965**. Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics* **155**: 115–155.
Brandt AJ, Leahy SC, Zimmerman NM, Burns JH. **2015**. Plant trait expression responds to establishment timing. *Oecologia* **178**: 525–536.
Breed MF, Stead MG, Ottewell KM, Gardner MG, Lowe AJ. **2013**. Which provenance and where? Seed sourcing strategies for revegetation in a changing environment. *Conservation Genetics* **14**: 1–10.
Broadhurst LM, Lowe A, Coates DJ, Cunningham S a., McDonald M, Vesk P a., Yates C. **2008**. Seed supply for broadscale restoration: maximizing evolutionary potential. *Evolutionary Applications*: 587–597.
Bu W, Huang J, Xu H, Zang R, Ding Y, Li Y, Lin M, Wang J, Zhang C. **2019**. Plant Functional Traits Are the Mediators in Regulating Effects of Abiotic Site Conditions on Aboveground Carbon Stock-Evidence From a 30 ha Tropical Forest Plot. *Frontiers in Plant Science* **9**: 1–10.
Bucharova A, Bossdorf O, Hölzel N, Kollmann J, Prasse R, Durka W. **2019**. Mix and match: regional admixture provenancing strikes a balance among different seed-sourcing strategies for ecological restoration. *Conservation Genetics* **20**: 7–17.
Burton PJ, Burton CM. **2002**. Promoting genetic diversity in the production of large quantities of native plant seed. *Ecological Restoration* **20**: 117–123.
Cahill J. **2002**. What evidence is necessary in studies which separate root and shoot competition along productivity gradients? *Journal of Ecology* **90**: 201–205.

Callaway RM, Al. E. **2002**. Positive interactions among alpine plants increase with stress. *Nature* **417**: 844–848.
Callaway RM, Walker LR. **1997**. Competition and facilitation: A synthetic approach to interactions in plant communities. *Ecology* **78**: 1958–1965.
Carmona CP, de Bello F, Azcárate FM, Mason NWH, Peco B. **2019**. Trait hierarchies and intraspecific variability drive competitive interactions in Mediterranean annual plants. *Journal of Ecology* **107**: 2078–2089.
Casper BB, Castelli JP. **2007**. Evaluating plant-soil feedback together with competition in a serpentine grassland. *Ecology Letters* **10**: 394–400.
Casper BB, Jackson RB. **1997**. Plant competition underground. *Annual Review of Ecology and Systematics* **28**: 545–570.
Chambers JC, Roundy BA, Blank RR, Meyer SE, Whittaker A. **2007**. What Makes Great Basin Sagebrush Ecosystems Invasible by Bromus tectorum? *Ecological Monographs* **77**: 117–145.
Chang CY, Marshall DJ. **2017**. Quantifying the role of colonization history and biotic interactions in shaping communities –a community transplant approach. *Oikos* **126**: 204–211.
Cheplick GP. **1997**. Responses to Severe Competitive Stress in a Clonal Plant: Differences between Genotypes. *Oikos* **79**: 581–591.
Cheplick GP, Gutierrez C. **2000**. Clonal growth and storage in relation to competition in genets of the rhizomatous perennial Amphibromus scabrivalvis. *Canadian Journal of Botany* **78**: 537–546.
Chesson P. **2000**. Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics* **31**: 343–366.
Chesson P. **2008**. Quantifying and testing species coexistence mechanisms. In: Unity in Diversity: Reflections on Ecology after the legacy of Ramon Margalef. 119–164.
Chivers I.H., Jones T.A., Broadhurst L.M., Mott I.W., Larson S.R. 2016. The merits of artificial selection for the development of restoration-ready plant materials of native perennial grasses. *Restoration Ecology* 24:174-183
Cipollini D, Purrington CB, Bergelson J. **2003**. Costs of induced responses in plants. *Basic and Applied Ecology* **4**: 79–85.
Cohn LD, Decker BJ. **2003**. How Meta-Analysis Increases Statistical Power. *Psychological Methods* **8**: 243–253.
Comstock JP, Ehleringer JR. **1992**. Plant adaptation in the Great Basin and Colorado Plateau. *Great Basin Naturalist* **52**: 195–215.
Cook-Patton SC, Hastings AP, Agrawal AA. **2016**. Genotypic diversity mitigates negative effects of density on plant performance: a field experiment and life-cycle analysis of common evening primrose Oenothera biennis. *Journal of Ecology* **105**: 726–735.
Coomes DA, Grubb PJ. **2000**. Impacts of root competition in forests and woodlands: a theoretical framework and review of experiments. *Ecological Monographs* **70**: 171–207.
Crawford KM, Rudgers J a. 2012. Plant species diversity and genetic diversity within a dominant species interactively affect plant community biomass (J Cahill, Ed.). *Journal of Ecology* **100**: 1512–1521.
Crawford K, Rudgers J. **2013**. Genetic diversity within a dominant plant outweighs plant species diversity in structuring an arthropod community. *Ecology* **94**: 1025–1035.
Crawley MJ. **2005**. *Statistics: An Introduction Using R*. John Wiley & Sons Ltd, Chichester.

CRB. **2013**. Conservation Release Brochure for ‘Arriba’ western wheatgrass (Pascopyrum smithii). *USDA-Natural Resources Conservation Service, Los Lunas Plant Materials Center* **Los Lunas,**.

Crutsinger GM, Collins MD, Fordyce J a, Gompert Z, Nice CC, Sanders NJ. **2006**. Plant genotupic diversity predicts community structure and governs ecosystem processes. *Science* **647**: 966–968.

Crutsinger GM, Souza L, Sanders NJ. **2008**. Intraspecific diversity and dominant genotypes resist plant invasions. *Ecology Letters* **11**: 16–23.

Dauro D, Mohamed-Saleem M. **1995**. Shoot and root interactions in intercopped wheat and clover. *Tropical Agriculture (Trinidad)* **72**: 170–172.

Diaz, S., Hodgson, J.G.; Thompson, K.; Cabido, M.; Cornelissen, J.H.C.; Jalili, A; Montserrat-Marti, G.; Grime JP, Zarrinkamar F, Asri Y, Band SR, Basconcelo S, Funes G, Hamzehee B, Khoshnevi M, Shirvany A, Vendramini F, *et al.* 2004. The plant traits that drive ecosystems : Evidence from three continents. *Journal of Vegetation Science* **15**: 295–304.

Dong X, Patton J, Wang G, Nyren P, Peterson P. **2012**. Effect of drought on biomass allocation in two invasive and two native grass species dominating the mixed-grass prairie. *Grass and Forage Science* **69**: 160–166.

Dorner J. **2002**. An introduction to using native plants in restoration projects . : 1–66.

Drenovsky RE, Khasanova A, James JJ. **2012**. Trait convergence and plasticity among native and invasive species in resource-poor environments. *American journal of botany* **99**: 629–39.

Dyer AR, Knapp EE, Rice KJ. **2016**. Unintentional Selection and Genetic Changes in Native Commercial Seed Production. *Ecological Restoration* **34**: 39–48.

Eckberg JO, Johnson GA, Seefeldt LL, Felton AJ, Casler MD, Shaw RG. **2018**. Competitive effects of cultivar and wild switchgrass on other native grasses. *Biological Invasions* **20**: 2439–2449.

Eilers EJ, Heger T. **2019**. Past Competition Affects Offspring Foliar Terpenoid Concentrations, Seed Traits, and Fitness in the Invasive Forb Erodium cicutarium (Geraniaceae). *Frontiers in Ecology and Evolution* **7**: 1–12.

Ensslin A, Van de Vyver A, Vanderborght T, Godefroid S. **2018**. Ex situ cultivation entails high risk of seed dormancy loss on short-lived wild plant species. *Journal of Applied Ecology* **55**: 1145–1154.

Espeland EK, Emery NC, Mercer KL, Woolbright SA, Kettenring KM, Gepts P, Etterson JR. **2017**. Evolution of plant materials for ecological restoration: insights from the applied and basic literature. *Journal of Applied Ecology* **54**: 102–115.

Evans RA. **1961**. Effects of Different Densities of Downy Brome (Bromus tectorum) on Growth and Survival of Crested Wheatgrass (Agropyron desertorum) in the Greenhouse. *Weeds* **9**: 216–223.

Falconer DS, MacKay TFC. 1996. Introduction to Quantitative Genetics. Addison Wesley Longman Limited, Essex England.

Fay MP. **2018**. asht: Applied Statistical Hypothesis Tests. *R package version 0.9.4*.

Fenner M. **1987**. Seedlings. *New Phytologist* **106**: 35–48.

Ferguson SD, Leger E a., Li J, Nowak RS. **2015**. Natural selection favors root investment in native grasses during restoration of invaded fields. *Journal of Arid Environments* **116**: 11–17.

Ferrero-Serrano Á, Hild AL, Mealor B a. 2011. Can Invasive Species Enhance Competitive Ability and Restoration Potential in Native Grass Populations? *Restoration Ecology* **19**: 545–

551.
Flint-Garcia SA. **2015**. Genetics and consequences of crop domestication. *Journal of Agricultural and Food Chemistry* **61**: 8267–8276.
Fort F, Cruz P, Catrice O, Delbrut A, Luzarreta M, Stroia C, Jouany C. **2015**. Root functional trait syndromes and plasticity drive the ability of grassland Fabaceae to tolerate water and phosphorus shortage. *Environmental and Experimental Botany* **110**: 62–72.
Fort F, Cruz P, Jouany C. **2014a**. Hierarchy of root functional trait values and plasticity drive early-stage competition for water and phosphorus among grasses. *Functional Ecology* **28**: 1030–1040.
Fort F, Cruz P, Jouany C. **2014b**. Hierarchy of root functional trait values and plasticity drive early-stage competition for water and phosphorus among grasses. *Functional Ecology* **28**: 1030–1040.
Fortunel C, Valencia R, Wright SJ, Garwood NC, Kraft NJB. **2016**. Functional trait differences influence neighbourhood interactions in a hyperdiverse Amazonian forest. *Ecology letters* **19**: 1062–70.
Foster BL. **1999**. Establishment, competition and the distribution of native grasses among Michigan old-fields. *Journal of Ecology* **87**: 476–489.
Fowler N. **1986**. The Role of Competition in Plant Communities in Arid and Semiarid Regions. *Annual Review of Ecology and Systematics* **17**: 89–110.
Fox J, Weisberg S. **2019**. An {R} Companion to Applied Regression. : Thousand Oaks, CA. https://socialsciences.mcmaster.
Foxx A, Fort F. **2019**. Root and shoot competition lead to contrasting competitive outcomes under water stress: A systematic review and meta-analysis. *PLoS ONE* **14**: e0220674.
Freckleton RP, Watkinson AR. **2001**. Asymmetric competition between plant species. **15**: 615–623.
Funk JL, Cleland EE, Suding KN, Zavaleta ES. **2008**. Restoration through reassembly: plant traits and invasion resistance. *Trends in Ecology and Evolution* **23**: 695–703.
García EH, Peña-Valdivia CB, Aguirre JRR, Muruaga JSM. **1997**. Morphological and agronomic traits of a wild population and an improved cultivar of common bean (Phaseolus vulgaris L.). *Annals of Botany* **79**: 207–213.
Gargallo-Garriga A, Sardans J, Perez-Trujillo M, Rivas-Ubach A, Oravec M, Vecerova K, Urban O, Jentsch A, Kreyling J, Beierkuhnlein C, *et al.* **2014**. Opposite metabolic responses of shoots and roots to drought. *Scientific Repors* **4**: 1–7.
Gedroc J, McConnaughay D, Coleman J. **1996**. Plasticity in root/shoot partitioning: optimal, ontogenetic, or both? *Functional Ecology* **10**: 44–50.
van Gelderen K, Kang C, Pierik R. **2017**. Light signaling, root development and plasticity. *Plant Physiology* **176**: pp.01079.2017.
Godoy O, Kraft N LJ. **2014**. Phylogenetic relatedness and the determinants of competitive outcomes. *Ecology letters* **17**: 836–844.
Goergen EM, Leger E a, Espeland EK. **2011**. Native perennial grasses show evolutionary response to Bromus tectorum (cheatgrass) invasion. *PloS one* **6**: e18145.
Gross N, Liancourt P, Butters R, Duncan RP, Hulme PE. **2015**. Functional equivalence, competitive hierarchy and facilitation determine species coexistence in highly invaded grasslands. *New Phytologist* **206**: 175–186.
Grossman JD, Rice KJ. **2012**. Evolution of root plasticity responses to variation in soil nutrient

distribution and concentration. *Evolutionary Applications* **5**: 850–857.

Group S f. ERISPW. **2002**. SER International Primer on Ecological Restoration. *Soceity for Ecological Restoration International*.

Gundel PE, Pierik R, Mommer L, Ballaré CL. **2014**. Competing neighbors: Light perception and root function. *Oecologia* **176**: 1–10.

Haidet M, Olwell P. **2015**. Seeds of Success: A National Seed Banking Program Working to Achieve Long-Term Conservation Goals. *Natural Areas Journal* **35**: 165–173.

Hanke JM, Ludewig K, Jensen K. **2015**. Effects of water level and competition on the endangered river corridor plant Cnidium dubium in the context of climate change. *Wetlands Ecology and Management* **23**: 215–226.

Harpole WS, Suding KN. **2007**. Frequency-dependence stabilizes competitive interactions among four annual plants. *Ecology Letters* **10**: 1164–1169.

Harris GA. **1967**. Some Competitive Relationships between Agropyron spicatum and Bromus tectorum. *Ecological Monographs* **37**: 89–111.

Hart MM, Reader RJ, Klironomos JN. **2003**. Plant coexistence mediated by arbuscular mycorrhizal fungi. *Trends in Ecology and Evolution* **18**: 418–423.

Hart S, Schreiber S, Levine J. **2016**. How variation between individuals affects species coexistence. *Ecology Letters* **19**: 825–838.

Haugland E, Froud-Williams R. **1999**. Improving grasslands: the influence of soil moisture and nitrogen fertilization on the establishment of seedlings. *Journal of Applied Ecology* **36**: 263–270.

Hedges L V, Gurevitch J, Curtis PS. **1999**. The Meta-Analysis of Response Ratios in Experimental Ecology. *Ecology* **80**: 1150–1156.

Heger T, Jacobs BS, Latimer AM, Kollmann J, Rice KJ. **2014**. Does experience with competition matter? Effects of source competitive environment on mean and plastic trait expression in Erodium cicutarium. *Perspectives in Plant Ecology, Evolution and Systematics* **16**: 236–246.

Herman JJ, Sultan SE, Horgan-Kobelski T, Riggs C. **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**: 77–88.

Hernández-Terán A, Wegier A, Benítez M, Lira R, Escalante AE. **2017**. Domesticated, Genetically Engineered, and Wild Plant Relatives Exhibit Unintended Phenotypic Differences: A Comparative Meta-Analysis Profiling Rice, Canola, Maize, Sunflower, and Pumpkin. *Frontiers in Plant Science* **8**: 1–10.

HilleRisLambers J, Adler PB, Harpole WS, Levine JM, Mayfield MM. **2012**. Rethinking Community Assembly through the Lens of Coexistence Theory. *Annual Review of Ecology, Evolution, and Systematics* **43**: 227–248.

Hothorn T, Bretz F, Westfall P. **2008**. Simultaneous Inference in General Parametric Models. *Biometrical Journal* **50**: 346–363.

Howard TG, Goldberg DE. **2001**. Competitive response hierarchies for germination, growth, and survival and their influence on abundance. *Ecology* **82**: 979–990.

Hsiao TC. **1973**. Plant Responses to Water Stress. *Annual Review of Plant Physiology* **24**: 519–570.

Hsiao TC, Xu LK. **2000**. Sensitivity of with of roots versus leaves to water stress: Biophysical analysis and relation to water. *Journal of Experimental Botany* **51**: 1595–1616.

Hughes R, Inouye BD, Johnson MTJ, Underwood N, Vellend M. **2008**. Ecological consequences of genetic diversity. *Ecology Letters* **11**: 609–623.

Hughes R, Stachowicz JJ. **2004**. Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *Proceedings of the National Academy of Sciences of the United States of America* **101**: 8998–9002.

IPCC. **2014**. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. In: Intergovernmental Panel on Climage Change In TF Stocker, D Qin, GK Plattner, MMB Tignor, SK Allen, J Boschung, A Nauels, Y Xia, V Bex, PM Midgley, eds, Climate Change 2013: The Physical Science Basis. Cambridge University Press, New York. 1335.

James JJ, Svejcar TJ, Rinella MJ. **2011**. Demographic processes limiting seedling recruitment in arid grassland restoration. *Journal of Applied Ecology* **48**: 961–969.

Jentsch A, Kreyling J, Elmer M, Gellesch E, Glaser B, Grant K, Hein R, Lara M, Mirzae H, Nadler SE, *et al.* **2011**. Climate extremes initiate ecosystem-regulating functions while maintaining productivity. *Journal of Ecology* **99**: 689–702.

Jones T, Larson S, Nielson D, Young S, Chatterton N, Palazzo A. **2002**. Registration of P-7 bluebunch wheatgrass germplasm (Registrations of Germplasm).

Jupp A, Newman I. **1987**. Morphological and anatomical effects of severe drought on the roots of Lolium perenne L. *New Phytologist* **105**: 393–402.

Kembel SW, Cahill J. **2011**. Independent Evolution of Leaf and Root Traits within and among Temperate Grassland Plant Communities. *PLoS ONE* **6**: 2–10.

Kiaer LP, Weisbach AN, Weiner J. **2013**. Root and shoot competition: a meta-analysis (D Gibson, Ed.). *Journal of Ecology* **101**: 1298–1312.

Klopf RP, Baer SG. **2011**. Root Dynamics of Cultivar and Non-Cultivar Population Sources of Two Dominant Grasses during Initial Establishment of Tallgrass Prairie. *Restoration Ecology* **19**: 112–117.

Knutson KC, Pyke DA, Wirth TA, Arkle RS, Pilliod DS, Brooks ML, Chambers JC, Grace JB. **2014**. Long-term effects of seeding after wildfire on vegetation in Great Basin shrubland ecosystems. *Journal of Applied Ecology* **51**: 1414–1424.

Koricheva J, Gurevitch J. **2014**. Uses and misuses of meta-analysis in plant ecology. *Journal of Ecology* **102**: 828–844.

Kormanik PP, Sung S, Kass DJ, Schlarbaum S. **1997a**. Effect of seedling size and first-order-lateral roots on early development of Northern Red Oak Seedlings. In: Proceedings of the Ninth Biennial Southern Silvicultural Research Conference. 194–200.

Kormanik PP, Sung SJS, Kormanik TL, Zarnock SJ, Schlarbaum S. **1997b**. Heritability of first-order-lateral roots in five. In: Proccedings of the second meeting of working party 2.08.08, Genetics of Quercus, of the International Union of Forest Research Organizations. 194–200.

Kraft NJB, Crutsinger GM, Forrestel EJ, Emery NC. **2014**. Functional trait differences and the outcome of community assembly: an experimental test with vernal pool annual plants. *Oikos* **123**: 1391–1399.

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

Kramer, AT, Foxx, AJF. 2016. Propagation protocol for production of propagules (seeds, cutting, poles, etc.) *Pascopyrum smithii* Seeds Chicago Botanic Garden – Research Glencoe, Illinois. In: Native Plant Network. URL: http://NativePlantNetwork.org (accessed 2018/12/12). *US Department of Agriculture, Forest Service, National Center for Reforestation, Nurseries, and Genetic Resources*.

Kulpa SM, Leger E a. **2013**. Strong natural selection during plant restoration favors an unexpected suite of plant traits. *Evolutionary applications* **6**: 510–23.

Kumar B, Abdel-Ghani AH, Reyes-Matamoros J, Hochholdinger F, Lübberstedt T. **2012**. Genotypic variation for root architecture traits in seedlings of maize (Zea mays L.) inbred lines. *Plant Breeding* **131**: 465–478.

Kunstler G, Lavergne S, Courbaud B, Thuiller W, Vieilledent G, Zimmermann NE, Kattge J, Coomes D. **2012**. Competitive interactions between forest trees are driven by species' trait hierarchy, not phylogenetic or functional similarity: implications for forest community assembly. *Ecology Letters* **15**: 831–840.

Ladouceur E, Jiménez-Alfaro B, Marin M, De Vitis M, Abbandonato H, Iannetta PPM, Bonomi C, Pritchard HW. **2018**. Native Seed Supply and the Restoration Species Pool. *Conservation Letters* **11**: 1–9.

Lajeunesse MJ. **2013**. Recovering Missing or Partial Data from Studies: A Survey of Conversions and Imputations for Meta-analysis. In: Handbook of Meta-analysis in Ecology and Evolution. 195–206.

Lamb EG, Kembel SW, Cahill JF. **2009**. Shoot, but not root, competition reduces community diversity in experimental mesocosms. *Journal of Ecology* **97**: 155–163.

Lamb JFS, Samac DA, Barnes DK, Henjum KI. **2000**. Increased herbage yield in alfalfa associated with selection for fibrous and lateral roots. *Crop Science* **40**: 693–699.

Lamb EG, Shore BH, Cahill JF. **2007**. Water and nitrogen addition differentially impact plant competition in a native rough Fescue grassland. *Plant Ecology* **192**: 21–33.

Lanta V, Doležal J, Zemková L, Lepš J. **2012**. Communities of different plant diversity respond similarly to drought stress: Experimental evidence from field non-weeded and greenhouse conditions. *Naturwissenschaften* **99**: 473–482.

Larson S, Jones T, HU Z, McCracken C, Palazzo A. **2002**. Genetic diversity of bluebunch wheatgrass cultivars and a multiple-origin polycross. *Crop Sciece* **40**: 2002.

Larson JE, Sheley RL, Hardegree SP, Doescher PS, James JJ. **2015**. Seed and seedling traits affecting critical life stage transitions and recruitment outcomes in dryland grasses. *Journal of Applied Ecology* **52**: 199–209.

Lazof DB, Rufty TW, Redinbaugh MG. **1992**. Localization of Nitrate Absorption and Translocation within Morphological Regions of the Corn Root. *Plant Physiology* **100**: 1251–1258.

Leger EA, Baughman OW. **2015**. What seeds to plant in the Great Basin? Comparing traits prioritized in native plant cultivars and releases with those that promote survival in the field. *Natural Areas* **35**: 54–68.

Leguizamón E, Yanniccari M, Guiamet J, Acciaresi H. **2011**. Growth, gas exchange and competitive ability of Sorghum halepense populations under different soil water availability. *Canadian Journal of Plant Science* **91**: 1011–1025.

Liancourt P, Lavorel S. **2013**. Importance and intensity of competition along a fertility gradient and across species. **17**: 455–464.

Lichstein JW, Dushoff J, Levin SA, Pacala SW. **2007**. Intraspecific Variation and Species

Coexistence. *The American Naturalist* **170**: 807–818.

Lloret F, Casanovas C, Penuelas J. **1999**. Seedling survival of Mediterranean shrubland species in relation to root : shoot ratio , seed size and water and nitrogen use. *Functional Ecology* **13**: 210–216.

Lyu S, Liu X, Venail P, Zhou S. **2017**. Functional dissimilarity, not phylogenetic relatedness, determines interspecific interactions among plants in the Tibetan alpine meadows. *Oikos* **126**: 381–388.

Macarthur R, Levins R. **1967**. The Limiting Similarity, Convergence, and Divergence of Coexisting Species. *The American Naturalist* **101**: 377–385.

Mangan SA, Schnitzer SA, Herre EA, MacK KML, Valencia MC, Sanchez EI, Bever JD. **2010**. Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. *Nature* **466**: 752–755.

Mathew I, Shimelis H, Mwadzingeni L, Zengeni R, Mutema M, Chaplot V. **2018**. Variance components and heritability of traits related to root: shoot biomass allocation and drought tolerance in wheat. *Euphytica* **214**: 1–12.

Mayfield MM, Levine JM. **2010**. Opposing effects of competitive exclusion on the phylogenetic structure of communities. *Ecology Letters* **13**: 1085–1093.

McCluney KE, Belnap J, Collins SL, González AL, Hagen EM, Nathaniel Holland J, Kotler BP, Maestre FT, Smith SD, Wolf BO. **2012**. Shifting species interactions in terrestrial dryland ecosystems under altered water availability and climate change. *Biological Reviews* **87**: 563–582.

Mealor B a, Hild AL. **2006**. Potential selection in native grass populations by exotic invasion. *Molecular ecology* **15**: 2291–300.

Melgoza G, Nowak RS. **1991**. Competition between cheatgrass and two native species after fire: Implications from observations and measurements of root distribution. *Journal of Range Management* **44**: 27–33.

Miner BG, Sultan SE, Morgan SG, Padilla DK, Relyea R a. **2005**. Ecological consequences of phenotypic plasticity. *Trends in ecology & evolution* **20**: 685–92.

Moher D, Liberati A, Tetzlaff J, Altman DG TPG. **2009**. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Med* **6**: e1000097.

Mommer L, van Ruijven J, Jansen C, van de Steeg HM, de Kroon H. **2012**. Interactive effects of nutrient heterogeneity and competition: implications for root foraging theory? *Functional Ecology* **26**: 66–73.

Montalvo AM, Williams SL, Rice KJ, Buchmann SL, Cory C, Handel SN, Nabhan GP, Primack R, Robichaux RH. **1997**. Restoration Biology : A Population Biology Perspective. **5**: 277–290.

Murphy GP, Dudley SA. **2007**. Above- and below-ground competition cues elicit independent responses. *Journal of Ecology*: 261–272.

Nagel R, Durka W, Bossdorf O, Bucharova A. **2019**. Rapid evolution in native plants cultivated for ecological restoration: not a general pattern. *Plant Biology* **21**: 551–558.

National Oceanic and Atmospheric Administration. Date accessed: 11 January, 2020. URL: https://w2.weather.gov/climate/xmacis.php?wfo=slc

Nissim Y, Jinggui F, Arik S, Neta P, Uri L, Avner C. **2004**. Phenotypic and genotypic analysis of a commercial cultivar and wild populations of Anemone coronaria. *Euphytica* **136**: 51–62.

Nooten SS, Hughes L. **2017**. The power of the transplant: direct assessment of climate change impacts. *Climatic Change* **144**: 237–255.

Oduor AMO. **2013**. Evolutionary responses of native plant species to invasive plants: A review. *New Phytologist* **200**: 986–992.

Ogle D, St. John L, Jones T. **2010**. Plant Guide: Bluebunch wheatgrass: Pseudoroegneria spicata. *U.S. Department of Agriculture, National Resources Conservation Service, Aberdeen Plant Materials Center.*

Oldfield S, Olwell P. **2015**. The Right Seed in the Right Place at the Right Time. *BioScience* **65**: 955–956.

Osmont KS, Sibout R, Hardtke CS. **2007**. Hidden branches: developments in root system architecture. *Annual review of plant biology* **58**: 93–113.

Owen SM, Sieg CH, Johnson NC, Gehring CA. **2013**. Exotic cheatgrass and loss of soil biota decrease the performance of a native grass. *Biological Invasions* **15**: 2503–2517.

Padilla FM, Miranda JDD, Pugnaire FI. **2007**. Early root growth plasticity in seedlings of three Mediterranean woody species. *Plant and Soil* **296**: 103–113.

Pakeman RJ, Beaton JK, Thoss V, Lennon JJ, Campbell CD, White D, Iason GR. **2006**. The extended phenotype of Scots pine Pinus sylvestris structures the understorey assemblage. *Ecography* **29**: 451–458.

Parkinson H, Zabinski C, Shaw N. **2013**. Impact of Native Grasses and Cheatgrass (Bromus tectorum) on Great Basin Forb Seedling Growth. *Rangeland Ecology & Management* **66**: 174–180.

Patel DK. **2013**. Early Warning System for Plant Nutrient Deficiency: Future Toolbox. *Agrotechnology* **02**: 9881.

Phillips A, Leger E. **2015**. Plastic responses of native plant root systems to the presence of an invasive annual grass. *American Journal of Botany* **102**: 73–84.

Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L. **2012**. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *The New phytologist* **193**: 30–50.

Prieto I, Padilla FM, Armas C, Pugnaire FI. **2011**. The role of hydraulic lift on seedling establishment under a nurse plant species in a semi-arid environment. *Perspectives in Plant Ecology, Evolution and Systematics* **13**: 181–187.

Putz F, Canham C. **1992**. Mechanisms of arrested succession in shrublands: root and shoot competition between shrubs and tree seedlings. *Forest Ecology and Management* **49**: 267–275.

Qi W-Z, Liu H-H, Liu P, Dong S-T, Zhao B-Q, So HB, Li G, Liu H-D, Zhang J-W, Zhao B. **2012**. Morphological and physiological characteristics of corn (Zea mays L.) roots from cultivars with different yield potentials. *European Journal of Agronomy* **38**: 54–63.

R Core Team. **2019**. R: A language and environment for statistical computing. R Statistical Computing,. *Vienna, Austria. URL https://www.R-project.org/.*

Rajaniemi TK, Allison VJ, Goldberg DE. **2003**. Root competition can cause a decline in diversity with increased productivity. *Journal of Ecology* **91**: 407–416.

Reichenberger G, Pyke DA. **1990**. Impact of early root competition on fitness components of four semiarid species. *Oecologia* **85**: 159–166.

Relyea R. **2002**. Competitor-induced plasticity in tadpoles: consequences, cues, and connections to predator-induced plasticity. *Ecological Monographs* **72**: 523–540.

Del Río M, Condés S, Pretzsch H. **2014**. Analyzing size-symmetric vs. size-asymmetric and intra- vs. inter-specific competition in beech (Fagus sylvatica L.) mixed stands. *Forest Ecology and Management* **325**: 90–98.

Rohatgi A. **2015**. WebPlotDigitizer. *https://automeris.io/WebPlotDigitizer/*: Accessed 3-15-19.

Roscher C, Schumacher J, Schmid B, Schulze E-D. **2015**. Contrasting Effects of Intraspecific Trait Variation on Trait-Based Niches and Performance of Legumes in Plant Mixtures. *Plos One* **10**: e0119786.

Rottstock T, Kummer V, Fischer M, Joshi J. **2017**. Rapid transgenerational effects in Knautia arvensis in response to plant community diversity. *Journal of Ecology* **105**: 714–725.

Roundy BA. **1999**. Lessons from Historical Rangeland Revegetation for Today ' s Restoration. : 33–38.

Rowe CLJ, Leger E. **2011**. Competitive seedlings and inherited traits: a test of rapid evolution of Elymus multisetus (big squirreltail) in response to cheatgrass invasion. *Evolutionary Applications* **4**: 485–498.

Salinger S, Bornkamm R. **1982**. Production of organic matter and interference of two grasses at different levels of water supply. *Agro-Ecosystems* **7**: 277–292.

Schamp BS, Aarssen LW. **2009**. The assembly of forest communities according to maximum species height along resource and disturbance gradients. *Oikos* **118**: 564–572.

Schenk HJ, Jackson RB. **2002**. Rooting depths, lateral root spreads and below-ground/above-ground allometries of plants in water-limited ecosystems. *Journal of Ecology* **90**: 480–494.

Schiffers K, Tielborger K, Tietjen B, Jeltsch F. **2011**. Root plasticity buffers competition among plants: theory meets experimental data. *Ecology* **92**: 610–620.

Schröder R, Prasse R. **2013**. Do Cultivated Varieties of Native Plants Have the Ability to Outperform Their Wild Relatives? *PLoS ONE* **8**.

Schroeder-Georgi T, Wirth C, Nadrowski K, Meyer ST, Mommer L, Weigelt A. **2016**. From pots to plots: Hierarchical trait-based prediction of plant performance in a mesic grassland. *Journal of Ecology* **104**: 206–218.

Seager R, Ting M, Held I, Kushnir Y, Lu J, Vecchi G, Huang H, Al. E. **2007**. Model Projections of an Imminent Transition to a More Arid Climate in Southwestern North America. *Science* **316**: 1181–1185.

Semere T, Froud-Williams RJ. **2001**. The effect of pea cultivar and water stress on root and shoot competition between vegetative plants of maize and pea. *Journal of Applied Ecology* **38**: 137–145.

Semple J. **1996**. Heterotheca villosa. In: Flora of North America Editorial Committee, eds. Vol 20. New York and Oxford. 231–248.

Sharma, RB and Ghidyal B. **1977**. Soil water-root relations in wheat: water extraction rate of wheat roots that developed under dry and moist conditions. *Agronomy journal* **69**: 231–233.

Sharma R, Ghidyal B. **1977**. Soil water-root relations in wheat: water extraction rate of wheat roots that developed under dry and moist conditions. *Agronomy Journal* **69**: 231–233.

Sharp RE, Davies WJ. **1979**. Solute regulation and growth by roots and shoots of water-stressed maize plants. *Planta* **147**: 43–49.

Sharp R, Davies W. **1985**. Root growth and water uptake by maize plants in drying soil. *Journal of Exp* **36**: 1441–1456.

Silva DD, Kane ME, Beeson RC. **2012**. Changes in root and shoot growth and biomass partition resulting from different irrigation intervals for Ligustrum japonicum Thunb. *HortScience* **47**: 1634–1640.

Silvertown J, Araya Y, Gowing D. **2015**. Hydrological niches in terrestrial plant communities: a review. *Journal of Ecology* **103**: 93–108.

Solomon JKQ. **2019**. Characterization of Adult Functional Traits of Local Populations and Cultivars of Sandberg Bluegrass and Bottlebrush Squirreltail Perennial Bunchgrasses. *Plants* **8**: 166.

Sorgonà A, Abenavoli MR, Cacco G. **2005**. A comparative study between two citrus rootstocks: Effect of nitrate on the root morpho-topology and net nitrate uptake. *Plant and Soil* **270**: 257–267.

Sorgonà A, Abenavoli MR, Gringeri PG, Cacco G. **2007**. Comparing morphological plasticity of root orders in slow- and fast-growing citrus rootstocks supplied with different nitrate levels. *Annals of botany* **100**: 1287–96.

Stevanato P, Trebbi D, Bertaggia M, Colombo M, Broccanello C, Concheri G, Saccomani M. **2011**. Root traits and competitiveness against weeds in sugar beet. *International Sugar Journal* **113**: 24–28.

Suding K, Goldberg D, Hartman K. **2003**. Relationships among species traits: separating levels of response and identifying linkages to abundance. *Ecological Monographs* **84**: 1–16.

Taiz L, Zeigler E. **2002**. *Plant Physiology (Third Edition)*.

Tang S, Knapp SJ. **2003**. Microsatellites uncover extraordinary diversity in native American land races and wild populations of cultivated sunflower. *Theoretical and Applied Genetics* **106**: 990–1003.

Taylor D, Aarssen L. **1990**. Complex competitive relationships among genotypes of three perennial grasses: implications for species coexistence. *The American Naturalist* **136**: 305–327.

Tilley D, Ogle D, St. John L. **2014**. Plant guide for hoary tansyaster (Machaeranthera canescens). *USDA Natural Resources Conservation Service, Idaho Plant Materials Center* **Aberdeen,**.

Treder K, Jastrz M, Kostrzewska MK, Wanic M. **2016**. Effect of competitive interactions and water stress on morphological characteristics of red clover (Trifolium pratense L .) cultivated with spring barley (Hordeum vulgare L .). *Acta Scienttiarum Polonorum seria Agricultura* **15**: 83–94.

Turcotte MM, Levine JM. **2016**. Phenotypic Plasticity and Species Coexistence. *Trends in Ecology & Evolution* **31**: 803–813.

Twolan-Strutt L, Keddy P. **1996**. Above- and Belowground Competition Intensity in Two Contrasting Wetland Plant Communities. *Ecology* **77**: 259–270.

USDA, Agricultural Research Service, National Plant Germplasm System. 2020. Germplasm Resources Information Network (GRIN - Taxonomy). National Germplasm Resources Laboratory, Beltsville, Maryland. URL: https://npgsweb.ars-grin.gov/gringlobal/taxonomydetail.aspx?. Accessed 11 January 2020.

Valentine JC, Pigott TD, Rothstein HR. **2010**. How Many Studies Do You Need? *Journal of Educational and Behavioral Statistics* **35**: 215–247.

Varney G, Canny M. **1993**. Rates of water uptake into the mature root systems of maize plants. *New Phytologist* **123**: 775–786.

Venables W, Ripley B. **2002**. *Modern Applied Statistics with S. Fourth Edition*.

Verwijmeren M, Rietkerk M, Bautista S, Mayor AG, Wassen MJ, Smit C. **2014**. Drought and grazing combined: Contrasting shifts in plant interactions at species pair and community level. *Journal of Arid Environments* **111**: 53–60.

Viechtbauer W. **2010**. Conducting Meta-Analyses in R with the metafor package. *Journal of Statistical Software* **36**.

Violle C, Navas ML, Vile D, Kazakou E, Fortunel C, Hummel I, Garnier E. **2007**. Let the concept of trait be functional! *Oikos* **116**: 882–892.

Wang X, Taub DR. **2010**. Interactive effects of elevated carbon dioxide and environmental stresses on root mass fraction in plants: a meta-analytical synthesis using pairwise techniques. *Oecologia* **163**: 1–11.

War AR, Sharma HC, Paulraj MG, War MY, Ignacimuthu S. **2011**. Herbivore induced plant volatiles: their role in plant defense for pest management. *Plant Signaling & Behavior* **6**: 1973–1978.

Washington State University. AgWeatherNet. Date accessed: 1-11-2020. http:weather.wsu.edu/?p=89550

Waters CM, Shaw NL. **2003**. Developing Native Grass Seed Industries for Revegetation in Australia and the Western United States: a Contrast in Production and Adoption. *Proceedings of the VIIth International Rangelands Congress*: 1152–1160.

Weigelt A, Röttgermann M, Steinlein T. **2000**. Sandy soils influence of water availability on competitive interactions between plant species. *Folia Geobotanica* **35**: 169–178.

Weigelt A, Steinlein T, Beyschlag W. **2005**. Competition among three dune species: the impact of water availability on below- ground processes. *Plant Ecology* **176**: 57–68.

Welbank PJ. **1961**. A Study of the Nitrogen and Water Factors in Competition with Agropyron repens (L.) Beauv. *Annals of Botany* **25**: 116–137.

White A, Fant JB, Havens K, Skinner M, Kramer AT. **2018**. Restoring species diversity: assessing capacity in the U.S. native plant industry. *Restoration Ecology* **26**: 605–611.

Wilkinson S, Gross C. **1964**. Competition for light, soil moisture and nutrients during Landino clover establishment in Orchardgrass Sod. *Agronomy journal* **56**: 389–392.

Wood TE, Doherty K, Padgett W. **2015**. Development of Native Plant Materials for Restoration and Rehabilitation of Colorado Plateau Ecosystems. *Natural Areas Journal* **35**: 134–150.

Young TP, Petersen D A., Clary JJ. **2005**. The ecology of restoration: Historical links, emerging issues and unexplored realms. *Ecology Letters* **8**: 662–673.

Zhang R, Schellenberg MP, Han G, Wang H, Li J. **2018**. Drought weakens the positive effects of defoliation on native rhizomatous grasses but enhances the drought-tolerance traits of native caespitose grasses. *Ecology and Evolution* **8**: 12126–12139.

APPENDIX ONE

Supplementary material from chapter one

SI Table 1.1. PRISMA checklist.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Root and shoot competition lead to contrasting competitive outcomes under water stress: A Meta-analysis	1
ABSTRACT			
Structured summary	2	**Background:** Competition is a critical process that shapes plant communities and interacts with environmental constraints. Though important to natural communities and agricultural systems, there are surprising knowledge gaps related to mechanisms that belie those processes: the contribution of different plant parts on competitive outcomes and the effect of environmental constraints on these contributions. **Objective:** Studies that partition competition into root-only and shoot-only interactions assess whether plant parts impose different competitive intensities using physical partitions and serve as an important way to fill knowledge gaps. Given predicted drought escalation due to climate change, we focused meta-analytic techniques on the effects of water supply and competitive outcomes. **Methods:** We searched Web of Science for peer-reviewed studies and found 2042 results. From which six suitable studies with 92 effect sizes on 10 species were identified to test these effects. **Results:** Water availability and competition treatment (root-only, shoot-only, and full plant competition) significantly interact to affect plant growth responses ($p < 0.0001$). Root-only and full plant competition are more intense in low water availability conditions than shoot-only competition. Shoot-only competition in high-water availability was the most intense showing the opposite pattern. These results also show that the intensity of full competition is similar to root-only competition and that low-water availability intensifies root competition while weakening shoot competition. **Conclusions:** These results emphasize the importance of root competition and these patterns of competition may shift in a changing climate, creating further urgency for further filling	2

		knowledge gaps to address issues of drought on plant interactions and communities.	
INTRODUCTION			
Rationale	3	A major question among plant ecologists is to understand plant competition mechanisms and their outcomes from different perspectives. Many contemporary ecological endeavors seek to elucidate the role of competition in community structure, processes, and species coexistence (Chesson, 2000, 2008; Mayfield & Levine, 2010; HilleRisLambers *et al.*, 2012; Godoy O, Kraft N, 2014; Kraft *et al.*, 2014). Evidence shows that competition impacts survival, and higher level processes such as community diversity and spatial structure (Schamp & Aarssen, 2009; Kunstler *et al.*, 2012) Past work dived deeply into understanding the role of pair-wise species competition on outcomes observed in communities and in field settings (Aerts, 1999; Acciaresi & Guiamet, 2010; Owen *et al.*, 2013; Parkinson *et al.*, 2013). But, only a small section of the literature describes the competitive contributions of roots and shoots separately (Fig. 1) and their interaction with environmental constraints - which is critical considering the contribution of roots and shoots to ecosystem processes and responses to environmental changes(Diaz, S., Hodgson, J.G.; Thompson, K.; Cabido, M.; Cornelissen, J.H.C.; Jalili, A; Montserrat-Marti, G.; Grime *et al.*, 2004; Bardgett *et al.*, 2014; Bu *et al.*, 2019). Most competition studies focus on competitive outcomes on shoots. But competitive behaviors resulting from shoot competition, may not influence competitive root responses in the same plant (Murphy & Dudley, 2007), thus the influence and outcome of roots interaction needs specific consideration. Traits can predict competitive ability and performance in environments (Violle *et al.*, 2007; Funk *et al.*, 2008), and Kembel & Cahill (Kembel & Cahill, 2011) showed that roots face different environments than shoots leading to variable correlation of above- and belowground traits in response to the environment. A meta-analysis on studies that physically partitioned roots and shoots during competition under nutrient stress found that roots imposed more intense competition than shoots reporting a 42% biomass reduction – indicating intense competition. (Kiaer *et al.*, 2013). A critical remaining question is on the role of water in competition. Water is a critical resource that allows plant growth, and related physiological processes such as cell growth and	3-5

		nutrient transport to shoots (Hsiao, 1973; Hsiao & Xu, 2000). In case of low water availability plants can close stomata to limit water loss and CO_2 capture (Taiz & Zeigler, 2002) . They can also respond to water stress by allocating more mass to roots to acquire the limited resource (Wang & Taub, 2010; Poorter *et al.*, 2012). Generally, while water stress reduces plant size, root allocation, branching, length, and uptake, increase to maintain soil water capture capacities (Sharp & Davies, 1979; Jupp & Newman, 1987; Berendse & Móller, 2009; Silva *et al.*, 2012) (Fig. 2). Conversely, water stress reduces shoot growth, leaf area, new leaf production, and photosynthetic light conversion (Sharp & Davies, 1979, 1985; Jentsch *et al.*, 2011; Silva *et al.*, 2012) (Fig. 2). Resulting diminished light interception and metabolic activity aboveground (Gargallo-Garriga *et al.*, 2014), coupled with increased absorptive root area under water stress should intensify competition between roots more than between shoots (e.g. (Casper & Jackson, 1997)), but the literature presents mixed evidence related to their outcomes. Despite established patterns of individual effects of water stress, water stress intensifies, decreases or produces no measured outcomes on root-only or shoot-only competition (e.g. (Dauro & Mohamed-Saleem, 1995; Weigelt *et al.*, 2005; Lamb *et al.*, 2007). The different physiological processes of roots and shoots to drought, may reduce resource acquisition need. These differing activity levels during drought may also have strong effects on above- compared to belowground performance that may affect the intensity of root and shoot competition in water limited environments. This is critical due to the predicted variable global precipitation patterns and increased regional aridity due to climate change (Seager *et al.*, 2007). Environmental constraints such as resource stress change the intensity of the competition among species (Rajaniemi *et al.*, 2003; Liancourt & Lavorel, 2013; Fort *et al.*, 2014a; Silvertown *et al.*, 2015). For example, low water availability can intensify (Weigelt *et al.*, 2000; Hanke *et al.*, 2015) or weaken competition (McCluney *et al.*, 2012) and, for example, water loss of a nurse shrub due to dry soil reduced mortality in a protégé shrub (Prieto *et al.*, 2011). Despite the substantial impacts water limitation imposes on competition and survival compared to nutrient stress (Coomes & Grubb, 2000), the literature pool on water and	

		competition is comparatively small so synthesis would advance our knowledge by elucidating patterns.	
Objectives	4	We conducted a meta-analysis to provide resolution on the intensity of root and shoot competition under water stress. We assessed whether roots and shoots impose different competitive intensities in studies that physically partitioning roots and shoots during competition experiments under different water availabilities (Fig. 1). We hypothesize that: 1) competitive intensity of root-only, shoot-only, and full competition will differ under varying water availability; 2) competitive intensity will differ between low – and high-water stress treatments; and 3) root competition will differ from shoot competition at varying water availabilities.	5
METHODS			
Protocol and registration	5	We sought peer reviewed literature using the ISI Web of Science searching platform. A search was performed on 2 May 2019	6
Eligibility criteria	6	Criteria: experimental designs that contained root-only, shoot-only, and or full competition, and a control group (Fig. 1), all under a high- and low-water availability treatments. Weigelt et al. (Weigelt *et al.*, 2005) lacked a shoot competition treatment but was included here.	6
Information sources	7	ISI Web of Science, contact with study authors to identify additional data.	6
Search	8	We used Boolaen terms to broaden the search: [(shoot* AND root*) OR (above AND below)] AND [(competit* OR interact*)], topic: “water stress.” Search results were refined by research areas of plant sciences, agriculture, genetics, heredity, forestry, and environmental sciences, and ecology.	6
Study selection	9	Abstracts were then evaluated for relevance and read if meet criteria	6
Data collection process	10	Studies were included in the analyses if we acquired response variables, standard deviation, and sample sizes, either from the study, the study authors, or from figures. When data were only available in graphics, those data were extracted from figures using the free web-based application WebPlotDigitizer v3.9 (Rohatgi, 2015). We extracted data from figures from three studies (Salinger & Bornkamm, 1982; Lamb *et al.*, 2007; Bartelheimer *et al.*, 2010).	6

Data items	11	We collected additional data such as study location, setting species, water treatment	6
Risk of bias in individual studies	12	Five suitable studies were not analyzed due to missing data. These studies are summarized in the discussion to compare their outcomes to studies analyzed. Standard deviations were imputed on three studies to include here. This reduces publication bias and improves variance estimates compared to when data from an incomplete study are excluded (Lajeunesse, 2013).	8-9
Summary measures	13	Log response ratio, sampling variance	7-8
Synthesis of results	14	Q, I^2, T^2 and sigma are reported herein	11

Page 1 of 2

Section/topic	**#**	**Checklist item**	**Reported on page #**
Risk of bias across studies	15	Factors across each study were held constant such as water level (lowest or highest levels are included when there were multiple levels included). Different species were used in many of the studies and phylogenetic independence may lead to correlation between species.	6
Additional analyses	16	Contrasts were performed to compare specific groups within treatments.	9
RESULTS			
Study selection	17	**PRISMA 2009 Flow** **gram** See figure 1.3	Fig 1.3
Study characteristics	18	Bartelheimer M, Gowing D, Silvertown J. Explaining hydrological niches: The decisive role of below-ground competition in two closely related Senecio species. J Ecol. 2010;98: 126–136. doi:10.1111/j.1365-2745.2009.01598.x Bornkamm R, Salinger S, Strehlow H. Productivity and Chemical Constituents of two Grasses under Pure and Mixed Cultivations. Flor Biodivers. 1975;164: 437–448. Dauro D, Mohamed-Saleem M. Shoot and root interactions in intercopped wheat and clover. Trop Agric. 1995;72: 170–	9

		172. Lamb EG, Shore BH, Cahill JF. Water and nitrogen addition differentially impact plant competition in a native rough Fescue grassland. Plant Ecol. 2007;192: 21–33. doi:10.1007/sll258-006-9222-4 Haugland E, Froud-Williams R. Improving grasslands: the influence of soil moisture and nitrogen fertilization on the establishment of seedlings. J Appl Ecol. 1999;36: 263–270. Putz F, Canham C. Mechanisms of arrested succession in shrublands: root and shoot competition between shrubs and tree seedlings. For Ecol Manage. 1992;49: 267–275. Salinger S, Bornkamm R. Production of organic matter and interference of two grasses at different levels of water supply. Agro-Ecosystems. 1982;7: 277–292. Semere T, Froud-Williams RJ. The effect of pea cultivar and water stress on root and shoot competition between vegetative plants of maize and pea. J Appl Ecol. 2001;38: 137–145. doi:10.1046/j.1365-2664.2001.00570.x Weigelt A, Steinlein T, Beyschlag W. Competition among three dune species: the impact of water availability on below-ground processes. Plant Ecol. 2005;176: 57–68. Welbank PJ. A Study of the Nitrogen and Water Factors in Competition with Agropyron repens (L.) Beauv. Ann Bot. 1961;25: 116–137. doi:10.1093/oxfordjournals.aob.a083737 Wilkinson S, Gross C. Competition for light, soil moisture and nutrients during Landino clover establishment in Orchardgrass Sod. Agron J. 1964;56: 389–392.	
Risk of bias within studies	19	The sample size within some studies is small and may introduce bias. For example, Bartelheimer et al. 2010 had the smallest treatment sample size of n = 3. The rank correlation test for funnel plot asymmetry to test for publication bias revealed low and non-significant correlation between studies (Kendall's tau = 0.153, p = 0.045) indicating publication bias.	SI Table 1
Results of individual studies	20	The model that best fit the data included an interaction between competition treatment and water treatments ($Q_{df = 5}$ = 395.5, $p < 0.001$) (Table 2), whereby competition and water treatments interacted to significantly affect plant growth. Root-only, shoot-only and full competition exhibited different responses to water treatments (Fig. 3). Root-only (-45%) and full (-53%) competition at low water availability was more intense than shoot-only (-14%) competition, while root-only (-51%) and full (-51%) competition similarly lead to similar mass suppression whereas shoot-only (-36%) competition had the	SI Table 1, p 10-11

		most intense competition outcome though was less suppressive under high water availability. Root only-competition significantly differed from shoot-only competition at low water availability (p = 0.0004) and under high water availability (p < 0.0001), where root-only competition was more intense under low water availability compared to high water availability. Though there are large confidence intervals for shoot-only competition at high water availability reduces our certainty of the true effect size. The heterogeneity between studies (Q_m on 5 df) is 395.5 indicating that heterogeneity between studies is high (given a Q > 100 we reject the null hypothesis that the variance component is 0 (Hedges *et al.*, 1999)) and there are differences between studies and unexplored sources of variation we did not capture in the analyses. This is reinforced by the high I^2 values (Table 2) denoting that a large part of the variation remains unexplained. Root-only and shoot-only competition had significantly different responses to water treatments (p <0.001) where root-only competition was more intense than shoot-only competition under low water availability and the opposite pattern at high water availability treatments (Fig. 3).The overall plant response was not significantly impacted by water availability (p = 0.1). Low water availability caused slightly weaker competition compared to compared to high water availability when aggregated over effect sizes of all treatments. The rank correlation test for funnel plot asymmetry to test for publication bias revealed low and non-significant correlation between studies (Kendall's tau = 0.153, p = 0.045) indicating publication bias.	
Synthesis of results	21	We found that shoot-only competition was more intense under high-water availability than in low-water availability treatments. To the contrary, the weakest competitive treatment was shoot-only competition in low-water availability. Root-only competition was weaker at high-water availability. Furthermore, root-only competition was more intense than shoot-only competition under low-water availability.	10-11
Risk of bias across studies	22	Three suitable studies were not included in the analysis which influences the risk of publication bias here and the rank correlation test for funnel plot asymmetry to test for publication bias revealed low and non-significant correlation between studies (Kendall's tau = 0.153, p = 0.045) indicating publication bias	16
Additional analysis	23	The overall plant response was not significantly impacted by water availability (p = 0.1).	11
DISCUSSION			

Summary of evidence	24	The impact of increasing drought in a changing climate (IPCC, 2014) and ever-present competition have large ramifications for natural plant communities and agricultural systems. Specifically, competition and water stress impacts community membership (Verwijmeren *et al.*, 2014; Kraft *et al.*, 2014) and crop yield (Acciaresi & Guiamet, 2010; Leguizamón *et al.*, 2011) and has global importance for plant conservation and food security. We demonstrate that water availability significantly modulates competitive outcomes where high-water availability intensified shoot-only competition while weakening root-only competition and the opposite patterns for low water availability. These results are important as short-term effects of competition were a top predictor of species' abundance in the field (Howard & Goldberg, 2001). This meta-analysis combines empirical evidence to reveal competitive patterns and influence future work to advance our knowledge. Given the climate change outcomes of increased drought leading to increased root allocation (Wang & Taub, 2010) this may have important competition-mediated community outcomes. We may see increases in root competition for water in communities (sensu (Casper & Jackson, 1997), (Wang & Taub, 2010)) that lead to plant diversity loss from drought (Lanta *et al.*, 2012). But more research is needed to assess these outcomes and in different biomes. Because we see contrasting outcomes in root-only and shoot-only competition, researchers should increase the assessment of belowground ecology to draw more accurate conclusions about competition particularly if environmental constraints would lead to a shift in biomass allocation (Cahill, 2002).	12,14
Limitations	25	These results show important interactions between plant competition and water availability. The fixed effects used in these models significantly explained variation in effect sizes but including other effects such as target species life history, non-target life-history, and experimental setting may reduce residual heterogeneity. Given the small number of studies, these factors could not be reliably tested without replication. Other sources of variation were in the differences in materials used to partition plants (e.g. mesh vs. solid aboveground dividers) and implementation of water stress where amounts that were considered "high" and "low" differed by study. Additionally, the adaptations of target species could have influenced competitive outcomes and responses to water stress. For example, Bartelheimer et al. (Bartelheimer *et al.*, 2010) used *Senecio aquaticus* – a wetland adapted species – which performed poorer than the terrestrial species in low water availability. Finally, there were	15-16

		known suitable studies that we excluded due to missing information. Authors should publish robust study results and parameters (e.g. sample size, responses, measures of variability) for future synthesis and knowledge advancement.	
Conclusions	26	The intensity of root-only and shoot-only competition showed opposing trends under differing water availability. Our results show that roots have major implication in competitive outcomes for plants when soil resource are limited. This suggests that root-dominated interactions should make coexistence more difficult and lead to more growth suppression in case of water shortage. Importantly, if we only record aboveground responses to water stress or competition, we may conclude weak competition when belowground responses may reveal contrasting evidence. Future research should tie in the role that root and shoot competition have on species coexistence in plant communities.	16
FUNDING			
Funding	27	This study was not funded directly, though A. Foxx is supported by the Robert Hevey and Constance M. Filling PhD Fellowship	NA

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for
Systematic Reviews and Meta-Analyses: The PRISMA Statement.
PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097
For more information, visit: **www.prisma-statement.org**.

SI Table 1.2. Study Data: Study dataset used to calculate effect sizes (lnRR) and sampling variances.

Study	Target species	Water	Comp	N	Cntrl Mean	Cntrl STD	LRR	Var
Bartelheimer et al. 2010	*Senecio acquaticus*	Ambient	Shoot	3	7.86	1.25	0.00905	0.008797
Bartelheimer et al. 2010	*Senecio acquaticus*	Ambient	Root	3	7.86	1.25	-0.33103	0.014943
Bartelheimer et al. 2010	*Senecio acquaticus*	Ambient	Full	3	7.86	1.25	-0.96281	0.041311
Bartelheimer et al. 2010	*Senecio acquaticus*	Stress	Shoot	3	2.63	11.75	-0.11441	6.728362
Bartelheimer et al. 2010	*Senecio acquaticus*	Stress	Root	3	2.63	11.75	-0.83833	7.493748
Bartelheimer et al. 2010	*Senecio acquaticus*	Stress	Full	3	2.63	11.75	-0.47542	7.490581
Bartelheimer et al. 2010	*Senecio jacobea*	Ambient	Shoot	3	2.93	1.25	-0.21706	0.064526
Bartelheimer et al. 2010	*Senecio jacobea*	Ambient	Root	3	2.93	1.25	-0.76913	0.172534
Bartelheimer et al. 2010	*Senecio jacobea*	Ambient	Full	3	2.93	1.25	-1.31568	0.539495
Bartelheimer et al. 2010	*Senecio jacobea*	Stress	Shoot	3	2.84	6.82	0.161268	1.955372
Bartelheimer et al. 2010	*Senecio jacobea*	Stress	Root	3	2.84	6.82	0	2.053518
Bartelheimer et al. 2010	*Senecio jacobea*	Stress	Full	3	2.84	6.82	-1.12393	4.533832
Bornkamm et al. 1975	*Arrhenatherum elatius*	Ambient	Full	7	1.72	0.024	-0.07859	8.89E-05
Bornkamm et al. 1975	*Arrhenatherum elatius*	Ambient	Root	7	1.72	0.024	0.051003	5.01E-05
Bornkamm et al. 1975	*Arrhenatherum elatius*	Ambient	Shoot	7	1.72	0.04	-0.26469	0.000873
Bornkamm et al. 1975	*Arrhenatherum elatius*	Stress	Full	7	1.77	0.21	-0.11355	0.038443
Bornkamm et al. 1975	*Arrhenatherum elatius*	Stress	Root	7	1.77	0.209708	0	0.002005
Bornkamm et al. 1975	*Arrhenatherum elatius*	Stress	Shoot	7	1.77	0.209708	-0.23451	0.002649
Bornkamm et al. 1975	*Bromus erectus*	Ambient	Full	7	1.47	0.004076	-0.83155	0.015497
Bornkamm et al. 1975	*Bromus erectus*	Ambient	Root	7	1.47	0.004076	-0.3557	0.000634
Bornkamm et al. 1975	*Bromus erectus*	Ambient	Shoot	7	1.47	0.004076	-0.74194	0.01115
Bornkamm et al. 1975	*Bromus erectus*	Stress	Full	7	0.76	0.014552	0.243978	0.000484
Bornkamm et al. 1975	*Bromus erectus*	Stress	Root	7	0.76	0.014552	0.254234	0.002356
Bornkamm et al. 1975	*Bromus erectus*	Stress	Shoot	7	0.76	0.014552	0.303996	0.000685
Lamb et al. 2007	*Artemesia frigida*	Ambient	Full	7	0.052497	0.015602	-1.31759	4.079984

Lamb et al. 2007	*Artemesia frigida*	Ambient	Root	7	0.052497	0.015602	-1.75399	0.619933
Lamb et al. 2007	*Artemesia frigida*	Ambient	Shoot	7	0.052497	0.015602	-0.81214	0.02186
Lamb et al. 2007	*Artemesia frigida*	Stress	Full	7	0.09284	0.058501	-2.12832	23.19363
Lamb et al. 2007	*Artemesia frigida*	Stress	Root	7	0.09284	0.058501	-2.30535	0.393603
Lamb et al. 2007	*Artemesia frigida*	Stress	Shoot	7	0.09284	0.058501	-0.71488	0.058992
Lamb et al. 2007	*Chenopodium leptophyllum*	Ambient	Full	7	0.049406	0.043016	-2.1814	27.57345
Lamb et al. 2007	*Chenopodium leptophyllum*	Ambient	Root	7	0.049406	0.043016	-1.00807	0.110764
Lamb et al. 2007	*Chenopodium leptophyllum*	Ambient	Shoot	7	0.049406	0.043016	-0.31399	0.14623
Lamb et al. 2007	*Chenopodium leptophyllum*	Stress	Full	7	0.092578	0.116589	-1.56994	7.576427
Lamb et al. 2007	*Chenopodium leptophyllum*	Stress	Root	7	0.092578	0.116589	-1.603	0.373681
Lamb et al. 2007	*Chenopodium leptophyllum*	Stress	Shoot	7	0.092578	0.116589	0.066103	0.22926
Weigelt et al. 2005	*Carex arenaria*	Ambient	Full	9	4.596222	3.390814	-0.52841	0.119402
Weigelt et al. 2005	*Carex arenaria*	Ambient	Root	9	5.530556	3.387683	-0.36864	0.081101
Weigelt et al. 2005	*Carex arenaria*	Stress	Full	8	4.202444	3.163751	-1.15927	0.124504
Weigelt et al. 2005	*Carex arenaria*	Stress	Root	8	3.110444	1.496866	0.089671	0.127769
Weigelt et al. 2005	*Carex arenaria*	Ambient	Full	9	4.596222	3.390814	-2.1408	0.232606
Weigelt et al. 2005	*Carex arenaria*	Ambient	Root	9	5.530556	3.387683	-2.58549	0.071626
Weigelt et al. 2005	*Carex arenaria*	Stress	Full	9	4.202444	3.163751	-2.84035	0.09657
Weigelt et al. 2005	*Carex arenaria*	Stress	Root	9	3.110444	1.496866	-2.61074	0.071843
Weigelt et al. 2005	*Carex arenaria*	Ambient	Full	9	4.596222	3.390814	-0.99271	0.093416
Weigelt et al. 2005	*Carex arenaria*	Ambient	Root	9	5.530556	3.387683	-1.22069	0.083925
Weigelt et al. 2005	*Carex arenaria*	Stress	Full	9	4.202444	3.163751	-2.24535	0.112159
Weigelt et al. 2005	*Carex arenaria*	Stress	Root	9	3.110444	1.496866	-1.90024	0.091949
Weigelt et al. 2005	*Corynephorus canescens*	Ambient	Full	8	19.49188	4.957775	-0.21294	0.025419
Weigelt et al. 2005	*Corynephorus canescens*	Ambient	Root	8	21.64729	2.616997	-0.29824	0.009223
Weigelt et al. 2005	*Corynephorus canescens*	Stress	Full	8	12.8665	5.064314	-0.12143	0.0332
Weigelt et al. 2005	*Corynephorus canescens*	Stress	Root	8	12.09657	4.168549	0.223325	0.028858
Weigelt et al. 2005	*Corynephorus canescens*	Ambient	Full	8	19.49188	4.957775	-1.53153	0.046423
Weigelt et al. 2005	*Corynephorus canescens*	Ambient	Root	8	21.64729	2.616997	-1.17259	0.005448
Weigelt et al. 2005	*Corynephorus canescens*	Stress	Full	8	12.8665	5.064314	-1.24792	0.0636

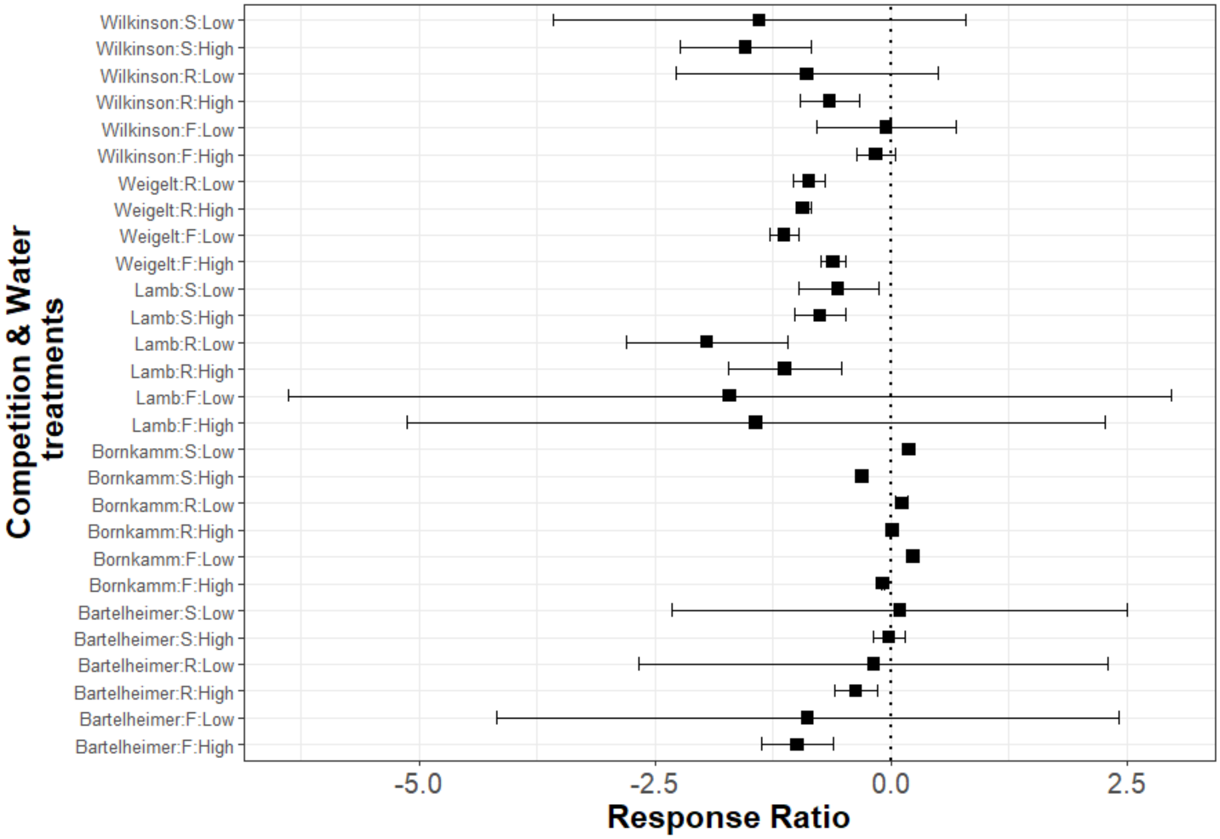
Weigelt et al. 2005	*Corynephorus canescens*	Stress	Root	8	12.09657	4.168549	-1.2265	0.086375
Weigelt et al. 2005	*Corynephorus canescens*	Ambient	Full	8	19.49188	4.957775	-0.34947	0.014878
Weigelt et al. 2005	*Corynephorus canescens*	Ambient	Root	8	21.64729	2.616997	-0.37287	0.012751
Weigelt et al. 2005	*Corynephorus canescens*	Stress	Full	8	12.8665	5.064314	-0.79627	0.065046
Weigelt et al. 2005	*Corynephorus canescens*	Stress	Root	8	12.09657	4.168549	-0.88276	0.054337
Weigelt et al. 2005	*Hieracium pilosella*	Ambient	Full	8	6.659875	2.536238	0.278324	0.040514
Weigelt et al. 2005	*Hieracium pilosella*	Ambient	Root	8	12.036	2.948714	-0.36944	0.023997
Weigelt et al. 2005	*Hieracium pilosella*	Stress	Full	8	9.403143	3.461359	-0.14418	0.038069
Weigelt et al. 2005	*Hieracium pilosella*	Stress	Root	8	10.918	4.373947	-0.36312	0.042909
Weigelt et al. 2005	*Hieracium pilosella*	Ambient	Full	8	6.659875	2.536238	-1.54307	0.046112
Weigelt et al. 2005	*Hieracium pilosella*	Ambient	Root	8	12.036	2.948714	-1.99168	0.023622
Weigelt et al. 2005	*Hieracium pilosella*	Stress	Full	8	9.403143	3.461359	-2.19488	0.046015
Weigelt et al. 2005	*Hieracium pilosella*	Stress	Root	8	10.918	4.373947	-1.80304	0.093072
Weigelt et al. 2005	*Hieracium pilosella*	Ambient	Full	8	6.659875	2.536238	-0.83619	0.050516
Weigelt et al. 2005	*Hieracium pilosella*	Ambient	Root	8	12.036	2.948714	-1.86524	0.040972
Weigelt et al. 2005	*Hieracium pilosella*	Stress	Full	8	9.403143	3.461359	-1.60791	0.06047
Weigelt et al. 2005	*Hieracium pilosella*	Stress	Root	8	10.918	4.373947	-1.51224	0.091446
Wilkinson & Gross 1964	*Trifolium repens*	Ambient	Full	12	7.29	1.877087	-0.07696	0.011969
Wilkinson & Gross 1964	*Trifolium repens*	Ambient	Full	12	7.29	1.877087	-1.95694	0.282291
Wilkinson & Gross 1964	*Trifolium repens*	Ambient	Root	12	7.29	1.877087	-0.64625	0.025647
Wilkinson & Gross 1964	*Trifolium repens*	Ambient	Shoot	12	7.29	1.877087	-1.53543	0.124646
Wilkinson & Gross 1964	*Trifolium repens*	Stress	Full	12	2.01	1.877087	0	0.145353
Wilkinson & Gross 1964	*Trifolium repens*	Stress	Full	12	2.01	1.877087	-2.30757	7.413209
Wilkinson & Gross 1964	*Trifolium repens*	Stress	Root	12	2.01	1.877087	-0.88446	0.498894
Wilkinson & Gross 1964	*Trifolium repens*	Stress	Shoot	12	2.01	1.877087	-1.39128	1.247162

SI Table 1.3. Koricheva & Gurevitch (2014) Meta-analysis Checklist.

Recommended Item	Performed
1. Has formal meta-analysis been conducted (i.e. combination of effect sizes using standard meta-analytical methodology) or is it simply a vote count?	Meta-analysis performed with in the systematic review
2. Are details of bibliographic search (electronic data bases used, keyword combinations, years) reported in sufficient detail to allow replication?	Yes, see materials and methods and PRISMA flow diagram
3. Are criteria for study inclusion/exclusion explicitly listed?	Yes, see PRISMA diagram
4. Have standard metrics of effect size been used or, if non-standard metrics have been employed, is the distribution of these parameters known and have the authors explained how they calculated variances for such metrics?	Yes, standard metrics – log response ratio (Hedges et al. 1999)
5. If more than one estimate of effect size per study was included in the analysis, has potential non-independence of these estimates been taken into account?	Not accounted for
6. Have effect sizes been weighted by study precision or has the rational for using unweighted approach been provided?	Variance of LRR is takes in account sample size and thus the precision
7. Have statistical model for meta-analysis and the software used been described?	Yes, see materials and methods
8. Has heterogeneity of effect sizes between studies been quantified?	Yes, see table 2
9. Have the causes of existent heterogeneity in effect sizes been explored by meta-regression?	Yes, see materials and methods
10. If effects of multiple moderators have been tested, have potential non-independence of and interactions between moderators been taken into account?	Not accounted for
11. If meta-analysis combined studies conducted on different species, has phylogenetic relatedness of species been taken into account?	Not accounted for given the 10 species
12. Have tests for publication bias been conducted?	Yes: Rank Correlation Test for Funnel Plot Asymmetry

	using the “ranktest” function in the “metafor” package. We identified publication bias (Kendall's tau = 0.153, p = 0.045)
13. If meta-analysis combines studies published over considerable time span, have possible temporal changes in effect size been tested?	There is a large time range (41 years), but this hasn’t been accounted for
14. Have sensitivity analysis been performed to test the robustness of results?	Sensitivity analyses were not performed
15. Have full bibliographic details of primary studies included in a meta-analysis been provided?	Yes, see PRISMA checklist
16. Has the data set used for meta-analysis, including effect sizes and variances/sample sizes from individual primary studies and moderator variables, been provided as electronic appendix?	Yes, see SI Table

SI Fig 1.1 Meta-estimates by study.



APPENDIX TWO

Supplementary material from chapter two

SI Table 2.1. Outcomes of tests for differences of means, medians, survival, and variation by collection type and accession source.

Test	Response	Test Statistic	Test Outcome	Trait type
		Trait variability		
Bartlett's Test	Root mass	Bartlett's K-squared = 1.1, 1 df	P = 0.3	Root
	RMF	Bartlett's K-squared = 5.1, 1 df	P = 0.02	Root
	Root length	Bartlett's K-squared = 0.03, 1 df	P = 0.9	Root
	Shoot mass	Bartlett's K-squared = 0.2, 1 df	P = 0.6	Shoot
	SMF	Bartlett's K-squared = 5.1, 1df	P = 0.02	Shoot
	Plant height	Bartlett's K-squared = 2.5, 1 df	P = 0.1	Shoot
Levene's Test	Root tip count	F = 3.4, 1 df	P = 0.07	Root
	Leaf count	F = 3.4, 1 df	P = 0.6	Shoot
		Trait comparisons		
Mann-Whitney U	RMF	Collection type M.W. estimate = 0.7	P = 0.0006	Root
	SMF	Collection type M.W. estimate = 0.7	P = 0.0006	Shoot
Kruskal-Wallis	RMF	Accession: χ^2 = 9.9	P = 0.08	Root
	SMF	Accession: χ^2 = 9.9	P = 0.08	Shoot
Generalized linear mixed model	Root tip count	Accession x Collection: F = 62, 5 df	P < 0.0001	Root
	Leaf count	F = 2.3, 1 df	P = 0.9	Shoot
Linear mixed effects model	Plant height	Null model F = 2.4, 1 df	P < 0.0001	Shoot
	Root mass	Collection type: F = 4.8, 1 df	P = 0.03	Root
	Shoot mass	Null model F = 2.1, 1 df	P < 0.0001	Shoot
	Root length	Null model, F = 12.7, 1 df	P < 0.0001	Root
		Survival		
Survival Proportion	Survival	Collection type: χ^2 = 3.9, 1 df	P = 0.05	
		Accession: χ^2 = 9.9, 5 df	P = 0.08	

APPENDIX THREE

Supplementary material from chapter four

SI Table 4.1. Lines of equations for the relationships between mass fraction (RMF and SMF) (Fig. 2) of the focal plant and hierarchical mass distance between focal and non-focal plants for the three focal populations and the interaction types. Equation parameters are calculated on raw values. ROS is whether the trait was derived from roots or shoots.

Type	Focal population	ROS	Slope	Intercept
Interspecific mixture	MACA-A	Root	0.01	0.2
Interspecific mixture	MACA-A	Shoot	0.001	0.8
Interspecific mixture	MACA-B	Root	0.02	0.2
Interspecific mixture	MACA-B	Shoot	0.009	0.8
Interspecific mixture	MACA-C	Root	0.03	0.1
Interspecific mixture	MACA-C	Shoot	0.01	0.8
Intraspecific mixture	MACA-A	Root	0.01	0.2
Intraspecific mixture	MACA-A	Shoot	-0.001	0.8
Intraspecific mixture	MACA-B	Root	0.03	0.1
Intraspecific mixture	MACA-B	Shoot	-0.007	0.8
Intraspecific mixture	MACA-C	Root	0.02	0.2
Intraspecific mixture	MACA-C	Shoot	-0.003	0.8
Monoculture	MACA-A	Root	0.01	0.2
Monoculture	MACA-A	Shoot	0.002	0.8
Monoculture	MACA-B	Root	0.01	0.2
Monoculture	MACA-B	Shoot	-0.0006	0.8
Monoculture	MACA-C	Root	0.03	0.2
Monoculture	MACA-C	Shoot	-0.01	0.8