

# Induced phenotypic plasticity alters intraspecific interactions

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April 27, 2020

## Abstract

Plant interactions play key roles in coexistence, where intraspecific neighbors must compete more intensely than interspecific neighbors to promote species coexistence. But because plastic responses can alter traits and interactions, including intraspecific interactions, plasticity can hinder or promote species coexistence. Whether plasticity induced by different types of competitors can impact mechanisms of coexistence remains unknown. To address this, I used a transplant experiment to induce plastic responses with intraspecific or interspecific interactions. Then, I investigated the effects of the induced phenotype on new intraspecific interactions. The interspecific interaction treatment induced plastic responses, producing facilitative outcomes. In the subsequent intraspecific interactions, interspecific-induced individuals exhibited more competitive intraspecific interactions than intraspecific-induced individuals, even though the initial induced effect was positive. This study demonstrates that interspecific interactions may play an indirect role in stabilizing niche mechanisms via induced plasticity, 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 (Chesson 2000). When individuals limit intraspecific growth more than interspecific 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, coexistence may also be altered (*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 plastic root growth led to decreased competitive intensity when *Bromus hordeaceus* was grown with intraspecific neighbors. Plants avoided overlapping root growth and nutrient uptake with intraspecific neighbors (Schiffers *et al.* 2011). While untested, species coexistence might be hindered if *B. hordeaceus* then competed more intensely with interspecific neighbors. Furthermore, plastic responses that increase resource uptake for interacting species can intensify interspecific competition (*e.g.* Acciaresi & Guaiamet, 2010), and 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). For example, Karban *et al.* (2000) showed that leaf damage in clipped sagebrush plants induced production of volatile compounds in nearby tomato plants and the induced volatile production reduced herbivory. Additionally, Relyea (2002) provided a clear example of plasticity that affected competition in *Rana sylvatica* tadpoles. 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 intraspecific competitors (Relyea 2002). However, empirical research on plant-plant induced responses impacting competitive interactions is currently lacking, though plasticity is a widespread phenomenon in plants and likely influence the interactions that form the mechanisms of species coexistence. Available observational data suggests previous interactions are important: Taylor & Aarssen (1990) noted that the identity of associates growing in the field 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; Galloway & Etterson 2007). 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; Ferrero-Serrano *et al.* 2011; Goergen *et al.* 2011; Oduor 2013), impacts the competitive ability and fitness of offspring compared to offspring from uninduced maternal plants. Transgenerational plasticity assessed in interaction studies help to elucidate effects of plasticity on interactions, but the evidence is potentially less direct due to factors such as 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 plants and communities change their interaction intensity under new conditions. The use of a transplant approach allows the impacts of induced plastic responses on subsequent interactions to be more directly quantified.

To elucidate whether intraspecific or interspecific interactions induce plastic responses, and to determine whether these induced responses impact subsequent intraspecific interactions, I performed transplant interaction experiments in a greenhouse and used above- and belowground traits to quantify interaction outcomes of intraspecific and interspecific treatments. I asked whether: traits of *Pascopyrum smithii* differ when grown with intraspecific or interspecific neighbors? And whether outcomes of subsequent intraspecific interactions differ for intraspecific-induced compared to interspecific-induced plants? Specifically, I assessed impacts of induced plasticity on subsequent intraspecific interactions, and thereby understanding impacts an important component of stabilizing niche mechanisms. Understanding the effects of induced plasticity on future competitive interactions has implications for 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. These plants may all interact with different associates over time and affect the outcome of subsequent interactions.

## Methods

I conducted two experiments and one supplemental study in the greenhouse between June 2017 and May 2018 at the Chicago Botanic Garden (Glencoe, IL, USA) The focal plant *Pascopyrum smithii* (Rydb) A. Löve, ('Arriba' cultivar (CRB 2013)) is a perennial rhizomatous bunchgrass native to the western United States. *Bromus tectorum* is a non-native invasive species that displaces native species and alters wildfire regimes (Chambers *et al.* 2007).

### Seed germination

Seeds of *Pascopyrum smithii* were purchased in July 2014 (Central Milling Wheatland, UT, USA), and *Bromus tectorum* seeds were wild collected from Grand County, UT (38.998@N, -110.177@W) in June 2013.

Seeds were stored in a seed dryer at 13°C at 15% relative humidity in the Dixon National Tallgrass Prairie Seed Bank (Glencoe, IL, USA) prior to use. I germinated seeds of both species 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 for seed dormancy break (Kramer & Foxx 2016). I germinated 50 seeds per dish for six dishes for both species. 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 one and two ran from 6 June – 18 September 2017 with an average of 21°C/ 16°C day/night with light supplementing ambient lighting from outdoors. I used 20 - 7.6 cm × 7.6 cm × 15.2 cm rectangular pots (Stuewe & Sons, OR, USA) per treatment for each experiment. For all studies, I organized pots into trays with 18 pots each and filled pots with fine commercial sand. I watered plants three times weekly, rotated trays weekly, and added Murashige-Skoog (Sigma Aldrich, MO, USA) half strength nutrients (4g/L) at week two of both experiments. The greenhouse was sprayed weekly with MainSpring GNL insecticides (MainSpring, MD, USA) for aphids, mites, and thrips.

### **One: Interactions to induce plasticity**

The treatments applied were intraspecific interactions with pots of three interacting *Pascopyrum smithii* seedlings; interspecific interactions with pots of one *Pascopyrum smithii* seedling surrounded by two *Bromus tectorum* seedlings; and pots with one *Pascopyrum smithii* growing alone and serving as the control group (Box 1, A). Each treatment had an initial sample size of 20 pots, for a total of 60 pots. Focal plants were tagged after emergence from the substrate surface. After four weeks of growth, plants were randomly designated to either be harvested for data collection or transplanted to new pots with two *Pascopyrum smithii* neighbors for the second part of the study. I induced plastic responses at an early life stage as seedlings are more sensitive to competition (Foster 1999; Young *et al.* 2005; James *et al.* 2011) as to capture resulting induced responses.

At harvest, I gently separated the plants and washed the roots of sand. I scanned each focal plant using an Epson expression 10000XL scanner (Epson, CA, USA) and counted the number of root tips from the scanned images. I recorded root mass and shoot mass after drying the plants in paper envelopes in a dryer at 95°C for five days. Lastly, I calculated the functional trait root mass fraction (RMF: root mass/ plant mass).

### **Two: Induced plasticity impacts on intraspecific interactions**

Focal plants were transplanted from the pots used in the first study after watering the pots to saturation and taking care to avoid disturbing the focal plant's roots and keeping the soil column around the roots intact. I transplanted focal seedlings to randomized pots with two *Pascopyrum smithii* plants of the same age. *Pascopyrum smithii* plants in the recipient pots were propagated and grown using the same procedure described for the first experiment. I placed a Ray Leach- cone-tainer (Stuewe & Sons, Inc., OR, USA) in the middle of the pot as a placeholder for the transplant next to the same-aged intraspecific neighbors (Box 1, B; see Appendix S1 in Supporting Information). I watered the pots to saturation following transplanting.

This experiment had two treatments: (i) intraspecific-induced plants transplanted with two same-age intraspecific plants, and (ii) interspecific-induced plants transplanted with two same-age intraspecific plants (Box 1, B). Sample sizes for intraspecific-induced ( $n = 14$ ) and interspecific-induced ( $n = 6$ ) treatments differed due to establishment issues of recipient same-age intraspecific plants. I washed plants of sand after harvesting; however, it was not possible to analyze the root system as roots were highly tangled with neighbors and mesh of the pots. Therefore, the two response variables for this experiment were shoot mass for each transplanted focal plant and cumulative number of rhizomes of all three plants in each pot. I placed plants in paper envelopes in a drier at 95°C for five days prior to weighing aboveground biomass.

### **Supplemental experiment: Impact of transplantation on growth**

To assess the impact of transplanting on growth of *Pascopyrum smithii*, I grew plants singly then excavated them to simulate transplantation. Plants grew from March 2019 to May 2019 at 16°C/21°C day/night temperatures and 56% relative humidity. Plants were watered three times weekly and fertilized weekly with a 237 ppm of nitrogen, phosphorous, and potassium containing solution. I germinated the same seedlot following the same procedure as in the previous experiments; then each germinant was planted to an individual pot in in commercial sand for four weeks. At four weeks of age, I excavated a subset of the plants ( $n = 14$ ) from the pots to simulate transplantation, and then returned the plants to the same pot. The other subset ( $n = 17$ ) served as the control group where I did not excavate the plants. Plants then grew to 8 weeks of age and I harvested then placed the plants in paper envelopes in a drier at 95°C for five days prior to weighing above- and belowground biomass.

## Analyses

All statistical analyses were performed 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 intraspecific-induced and interspecific-induced treatments differed from the control treatment. I used linear models with treatment (control, intraspecific and interspecific 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 the number of root tips. I made planned contrast comparisons between the control group and the treatment groups, and between the treatment groups.

For experiment two, I determined whether growth in interspecific-induced treatments differed from intraspecific-induced treatments. I tested if shoot mass and the number of rhizomes responses differed by treatments using separate linear and generalized linear models with a Poisson error distribution, respectively. Lastly, for the supplemental experiment, I determined the impact of focal plant excavation on plant growth using linear models to analyze the effect of excavation treatment on shoot mass and root mass.

## Results

### One: Interactions to induce plasticity

Plant interaction treatments led to differences in plant traits. Shoot mass of focal plants differed across the plant interaction treatments ( $R^2 = 0.37$ ,  $F_{2,34} = 11.5$ ,  $p = 0.0002$ , Fig. 1) with the interspecific treatment showing a higher value than the control ( $p < 0.0001$ ). On the other hand, plants in the intraspecific treatment showed a marginally higher value than the control ( $p = 0.06$ , Fig. 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 exhibited a similar pattern as shoot mass, whereby root mass was higher in the interspecific treatment than the control ( $p = 0.02$ ) but did not differ between the intraspecific treatment and the control ( $p = 0.4$ , Fig. 1B). Conversely, RMF was significantly lower than control ( $p = 0.003$ ) in the interspecific interaction treatment and similar between the control group and the intraspecific treatment ( $p = 0.2$ ; Fig. 1C). Number of root tips did not differ across all treatments ( $F_{1,35} = 49.3$ ,  $p = 0.9$ , Fig. 1D).

### Two: Induced plasticity impacts on intraspecific interactions

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

### Supplemental 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. 3A). Conversely, mean root mass was not affected by excavation ( $p = 0.30$ ; Fig. 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 *Pascopyrum smithii* through intraspecific and interspecific interactions with the invasive *Bromus tectorum*, and then test whether those induced responses impact subsequent intraspecific interactions. I found evidence that *Bromus tectorum* induced plastic, facilitative responses in *Pascopyrum smithii*. Importantly, I also found that these induced plastic responses altered intraspecific interactions, where interspecific-induced plants were more competitive with new intraspecific neighbors than were intraspecific-induced plants, even though the initial interspecific-induced interactions indicated facilitation. This is the first study to empirically demonstrate different plastic responses to intraspecific and interspecific interactions that influence subsequent interactions with intraspecific neighbors, to my knowledge and, therefore, is an important contribution to the study of species coexistence.

### Interspecific interactions induced plastic responses

Interspecific neighbors exerted greater impacts on the focal plants than did intraspecific neighbors. For example, shoot mass and root mass of the focal species were greatest in the interspecific interaction treatment, intermediate in the intraspecific treatment, and lowest in the control. If stabilizing niche mechanisms are at play in a population, intraspecific interactions should be more competitive than interspecific interactions that factor to self-limiting population growth (Chesson 2000; Adler *et al.* 2018). The intraspecific and interspecific interaction patterns of *Pascopyrum smithii* used here may factor up to stabilizing niche mechanisms, but more research is needed.

The facilitative effects of *Bromus tectorum* on *Pascopyrum smithii* biomass are surprising, given its invasive nature 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 another native grass (Owen *et al.* 2013). The colonization of arbuscular mycorrhizal fungi (AMF) may explain the mechanism for facilitation and the lack of plastic responses in number of root tips. AMF hyphae function to capture resources, reducing the need for greater root tip proliferation (Hodge *et al.* 2009). I observed emerging evidence that AMF colonization differed by treatment and was greater when *Pascopyrum smithii* interacted with *Bromus tectorum* than intraspecific individuals (*data not shown*), but further work is needed. Generally, microbiota strongly mediate plant species coexistence by impacting resource availability and interactions (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 more fully typify the belowground contribution to species coexistence.

The findings here are not the first evidence that the *Pascopyrum smithii* ‘Arriba’ cultivar collection used in this study competes less intensely with interspecific neighbors than with intraspecific neighbors, indeed this cultivar *P. smithii* has done so with native interspecific neighbors (Fox *unpublished data*). Generally, facilitation impacts plant interactions and the resulting structure and diversity of plant communities (Callaway & Walker 1997; Callaway *et al.* 2002; Prieto *et al.* 2011). Recent research showed facilitation to explain 40% of interactions 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. Facilitation is not currently integrated into this theory, but has some importance in interactions that determine whether species can coexist (*invasibility criterion*; Ellner *et al.* 2019).

For root mass fraction (RMF), intraspecific interactions were more competitive than interspecific interactions, and this pattern may be indicative of intense intraspecific competition important for stabilizing mechanisms

(Bennett *et al.* 2016). The RMF was greatest in the intraspecific treatment though not statistically different from the control, while RMF in the interspecific treatment was significantly lower than both the intraspecific 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).

### **Interspecific- and intraspecific-induced plasticity differentially impact subsequent interactions**

I found evidence that the outcome of new intraspecific interactions differed depending on whether the focal plant was previously induced by intraspecific or interspecific neighbors. Rhizome production in the intraspecific-induced treatment was significantly higher than in the interspecific-induced treatment. Rhizome production is known to decline under water stress and competition (Qi *et al.*, 2012), including for *Pascopyrum smithii* (Dong *et al.* 2012; Bam 2018; Zhang *et al.* 2018). However, because the number of rhizomes in this study was for all individuals in a pot, it is unclear whether lower number of rhizomes in the interspecific-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 interspecific-induced focal plant, or iii) a combination of both of these responses. Either interpretation provides evidence of a below-ground effect of induction by interspecific or intraspecific neighbors. The intraspecific-induced treatment produced two times as many rhizomes as the undisturbed control group (*data not shown; available upon deposition*), further indicating more facilitative interactions in intraspecific-induced treatment group. Furthermore, the aboveground results followed the same pattern, with a non-significant trend toward lower shoot mass of the focal plant in the interspecific-induced treatment.

This study suggests that interspecific interactions may play a role in stabilizing niche mechanisms. Interspecific-induced plasticity promoted stronger intraspecific competition in the subsequent experiment, thereby acting as an indirect stabilizing mechanism. That interspecific interactions can act to influence the intensity of intraspecific competition has roots in the idea that interspecific competition can alter traits through character displacement over time to increase niche differentiation (Slatkin 1980). Additionally, Hart *et al.* (2019) showed for aquatic plant species, that interspecific competition over generations impacted competitive ability, which in turn affected population growth important to stabilizing mechanisms. The mechanism for how previous interspecific interactions in the current study could lead to indirect stabilizing mechanisms may lie in trait hierarchies between induced plants and new intraspecific neighbors. Trait hierarchies are trait differences between a superior and inferior competitor that impact interactions (*fitness inequalities*; Chesson 2000; Mayfield & Levine 2010; HilleRisLambers *et al.* 2012). In this study, interspecific-induced plants were larger than intraspecific-induced plants. Thus, interspecific-induced plants were likely able to impose more intense competition on new intraspecific neighbors than intraspecific-induced plants. Fitness inequalities are usually assessed between species (Mayfield & Levine 2010; Kunstler *et al.* 2012; Fort *et al.* 2014; Kraft *et al.* 2014), and not between intraspecific neighbors (*but see* Abbott & Stachowicz 2016). However, trait differences between intraspecific neighbors should also influence interaction outcomes critical to stabilizing (*reviewed in* Ehlers *et al.* 2016) and equalizing (Barabas & D'Andrea 2016) mechanisms. Future work should assess fitness inequalities between interacting intraspecific neighbors and the impact of trait variation on outcomes to expand our understanding of how traits influence interactions influential to stabilizing niche mechanisms.

### **Practical implications and future work**

The finding that plasticity induced by different competitors can differentially impact subsequent intraspecific interactions is of concern to practitioners interested in conservation and restoration using native plants. These interactions can affect long-term persistence of restored populations. In particular, this study provides further

support to previous research showing that the identity of associates growing with plants prior to out-planting (e.g., in a nursery setting) 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 many intraspecific neighbors (Espeland *et al.* 2017), and plastic responses to intraspecific neighbors compared to interspecific neighbors can also be apparent over multiple generations such as in transgenerational plastic responses (Rottstock *et al.* 2017).

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 early or 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 one of the first of its kind to induce plastic responses in plants to assess impacts on subsequent intraspecific interactions - a concept on plasticity first reviewed by Turcotte & Levine (2016). The transplant approach helps to elucidate impacts of multiple biotic and abiotic environments on individuals (Alexander *et al.* 2015). It also has advantages over transgenerational plasticity experiments when drawing inferences about changes to interaction outcomes given factors such as recombination and outcrossing that lead to genetic differences between generations in these assessments. However, transplant experiments have the issue of distinguishing between natural changes in growth and ontogeny from experimental effects. Plants in the subsequent experiment are older than plants in the prior experiment, and traits in the subsequent experiment are due partially 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 intraspecific competition for the cycad *Dioon sonorense* decreased with age, whereas interspecific 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 early life-stage 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 the supplemental 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) like those from which seeds for this study were sourced. Future research should investigate ways to mitigate transplant impacts as researchers increase the use of transplant experiments.

## Conclusions

Using a two-part interaction study, I show that interactions induced plastic responses and that interspecific-induced plants had more competitive interactions with subsequent intraspecific neighbors than did intraspecific-induced plants. This study provides some mechanistic evidence of how plasticity induced by interactions may affect species coexistence, via alterations to intraspecific interactions that lay the foundation for the strength of stabilizing mechanisms that are based on 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

for the ecological consequences of plasticity.

## Acknowledgments

I extend gratitude to B. Allen, an NSF REU student on this project (NSF REU grant # DBI-1461007) and G. Lanzo, a College First high school student intern on this project. I thank Drs. A. Iler, A. Kramer, S. Wagenius, and F. Fort for input on study methodology. I thank T. Lichtenberger, A. Ernst and Drs. B. Barak and M. De Vitis for providing feedback on the manuscript, as well as Dr. J. Cahill Jr. for feedback at a poster session at the Ecological Society of America annual conference. I also extend gratitude for Northwestern University's Plant Biology and Conservation Research Award for funding support.

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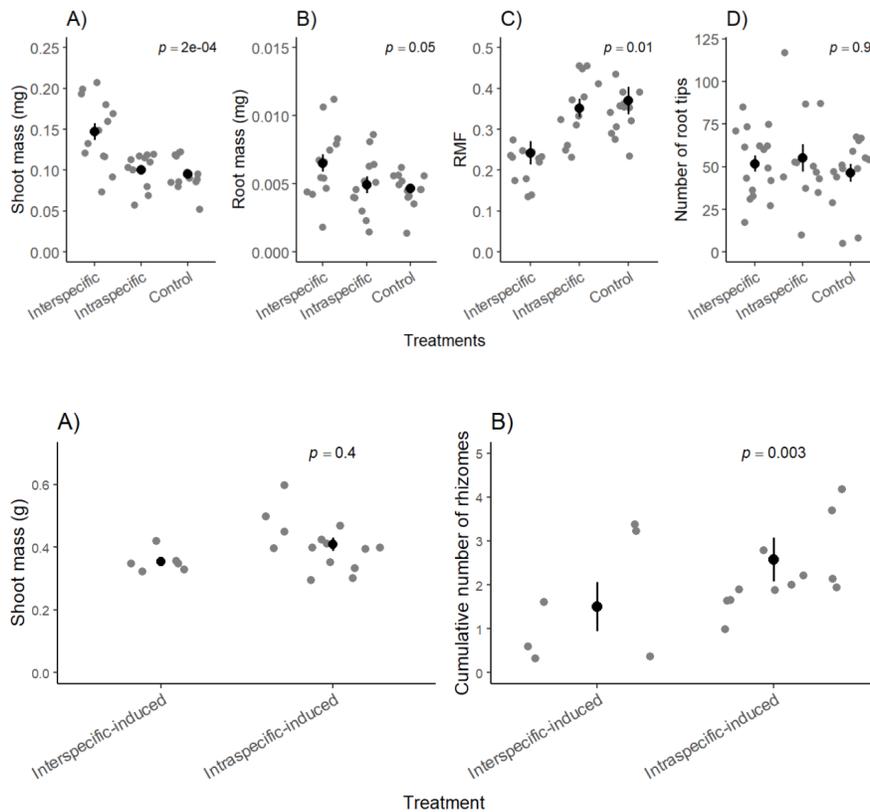
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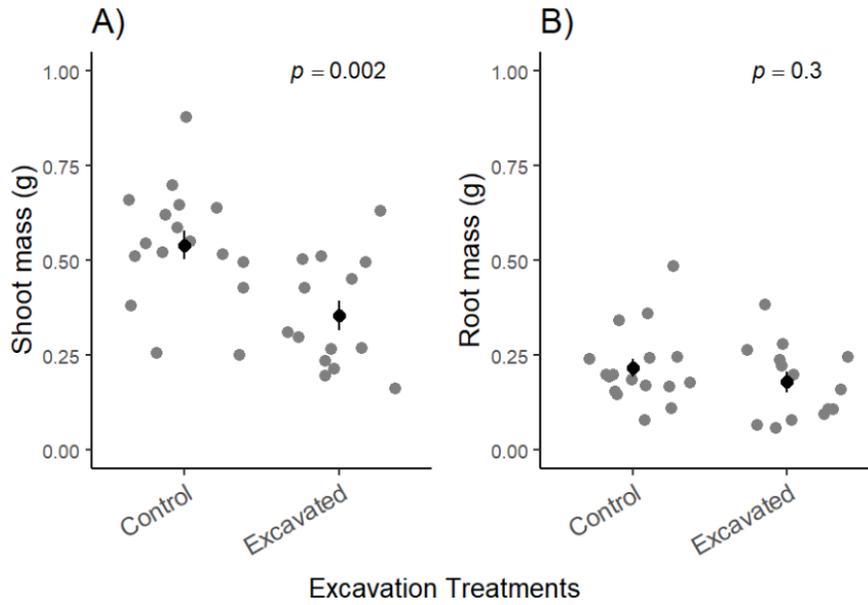
**Figure 1** . Mean  $\pm$  standard error of the treatment groups for *Pascopyrum smithii* plants growing alone, and with intraspecific and interspecific neighbors for A) shoot mass, B) root mass, C) RMF, and D) number of roots. Black dots indicate mean values and grey circles are individual plant values.

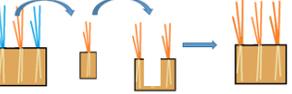
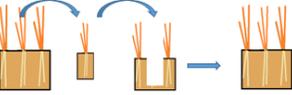
**Figure 2**. Mean  $\pm$  standard error of *Pascopyrum smithii* plants induced by intraspecific and interspecific neighbors for A) shoot mass, and B) cumulative rhizomes per pot. Black dots indicate treatment means, and grey circles are individual plant values.

**Figure 3**. Mean  $\pm$  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 circles indicate individual plant values.

**Box 1**. Treatment groups and final sample size for the two main greenhouse experiments with *Pascopyrum smithii* focal plant (orange). Interspecific interaction treatments are with *Bromus tectorum* (blue) neighbors. A) experiment one using interactions to induce plasticity, B) experiment two on the consequences of induced plasticity. Treatment sample sizes are in parentheses.





Experiment	Treatment	Treatment diagram
A) One: Interactions to induce 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: Induced plasticity impacts on intraspecific interactions	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)