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DISCERNING COMPETITIVE VS. FACILITATIVE RELATIONSHIPS OF WEEDS ON  
FOREST RESTORATION EFFORTS AT LA SAL DEL REY, TX

A Thesis  
by  
MYLEN A. ARIAS

Submitted to the Graduate College  
The University of Texas Rio Grande Valley  
In partial fulfillment of the requirements for the degree of  
MASTER OF SCIENCE

DECEMBER 2020

Major Subject: Biology



DISCERNING COMPETITIVE VS. FACILITATIVE RELATIONSHIPS OF WEEDS ON  
FOREST RESTORATION EFFORTS AT LA SAL DEL REY, TX

A Thesis  
by  
MYLEN A. ARIAS

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December 2020



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## ABSTRACT

Arias, Mylen A., Discerning Competitive vs. Facilitative Relationships of Weeds on Forest Restoration Efforts in La Sal del Rey, TX. Master of Science (MS), December 2020, 54 pp., 1 table, 11 figures, 65 references.

Dryland ecosystems need effective restoration strategies to address severe degradation. It is assumed that voluntary forb weeds compete with or have no effect on native seedlings. However, theory suggests a potential facilitative role for such weeds. We conducted a weed exclusion experiment at a semi-arid site in South Texas targeted for forest restoration to discern the effect of early successional weeds on growth and survival of thornscrub seedlings. Overall, weed presence did not significantly affect seedling mortality or net plant height growth after 6 months of weed exclusion, regardless of seedling growth habits. However, excluding seedlings with significant animal damage, we found that during abiotic stress, weed presence improved seedling growth rates, in accord with plant facilitation theory. This facilitative effect was driven by fast-growing species. During hot and dry conditions, light-saturated photosynthetic capacity and air temperature on seedlings adjacent to weeds were not significantly different from that of weed-excluded seedlings, but reduced afternoon light levels indicated improved microclimatic conditions. Results offer evidence to warrant further research into how to exploit plant-plant facilitation in forest restoration and management. Future work should consider the ecological strategies of pioneer weed species by planting clustered, multispecies restoration pockets in harsh environments to ameliorate microclimatic conditions and minimize effects of herbivory.





## DEDICATION

No podría haber llegado hasta aquí sin el esfuerzo y el apoyo de mi familia. A mis padres, Drs. Nicolás Arias González y Perla Mylen Vasquez Rodríguez, gracias por alentarme siempre y por nunca dudar de mí—llevo su ejemplo conmigo a cada momento y prometo que ningún sacrificio será en vano. A mis hermanos, Nicolás y Mauricio Andrés, quienes son lo más importante para mí y mi inspiración más grande para seguir adelante, gracias por compartir conmigo cada escalón de mi vida. A mi Wanda, que me llena de amor, gracias por las sonrisas que iluminan todos mis días.

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A mi tío Beto, a quien extraño con el alma. Nunca olvide que lo quiero.



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## CHAPTER I

### INTRODUCTION

The Lower Rio Grande Valley (LRGV), encompassing the southernmost Texas counties of Hidalgo, Cameron, Willacy, and Starr, extends for 2,986,240 acres and is characterized by both semi-arid and subtropical climates unique to this area of the United States (Leslie, 2016; Richardson & King, 2011). As a result of its distinctive habitat of subtropical and semi-arid climate, the ecoregion exhibits great biodiversity, with more than 500 vertebrate species dependent on its Tamaulipan thornscrub forest for food, cover, and nest sites (Leslie, 2016; Judd, Lonard, & Waggerman, 2002). As reported by Leslie (2016), over 35 of these species, including the ocelot (*Leopardus pardalis albescens*), Texas tortoise (*Gopherus berlandieri*), and Texas horned lizard (*Phrynosoma cornutum*), are critically endangered, as the ecosystem has been diminished by over 98% of its original range due to agricultural expansion, urbanization, and other anthropogenic activities associated with staggering population growth in the LRGV since the 1920s (Ewing & Best, 2004; Ricketts et al., 1999; Jahrsdoerfer & Leslie, 1988).

In response to these threats to biodiversity in the area, the United States Fish and Wildlife Service (USFWS) has committed to its continued restoration. Multiple reforestation events since 1984, in conjunction with the establishment of the USFWS Farmland Phase-Out and Revegetation Program, have led to the planting of over 3.1 million seedlings in old agricultural land with the purpose of creating a wildlife corridor, combatting land fragmentation across the region (Friends of the Wildlife Corridor, 2016). These events have focused on planting native

seedlings of trees and shrubs of approximately 50 woody species predominant in the thornscrub forest, including Texas ebony (*Ebenopsis ebano*), snake eyes (*Phaulothamnus spinescens*), and blackbrush (*Acacia rigidula*), amongst others. However, no records exist detailing the success of said reforestation measures, indicating a largely understudied area of research critical to the reestablishment of the thornscrub ecosystem and the survival of its endangered species. Notably, recent literature has focused on the identification of threats to seedlings in the initial stages of reforestation and potential strategies to maximize their survivorship in the LRGV's degraded soils (Alexander, Moczygemba, & Dick, 2016; Dick, Alexander, & Moczygemba, 2016; Ewig & Best, 2004). Still, the influence of herbaceous weeds in such efforts is often ignored or diminished, despite consensus regarding its importance for implementing appropriate and effective conservation tactics (Callaway, 1995; Bruno, Stachowicz, Bertness, 2003; Holmgren, Scheffer, & Huston, 1996).

### **Facilitative effects of plants**

Historically, understanding of communities has led most literature in plant ecology to overstress the effects of competition by grasses on target species, with little to no mention of positive interactions between flora (Callaway & Walker, 1997; Connell, 1983). In fact, most efforts in ecological restoration center around the concept of inter- and intraspecific competition, stemming from an understanding of plant communities as no more than a collection of species coincidentally sharing an environment for which they are similarly adapted, leading to the assumption that resource competition and facilitation act in isolation from each other (Callaway, 1997; Callaway & Walker, 1997). A practical outcome of a competition-centric view of plant-plant interactions is that researchers push for the use of herbicides, mowing, prescribed fire, and

other techniques to eliminate the presence of weeds in reforestation plots (Holmgren, Scheffer, & Huston, 1997). Though competition is an undoubtedly important aspect to consider when implementing conservation strategies, the global regard of herbaceous weeds as heavy competitors increases the economic and ecological cost of restoration. In contrast, recent experimental studies in systems ecology indicate that facilitative (mutualistic or commensal) interactions have a greater effect on community structure and dynamics than previously thought (Bruno, Stachowicz, & Bertness, 2003; Gómez-Aparicio et al., 2004).

Coined by Niering et al. (1963), “nurse-plant syndrome” thus refers to the positive influence of neighboring adult plants on seedlings by improving microclimatic conditions, retaining nutrients and water in the soil, protecting seedlings from herbivores, and attracting beneficial pollinators to target plants (as cited in Padilla & Pugnaire, 2006). This facilitative interaction could potentially promote the establishment of seedlings in harsh, degraded ecosystems that experience continued abiotic stress, such as the LRGV, where summer drought, erosion, and soil depletion limit the survivorship of seedlings (Ewing & Best, 2004). Competition between individuals in harsh environments via resource limitation can be offset by a close association with neighbors that may provide an improvement of these conditions, and in turn outweigh any competition arising from growing in close proximity (Olofsson, Moen, & Oksanen, 1999). In fact, plant facilitation on reforested areas has been documented in similar environments to the LRGV, such as the semi-arid Mediterranean and Duero Plateau of the Iberian Peninsula, where an increase in seedling growth and decrease of soil erosion were recorded, respectively (Gómez-Aparicio et al., 2004; Herrero & Gutiérrez, 2006). For instance, when focusing on the effects of shade on tree seedlings strategically planted under shrubs, Gómez-Aparicio et al. (2004) found survivorship of tree seedlings planted in such conditions

more than doubled in comparison to those planted in open areas without vegetation, as well as beneficial effects on seedling growth. These results have multiple implications for restoration of stressed ecosystems.

### **The potential role of nurse plants in restoration ecology**

First, discouraging the removal of adult weeds pre-planting due to its damaging potential eliminates the need to add labor and capital expenses to ecological rehabilitation programs, allowing for resources to be reallocated to the planting of larger areas (Castro, Zamora, Hódar, & Gómez, 2002). Therefore, the identification of specific, native weed species as “nurses” opens the possibility of exploiting facilitative relationships by providing agencies with a cost-free cover crop that could be propagated across reforested land patches. In this regard, more weed species could mimic leguminous nurse plants utilized in agriculture to improve soil nutrients due to their nitrogen-fixing qualities (Gómez-Aparicio et al., 2004). In addition, Blumenthal, Jordan, and Svenson (2003) suggest that increasing diversity in plant communities may offset invasibility, as diversity of native plants should limit the resources available to potential invaders. Under the threat of global warming, this technique also offers added benefits to the restoration of arid ecosystems, as expected increases in temperature further deplete soils of moisture that could be retained by a denser ground cover, but the role of cover crops or weeds on soil moisture is mixed (Gómez-Aparicio et al., 2004). Most notably, Joffre and Rambal (1988) point to facilitative effects of nurse plants on water storage in soil and decreasing the volume of water lost through evapotranspiration, with the amount of precipitation that reaches the soil as another factor affected by ground cover.

It is worth noting that, although promising, the effects of facilitation may be dependent on not only the environment, but species identity. Some benefactor (nurse) species may outlive

and eventually harm beneficiary (seedling) species, and vice versa, reinforcing the notion that facilitation and competition act simultaneously and their net effect ought to be considered when encouraging nurse-plant interactions (Callaway, 1995). The primary ecological strategies of plants in multispecies communities must also be considered, as species of different growth habits (fast vs. slow) will have different resource requirements that will affect their competitive ability (Grime, 1977). On this note, reforestation efforts ought to be guided by careful examination of sites and success of previous attempts at restoration. The complexities of species selection involve not only the harsh environmental conditions species are subject to, but species' own adaptation strategies and growth rates. Thus, it is important to prioritize the restoration of those with documented higher survivorship and account for potential tradeoffs based on species characteristics (Padilla Ruiz, Pugnaire de Idaola, Marín, Hervás Muñoz, & Ortega Oller, 2004).

To determine the nature of impact of weeds on native thornscrub tree and shrub seedlings of the LRGV, this study will employ a manipulative weed exclusion experiment at the LRGV National Wildlife Refuge (LRGV NWR) in La Sal del Rey, TX. It is worth noting that a laboratory or small-scale field experiment would allow for control of environmental variables, targeted simulation of biotic or abiotic damage, and an opportunity for replication. However, a manipulative weed exclusion *in situ* was chosen to provide a valid representation of responses target seedlings would exhibit in their natural habitat, thus directly informing current management practices (Morin et al., 2009). This experiment will be used to assess the effect of weeds on native seedling physiology, growth, and survivorship.

Note: Chapter II has undergone editorial review is in revision for *Ecological Solutions and Evidence* as Arias, M., Mendez, S., Chavana, J., Wahl, K., Kariyat, R., & Christoffersen, B. (2020). Do early successional weeds facilitate or compete with seedlings in forest restoration? Disentangling abiotic vs. biotic factors. *Ecological Solutions and Evidence*. Manuscript in revision.



## CHAPTER II

# DO EARLY-SUCCESSIONAL WEEDS FACILITATE OR COMPETE WITH SEEDLINGS IN FOREST RESTORATION? DISENTANGLING ABIOTIC VS. BIOTIC FACTORS

### **Abstract**

Dryland ecosystems, expected to increase to 56% of the global land area by 2100, need effective restoration strategies to address their severe degradation. It is often assumed that voluntary forb weeds, which dominate early successional stages at reforestation sites, either compete with or have no effect on native seedlings. In contrast, theory and empirical work suggest a potential facilitative role for such forb weeds, contingent on abiotic and biotic stressors, as well as the growth rate of native seedlings. We conducted a manipulative weed exclusion experiment at a semi-arid site in South Texas targeted for large scale forest restoration and subsequently dominated by early successional forb weeds to discern the net effect of these weeds on the growth and survival of target thornscrub tree and shrub seedlings. We assessed the roles of contrasting seedling growth habits (fast vs. slow), temporal variation in abiotic stress, microclimate, and mammalian herbivory in modulating weed-seedling interactions. Overall, weed presence did not significantly affect seedling mortality or net plant height growth after 6 months of weed exclusion, even when considering contrasting seedling growth habits. However, mammalian herbivory was prevalent in many seedlings. Excluding seedlings with significant animal damage, we found that during periods of significant abiotic stress (hot and dry), weed presence significantly improved native seedling growth rates, in accord with plant facilitation

theory. This facilitative effect was driven primarily by species with a fast growth habit. During hot and dry conditions, light-saturated photosynthetic capacity ( $A_{\text{sat}}$ ) and air temperature on seedlings adjacent to weeds were not significantly different from that of weed-excluded seedlings, but afternoon light levels were reduced by ~50%, possibly indicative of lower leaf temperatures and improved microclimatic conditions. Our results offer sufficient evidence to warrant further research into how best to exploit plant-plant facilitation in dryland forest restoration and management. Future work should consider the ecological strategies of pioneer weed species to maximize growth in secondary succession by strategically planting clustered, multispecies restoration pockets in harsh environments that serve to jointly ameliorate microclimatic conditions and minimize the effects of herbivory.

Keywords:  $A_{\text{sat}}$ , lower Rio Grande Valley, mammalian herbivory, nurse plants, plant facilitation, semi-arid reforestation, stress-gradient hypothesis

## **Introduction**

Dryland ecosystems, characterized by water scarcity and encompassed by arid, semi-arid, and dry-subhumid regions, occupy about 40% of the Earth's surface and support about a third of the global population (Millennium Ecosystem Assessment, 2005). As some form of severe degradation is present in 10-20% of these ecosystems, it is also estimated that the extent of drylands will increase to 56% of the global land area by 2100 (Prăvălie, 2016; Huang et al., 2016, Reynolds et al., 2007). While both the fraction of drylands with tree cover and the area which can be reforested has been debated as of late (Bastin et al., 2017; Veldman et al. 2019) there is no question that a significant fraction of forests has been lost (Lindquist et al., 2010), and

thus in need of restoration. Limited rainfall, combined with high temperatures and low nutrient levels and water holding capacity, present increasing challenges for the restoration of these highly degraded ecosystems under global climate change and increased land use for farming or urbanization (Cherlet, Hutchinson, Reynolds, Sommer, & von Maltitz, 2018). Despite the efforts to restore these ecosystems through native plant seeding or transplanting, managed grazing, prescribed fire treatments, and weed management, the success of these efforts remains low, indicating a need for different approaches to restoring these systems (James, Sheley, Erickson, Rollins, Taylor, & Dixon, 2013).

Herbaceous vegetation (hereafter, weeds) often dominates early successional stages (0 – 5 years) of restoration sites, particularly those which were previously agricultural (Falkowski, Chankin, & Diemont, 2020; Guariguata & Ostertag, 2001). Weeds may have either positive, negative, or neutral effects on target restoration plants. Negative consequences may arise due to the sharing of already limited resources such as nutrients and water, whereas positive effects generally occur through some form of habitat amelioration, or the minimization of otherwise harsh conditions (Bertness & Callaway, 1994). The potential exists, therefore, for weeds to act as nurse plants in restoration contexts by improving microclimatic conditions, retaining nutrients and water in the soil, protecting seedlings from herbivores, and attracting beneficial pollinators to target plants (Niering et al., 1963; Padilla & Pugnaire, 2006, Padilla & Pugnaire, 2009). It is therefore imperative to explore the facilitative potential of weeds as an alternative to costly management strategies (Chirino et al., 2009; Castro, Zamora, Hódar, & Gómez, 2002).

Though promising, facilitation is likely contingent on multiple factors (Padilla & Pugnaire, 2006; Callaway & Walker, 1997). The stress-gradient hypothesis posits that facilitation is more likely under harsh environmental conditions (Bertness & Callaway 1994; Callaway et al.

2002). In harsh conditions, because growth rates are less than optimal, competition is reduced, while the likelihood increases that one aspect of plant microclimate is favorably altered (shade, heat, moisture, nutrients). In addition, herbaceous weeds may further contribute to shielding target species from mammalian herbivory (Smit et al., 2007). Browsing by mammalian herbivores during restoration activities can be substantial and thus act as a significant constraint on forest restoration (Opperman & Merenlender, 2000). Third, species identity and growth rate, both of nurse weeds and target plants, is likely to play a role (Fagundes et al., 2017; Numata, 1982). Some nurse species may outlive and eventually harm target species, and vice versa, emphasizing the notion that facilitation and competition act simultaneously and their net effect ought to be considered when encouraging nurse-plant interactions (Callaway, 1995). Target seedling ecological strategy is an important consideration because of its dual role in succession and weed-seedling interactions. Different growth habits (e.g., the fast-slow continuum of Reich, 2014) or ecological strategies (e.g., Grime's adaptive strategy theory; Grime, 1977) likely underpin varying degrees of species survival in restoration settings (Martínez-Garza, Bongers, & Poorter, 2013), but also imply different responses to shading and other aspects microclimate that are modified by adjacent weeds.

We addressed these knowledge gaps by conducting a manipulative weed exclusion experiment at a semi-arid site recently targeted for large scale forest restoration (>100,000 woody seedlings planted annually over several years) and subsequently dominated by early-successional forb weeds. The objectives of this study are to 1) discern the net effect (positive, negative, neutral) of naturally occurring weeds on native thornscrub tree and shrub seedlings of contrasting growth rates, 2) disentangle the abiotic vs. biotic mechanisms associated with any effects or the lack thereof, and 3) understand how temporal variation in abiotic stress modulates

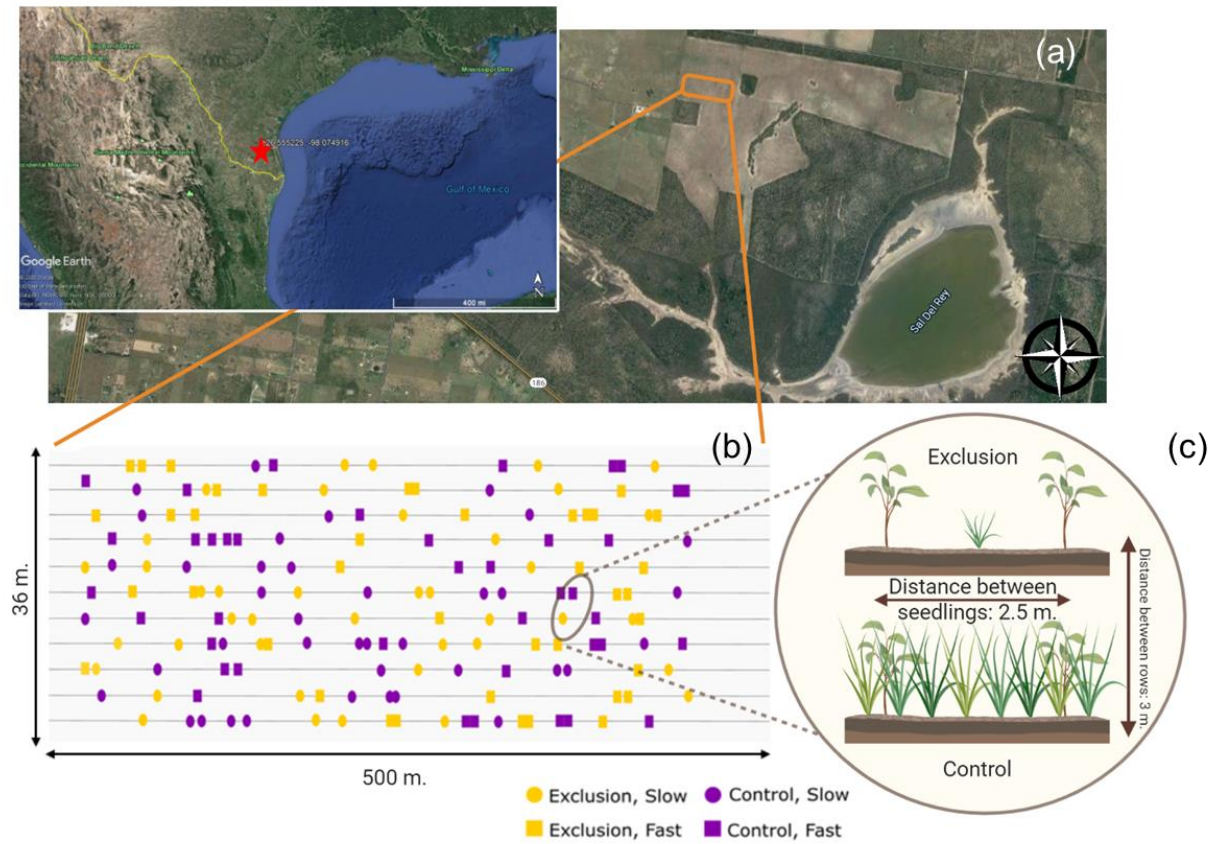
weed-seedling interactions. Overall, we hypothesize that the net effects of interactions between weed cover and target seedlings will be facilitative given the overall harsh arid conditions, and that facilitative effects will be most pronounced during the hottest and driest periods. We also hypothesize that fast-growing species of target seedlings will disproportionately benefit from weeds over their slow-growing counterparts, given that species stress tolerance tends to trade off with growth rate (Chapin et al., 1986).

## **Materials and Methods**

### **Study Site**

The study took place within the La Sal del Rey Tract (26.555225°N, -98.074916°W) of the Lower Rio Grande Wildlife Refuge, near Linn, TX (Figure 1), at the northern margin of Tamaulipan thorn forest (Jahrsdoerfer & Leslie, 1988; Richardson & King, 2011; NRCS Web Soil Survey). The closest site of undisturbed thorn forest, ca. 15 km to the south, consists of continuous closed canopy of trees 3-4 m in height and a variety of understory shrubs (Flores 2019). Between 1939 and 1959, most of the La Sal del Rey tract was cleared for agricultural dryland crop production (e.g., sorghum) with no irrigation. The U.S. Fish and Wildlife Service (USFWS) purchased the land in 1992, and since 1993, has reforested sub-tracts through direct seeding and tree seedling transplanting. Air temperatures in the summer can reach upwards of 40°C, with mild winters and occasional freezes. The site receives 682 mm annually on average, with peak precipitation in the months of September and October. Several browsing mammalian species are present on site, such as white-tailed deer (*Odocoileus virginianus* Zimmerman) and feral hogs (*Sus scrofa* Linnaeus), as well as exotic species such as the nilgai antelope

(*Boselaphus tragocamelus* Pallas) (Leslie, 2016). In October – November 2018, over 100,00 native tree and shrub seedlings of 24 species were planted as part of a joint effort between Land Life Company ([www.landlifecompany.com](http://www.landlifecompany.com)) and the USFWS. Linear rows were ~3 m apart and seedlings were spaced 2-3 m within each row, resulting in a planting density of ~988 seedlings/ha. The rows were ripped to a depth of 30 cm and seedlings were planted at 20 cm depth.



**Figure 1.** Study site & experimental design. a) Study location map; red star denotes study site (credit: Google Earth). b) Layout of study plot showing study seedling individuals by treatment (control, weed exclusion) and species growth habit (fast, slow). c) Close-up showing average distance between planted seedlings and inter-row distance. Actual distance between study individuals was often greater than 2.5 m because not all seedlings were part of study (see Methods). A total of 160 target seedlings (8 species x 2 treatments x 10 replicate individuals) were studied. b) and c) created with Inkscape 1.0 (<https://inkscape.org/>) and BioRender.com, respectively.

## **Species Selection and Experimental Design**

A subset of 3600 individuals (150 individuals per each of 24 species) were assessed in intensive seedling demography plots for growth and mortality over one year (Mohsin et al., unpublished data). In May 2019 we opportunistically designed the present experiment using preexisting planted seedlings and preliminary data from the intensive seedling demography plots. We selected a subset of eight focal species that were planted at sufficiently high densities within relatively close proximity to each other in an adjacent 500 by 36 m plot (Figure 1b), and which spanned a wide range of growth rates and survival. After initiating the experiment, we later classified our selected species into fast- and slow-growing based on a full year of growth and survival from the intensive seedling demography plots (Table 1 and Supplemental Figure S1).



**Table 1.** Species traits, growth habit classification, RGR<sub>h</sub> and survival from Mohsin et al., unpublished manuscript for the eight focal species in the present study. Initial height (cm) and total height growth (cm) are for the duration of the present study.

Species	Family	Growth Habit	RGR <sub>h</sub> (weeks <sup>-1</sup> )	Survival	Initial height (cm)	Total Growth (cm)
<i>Forestiera angustifolia</i>	Oleaceae	Slow	0.27 ± 0.11	0.66	28 ± 2	10 ± 4
<i>Celtis ehrenbergiana</i>	Ulmaceae	Slow	0.51 ± 0.17	0.74	30 ± 2	11 ± 3
<i>Senegalia wrightii</i>	Fabaceae	Slow	0.47 ± 0.06	0.86	49 ± 5	14 ± 2
<i>Amyris texana</i>	Rutaceae	Slow	0.73 ± 0.15	0.82	13 ± 2	12 ± 2
<i>Zanthoxylum fagara</i>	Rutaceae	Slow	0.78 ± 0.13	0.94	17 ± 2	20 ± 4
<i>Havardia pallens</i>	Fabaceae	Fast	1.18 ± 0.09	0.76	24 ± 3	38 ± 4
<i>Chromolaena odorata</i>	Asteraceae	Fast	1.28 ± 0.18	0.7	41 ± 4	54 ± 11
<i>Vachellia rigidula</i>	Fabaceae	Fast	1.54 ± 0.07	0.9	23 ± 2	44 ± 3

We identified three focal fast-growing species: tenaza (Fabaceae: *Havardia pallens* (Benth.) Britton & Rose, tree), crucita (Asteraceae: *Chromolaena odorata* (L.) R.M. King & H. Rob, shrub), and blackbrush (Fabaceae: *Vachellia rigidula* (Benth.) Seigler & Ebinger, shrub), and five slow-growing species: elbowbush (Oleaceae: *Forestiera angustifolia* (Torr.), shrub), granjeno (Ulmaceae: *Celtis ehrenbergiana* (Klotzsch) Liebm., shrub), Wright's acacia (Fabaceae: *Senegalia wrightii* (Benth.) Britton & Rose, tree), chapotillo (Rutaceae: *Amyris texana* (Buckley) P. Wilson, shrub), and colima (Rutaceae: *Zanthoxylum fagara* (L.) Sarg., shrub). We verified growth habit classifications with two independent sources, that of Flores & Jurado (1998) and an unpublished classification based on anecdotal observations (K. Wahl, unpublished dataset). Notably, all three species classified by our field data as fast-growing were also classified as such by one or the other of the two sources, and our five slow-growing species had classifications other than 'fast-growing' by the unpublished dataset. These five species likely represent a range of growth habits from slow to moderate, and thus should be thought of as slow-growing relative to the three fast-growing species (Suppl. Figure S1), not in an absolute sense.

We used an iterative approach for selecting  $n = 10$  pairs of experimental individuals per species (160 individuals total) in order to distribute species, growth habits, and treatments in a homogenous way throughout the study plot. First, we identified all planted seedlings to species and assigned x/y coordinates (1540 seedlings in total). Then, starting with the least abundant species and proceeding in order of increasing species relative abundance, we selected pairs of closely planted individuals of each species distributed across the domain of our plot. For each successive species, we selected the next closest pair to already selected individuals. The individuals of each pair were randomly assigned to weed exclusion or control. Our 160 target seedlings (8 species x 2 treatments x 10 replicate individuals) were thus distributed across our

study plot (Figure 1b). We accomplished weed exclusion by mowing a six foot radius around weed exclusion seedlings using a FS 560 C-EM model brush cutter (STHIL Incorporated; Virginia Beach, VA) to guarantee close cutting of grasses and defined areas, and as needed to prevent the incidence of grasses in exclusion areas. Mowing was completed in July 2019 and September 2019, as needed to guarantee full exposure of exclusion seedlings to sunlight.

### **Post-Planting Weed Cover Assessments**

Weed cover assessments (see site photo; Suppl. Figure S2) were conducted by three independent observers in June 2019 (baseline), a month post-treatment in August 2019, and three months post-treatment in October 2019. Weed crown projections for twenty-four sample points were observed with use of a PVC quadrat with an area of 1 m<sup>2</sup>, and percent cover estimations recorded. These assessments indicated cowpen daisy (Asteraceae: *Verbesina encelioides* (Cav.) Benth. & Hook.), doveweed (Euphorbiaceae: *Croton texensis* (Klotzsch) Müll. Arg.), and grasses such as Texas signalgrass (Poaceae: *Urochloa texana* (Buckley) R. Webster) as predominant weeds on site. Honey mesquite (Fabaceae: *Prosopis glandulosa* Torr.) was also present with early predominance on site. Its percent cover, however, did not exceed 7% in sampled areas (Supplemental Figure S3).

### **Response variables – seedling vital rates and physiology**

We conducted monthly to bi-monthly seedling demographic surveys and recorded plant vigor on a 0-3 scale (0 = dead, 3 = >75% green leaves), plant height, numbers of main branches, and animal damage on a 0-3 scale (0 = no damage, 3 = severe damage), following a similar approach used by others (Muiruri, Barantal, Iason, Salminen, Perez-Fernandez, & Koricheva, 2018). In addition, in July, August, and October 2019, we measured net photosynthetic rate on seedlings using a Licor 6400 XT infrared gas analyzer (LI-COR Biosciences; Lincoln, NE) with

a 6400-22 opaque conifer chamber attachment in lieu of the standard 2x3 cm chamber, because the majority of our species had a very limited leaf area, which was often compound leaves with short petioles and petiolules. The short stature and limited branching of many seedlings precluded illuminating branches from above. We instead illuminated seedlings from the side, placing the seedling stem base at one side and we recorded the length of stem inside the chamber. Photosynthetic measurements were conducted with reference CO<sub>2</sub> of 400 ppm and a light intensity of 2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR in 5-15 minutes intervals to allow for stabilization. We did not harvest leaf area inside the chamber to avoid damaging the seedlings. While this prevented us from determining photosynthesis on a per unit leaf area basis, we controlled for differences in plant size by normalizing photosynthesis by dividing by stem length present inside the chamber.

### **Response variables – seedling microclimate**

We measured soil temperature and moisture in September 2019 using a TEROS 12 sensor (METER Group Inc.; Pullman, WA) and ProCheck C device (Edaphic Scientific; AU), under 16 replicate control and exclusion seedlings. For a more complete picture of how weeds alter seedling microclimate during hot, dry conditions, in May 2020 we made additional microclimate measurements. We measured incoming afternoon photosynthetically active radiation (PAR  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), relative humidity (%) and air temperature (°C) over a period of 7 minutes at a constant height of 35cm under each of n=4 weed canopies and adjacent to seedlings in n = 5 exclusion areas using a Licor 6400 XT held level to ground level and with its chamber left open. Because the weed composition in May 2020 was no longer the same, weed canopy estimates were replicated under the shade of the most prominent weed, *Prosopis glandulosa* (Torr.).

## Data Analyses

All data analyses were performed in R v3.6.0 (R Core Team, 2019). Because a small number of tagged experimental individuals died ( $n = 6$  total) prior to initiation of the first mowing treatment in July 2019 ( $t_0$ ), we estimated mortality (unitless) at a given time  $t$  for each experimental unit  $i$  as the number of newly dead individuals since the first mowing ( $n_{dead,i,t} - n_{dead,i,t_0}$ ) divided by the number of live individuals at the time of the first mowing ( $n_{live,i,t_0}$ ):

$$mortality_{i,t} = \frac{n_{dead,i,t} - n_{dead,i,t_0}}{n_{live,i,t_0}}$$

We calculated relative height growth rate for each experimental unit  $i$  at any given time  $t$  ( $RGRh_{i,t}$ :  $\text{cm cm}^{-1} \text{ month}^{-1}$ ) as

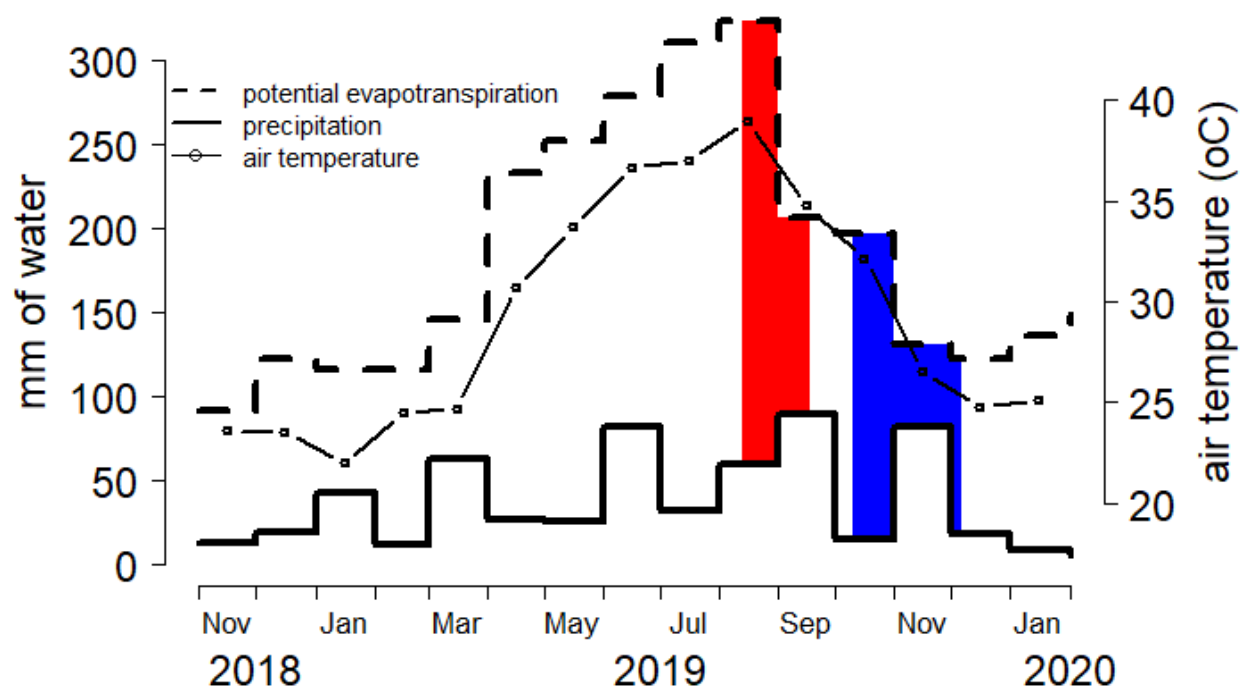
$$RGRh_{i,t} = \frac{\ln \frac{H_{i,t_2}}{H_{i,t_1}}}{t_2 - t_1}$$

where  $H_{i,t_2}$  and  $H_{i,t_1}$  are seedling height at the current and previous census, respectively, and  $t_2$  and  $t_1$  indicate time (months) of the current and previous census, respectively.

For statistical inference related to mortality, we used a generalized linear model, with treatment and species growth habit (fast vs. slow) as factors, using a binomial logit link function. Factors were sequentially removed until only significant factors at the  $\alpha = 0.05$  level remained (Crawley, 2013b). For seedling height and  $RGRh$ , we used a two-way ANOVA with treatment and species growth habit (fast vs. slow) as factors. We experimented with more complex statistical linear mixed models suitable for repeated measures using the lme4 package, but they failed to converge. Hence, we present analyses for specific time points of interest corresponding

to the end of the experiment (January 2020), and relatively high (Aug – Sept 2019) and low (Oct – Dec 2019) periods of abiotic stress as indicated by water deficit.

Abiotic stress was inferred according to the ecosystem water deficit, estimated as the difference between potential evapotranspiration (PET; mm) and incoming precipitation (mm). PET was estimated at the daily timescale using the R package ‘Evapotranspiration’ (Guo, Westra, & Peterson, 2019), which estimates PET according to the Penman-Monteith equation using daily minimum and maximum air temperature ( $^{\circ}\text{C}$ ) and relative humidity (%), wind speed ( $\text{m s}^{-1}$ ), and solar radiation ( $\text{W m}^{-2}$ ) as inputs. Meteorological data were sourced from a nearby met station ( $\sim 1$  mile from study plot) within the refuge, accessed from the MesoWest network API (<https://mesowest.utah.edu/>) using the R package ‘mesowest’ (Fick, 2019). Figure 2 highlights periods of high and low abiotic stress according to the ecosystem water deficit.



**Figure 2.** Experimental design and climate data during the study period ~ 1 mi from study site (MesoWest station ID ‘LSRT2’). Blue and red step lines indicate monthly precipitation (mm) and potential evapotranspiration (PET) following the Penman-Monteith equation (mm), respectively. Gray shaded region indicates climatic water deficit (mm). The black dashed line indicates monthly mean air temperature. Arrows indicate when mowing occurred. Pink and blue shaded regions denote ‘stressful’ (high water deficit) and ‘non-stressful’ (low water deficit) periods, corresponding to Figure 4.

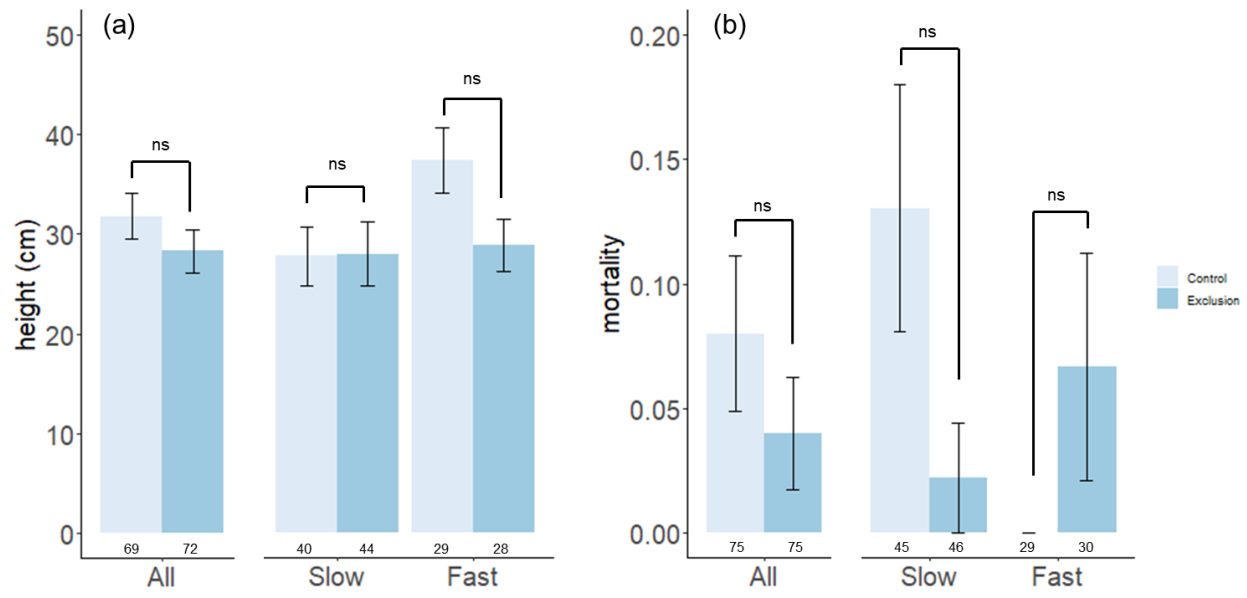
Finally, we separated out plants with and without significant animal damage and analyzed their  $RGR_h$  separately during periods of high and low abiotic stress. We used both our field 0-3 score as well as a post-hoc analysis of changes in seedling height, since animal damage from browsing (e.g., snapped apical meristem) was not always apparent in the field. Browsing can be evidenced either as a large one-time reduction in height, or as a persistent low-level of browsing, evidenced by frequent, small reductions in height. To identify individuals identified by both such causes, we computed the cumulative sum of all reductions in height growth over the entire study period (Jun 2019 – Jan 2020), or the total height reduction (THR; cm). We then determined individuals to have significant animal damage as those with a THR falling below the 50<sup>th</sup> percentile (-9 cm) over the course of the study and with a mean value of animal damage of  $\geq 1$  (Suppl. Figure S4). We additionally eliminated all plants with any negative height reduction (height growth  $< 0$ ) and any value of animal damage  $> 0$  over the analysis period of interest.

## Results

At the start of the experiment, no pre-existing significant effects on mean plant height or total mortality among treatments were present (Table 1; Suppl. Figure S5). Weed composition assessments indicated a dominant cover of cowpen daisy (*Verbesina encelioides* (Cav.) Benth. & Hook.) during non-stressful months, which gradually decreased and turned into a mixed dominant cover of doveweed (*Croton texensis* (Klotzsch) Müll. Arg.) and Texas signalgrass (*Urochloa texana* (Buckley) R. Webster) during periods of water-deficit. Bare ground fluctuated from a 30% to 55% from June to October and no invasive grass species were recorded (Suppl. Figure S3).

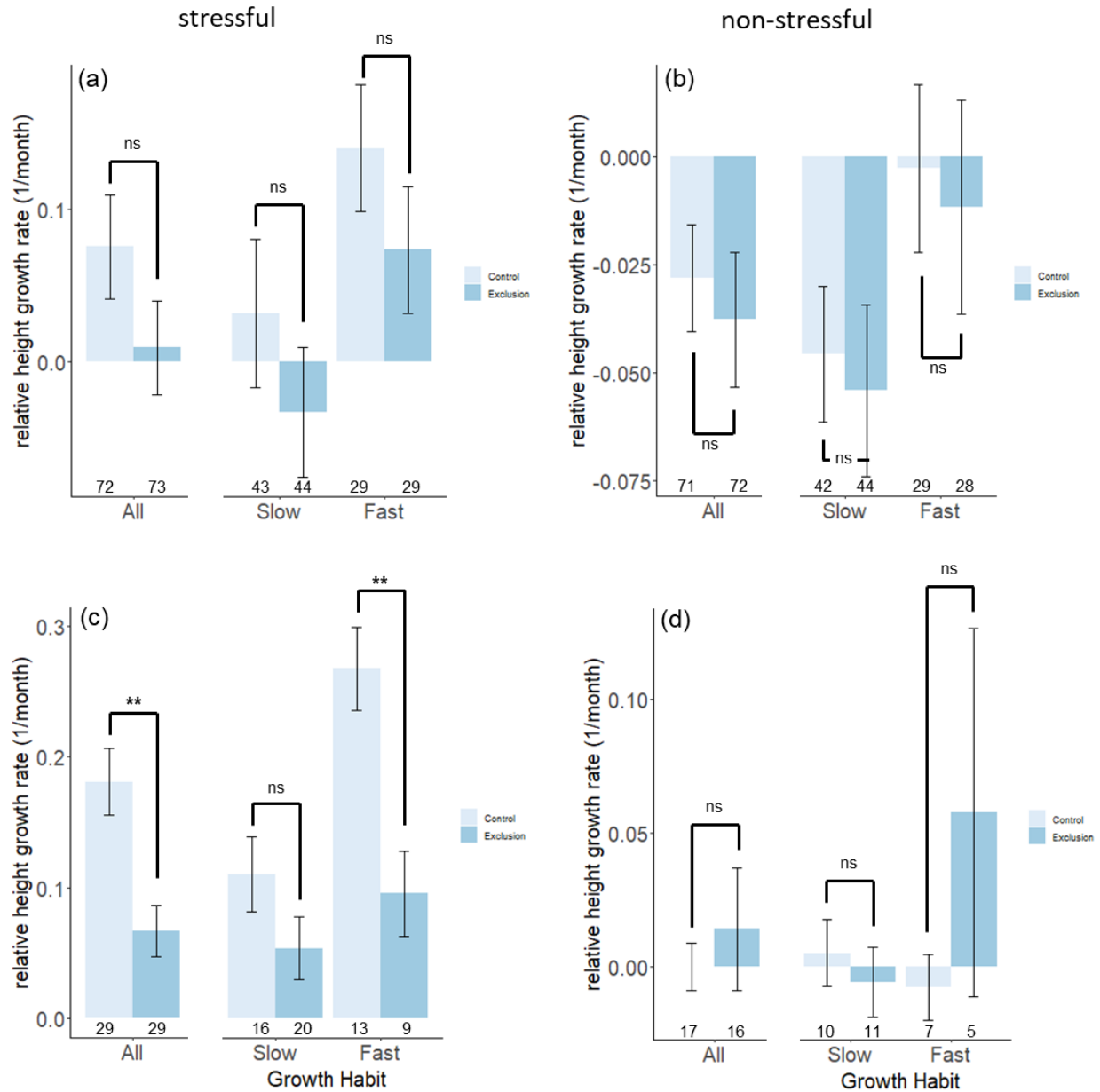


Overall, there was no significant effect of weed exclusion on seedling height (Figure 3a) or mortality (Figure 3b) at the end of the experiment (two-way ANOVA; height:  $F = 1.464$ ,  $p = 0.228$ ; two-way binomial GLM; mortality:  $\text{dev} = 13.130$ ,  $p = 0.297$ ). While Figure 3b and the two-way GLM (Table 2a) suggested that slow-growing species might experience reduced mortality in response to weed exclusion, post-hoc tests did not indicate that this difference was significant (Tukey post-hoc test,  $p = 0.166$ ). On average, slow-growing plants grew 13 cm in height as opposed to 45 cm in the case of fast-growing plants over the six-month period.



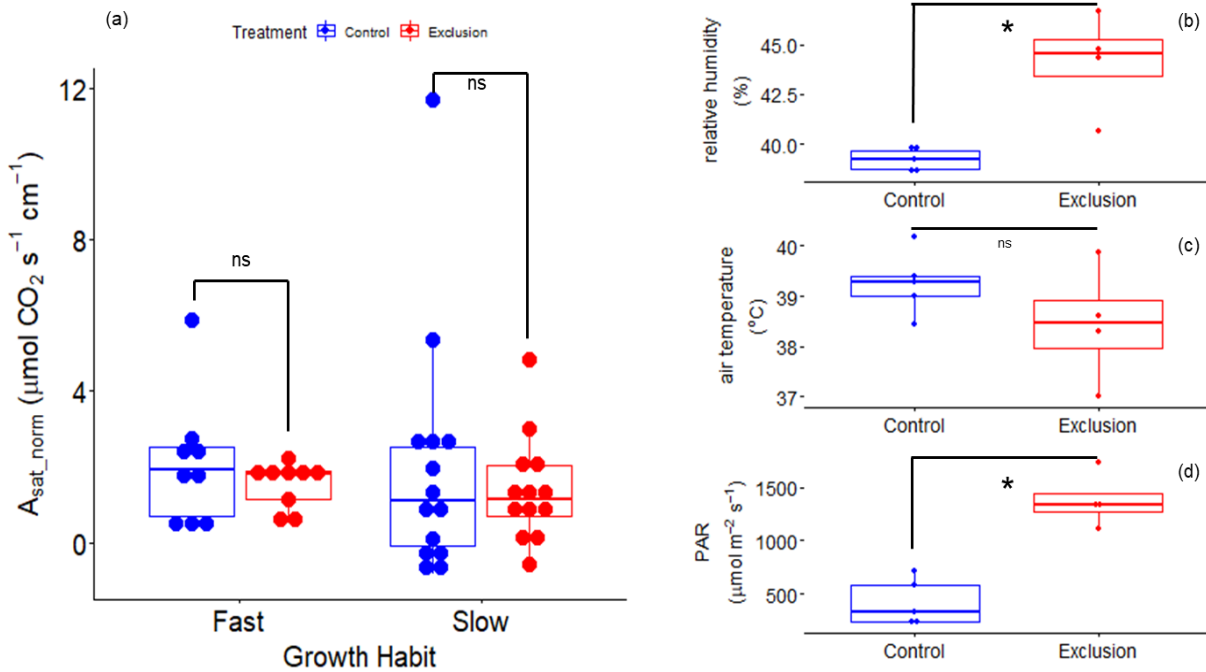
**Figure 3.** Mean seedling height (a) and total mortality (b)  $\pm$  sem (error bars) in January 2020, six months post-treatment. Numbers beneath each bar correspond to the number of individuals in (a), and the total number of live individuals in July 2019 for (b); mortality represents the fraction of new deaths relative to live individuals in July 2019. Significance codes: ‘\*\*\*’  $p < 0.001$ ; ‘\*\*’  $p < 0.01$ ; ‘\*’  $p < 0.05$ ; ‘ns’  $p > 0.05$ .

There was no significant effect of weed exclusion on relative height growth rate during periods of high and low moisture stress across all plants regardless of the degree of animal damage (Figure 4). However, we found during the stressful period of water deficit of August to September, weed exclusion did significantly reduce relative height growth rate particularly for plants without significant animal damage (Figure 4c), which appears to be driven primarily by a large reduction of height on fast growing species (Tukey HSD post-hoc test;  $t = 3.619$ ,  $p = 0.001$ ). In contrast, during periods of low moisture stress, the beneficial effect of weeds was lost (two-way ANOVA;  $F = 0.669$ ,  $p = 0.42$ ). However, this period also corresponded to a period when most canopy-forming weeds had fully senesced (suppl. Fig S2).



**Figure 4.** Relative seedling height growth rate (RGR<sub>h</sub>) +/- sem (error bars) in relation to animal damage and contrasting abiotic stress. (a) and (b) are all individuals regardless of degree of animal damage and (c) and (d) are only individuals unaffected by significant animal damage (see Methods). (a) and (c) correspond to large water deficit (Aug – Sept 2019) and (b) and (d) correspond to small water deficit (Oct – Dec 2019); Fig 1). Number of individuals given beneath each bar. Significance codes: '\*\*\*' p < 0.001; '\*\*' p < 0.01; '\*' p < 0.05; 'ns' p > 0.05.

Weed exclusion did not have an effect on light-saturated photosynthetic rate per unit stem length of slow- or fast-growing seedlings (Figure 5a; two-way ANOVA;  $F = 0.547$ ,  $p = 0.473$ ). In contrast, weeds had a significant effect on the microclimatic conditions of adjacent seedlings, with relative humidity (%) and photosynthetically active radiation (PAR  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) significantly higher in exclusion areas relative to control (Figure 5a, 5b; Wilcoxon rank sum test;  $W = 0$ ;  $p = 0.016$ ). Midday air temperature ( $^{\circ}\text{C}$ ), however, is not altered by the presence of weeds near fast- or slow-growing seedlings (Wilcoxon rank sum test;  $W = 15$ ;  $p = 0.286$ ).



**Figure 5.** Seedling photosynthetic capacity and comparable microclimate conditions adjacent to saplings (see Methods). **a)** Light-saturated photosynthetic rate per unit stem length ( $A_{sat\_norm}$ ;  $\mu\text{mol CO}_2 \text{ s}^{-1} \text{ cm}^{-1}$ ) for fast- and slow-growing species in August 2019 when water deficit is high (Fig 1). **b)** relative humidity (%). **c)** air temperature ( $^{\circ}\text{C}$ ). **d)** photosynthetically active radiation (PAR) ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ). In **a)**, measurements were conducted with reference  $\text{CO}_2$  of 400 ppm and a light intensity of  $2000 \mu\text{mol m}^{-2} \text{ s}^{-1}$  PAR. In **b)-d)**, Results of unpaired two-sample Wilcoxon rank sum tests are shown above. Significance codes: \*  $p < 0.05$ ; ns – not significant. In all boxplots, horizontal line, lower and upper hinge, and whiskers indicate median, 25<sup>th</sup> and 75<sup>th</sup> percentile, and data points closest to (but not exceeding) 1.5 times the inter-quartile range (IQR), respectively.

## Discussion

Overall, our results indicate that, absent the inhibition of substantial mammalian herbivory pressure, naturally occurring early successional herbaceous weeds on average do not significantly benefit seedling net height growth or reduce mortality over a six-month period, even when considering contrasting growth habits (fast vs. slow) of target seedlings (Figure 3). Thus, we reject the initial hypothesis that the net effects of interactions between weeds and seedlings would be facilitative. However, we also found no evidence to suggest that weeds compete with seedlings: herbaceous weeds at no point in our study significantly reduced height growth or increased mortality. Rather, we found specific instances in our study in which seedlings were intermittently benefitted by the presence of weeds, suggesting that other biotic or abiotic factors must be at play in the interaction of weeds and seedlings. We argue, therefore, that sufficient evidence exists to warrant further research into how best to exploit plant-plant facilitation in dryland forest restoration and management.

### **Minimizing mammalian herbivory is critical for realizing the nurse potential of early successional weeds**

We found a dominant role for mammalian herbivory modulating plant-plant facilitation in our study (Figure 4a vs 4c). Therefore, while weeds do facilitate certain species during stressful conditions in accordance with expectations, they may not necessarily shield them from herbivores as initially assumed (Smit et al., 2007; Opperman & Merenlender, 2000). Interestingly, previous work has shown that grazing-mediated facilitation occurs under high abiotic stress rather than in non-stressful periods (Soliveres et al., 2012; Callaway, 2007; Gómez-

Aparicio et al., 2004). Future work should focus on mitigating the drivers of animal damage and the effects of seedling palatability on the success of restoration efforts.

In particular, future work should screen native forbs in terms of their ability to repel herbivores, either through visual shielding or other means, in order to offset mammalian herbivory. Invasive herbivores remain a significant force in structuring novel ecosystems in semi-arid areas, and the Rio Grande Valley of Texas in particular (e.g., feral hogs, nilgai antelopes, and other small mammals; Leslie, 2016). Previous work has shown that native shrub and forb vegetation can play an important role in shielding target restoration species from herbivory; however, more work is needed to determine if facilitative effects from nurses are enough to mitigate the effects of herbivory, or if more drastic shielding measures are necessary (Cushman, Lortie, & Christian, 2011). Applying cluster reforestation of simple or complex plantings (Saha et al., 2012) could provide a seed source for further expansion of target species. Such aggregated plantings could, simultaneously, place fast-growing individuals and native forb weeds as a barrier against mammals while shielding slow-growing species from herbivory (Stanturf, Palik, & Dumroese, 2014). In addition, such clustered plantings could attract various beneficial or negative herbivores, and the effects of those and forb weed cover should be investigated with respect to the arthropod community (Losapio et al., 2019).

### **Facilitation increases with the harshness of abiotic conditions**

Our analysis shows that weeds facilitated growth of seedlings without significant animal damage during hot, dry periods with large water deficits (August-September; Figure 4c), but conferred no net benefit during cooler periods with smaller water deficits (October-December; Figure 4d), supporting our hypothesis that the facilitative effects of weeds would predominate during periods of intense abiotic stress (Fagundes et al., 2017). However, the lack of a treatment



effect during this latter period may be due more to the lack of weed cover than a treatment effect *per se*, as a significant fraction of forb weed canopy leaf area was lost during this time, leaving > 50% bare ground (Suppl. Fig S2). Regardless, the senescing forb weeds and grasses during unstressful periods did not significantly impact seedling growth. Our results therefore tentatively support a growing body of evidence in support of the stress-gradient hypothesis of plant facilitation (Maestre, Callaway, Valladares, & Lortie, 2009; Bertness & Callaway, 1994), and in particular, underscores the importance of temporal variation in the balance of competition and facilitation, which could inform restoration management practices, such as termination of nurse cover crops (Liu et al., 2013).

The expected improvement in abiotic conditions associated with weed cover was mixed: under weed canopies relative to exclusion areas, air temperature was not significantly cooler (Fig 5c), and relative humidity was even slightly lower (Fig 5b). Only light conditions were significantly more shaded under weed canopies, by ca. 50% (Fig 5d). A lower radiation load, however, almost certainly implies a lower leaf temperature, even if overall air temperature is unaffected (Blonder & Michaletz, 2018, but see Cavieres et al., 2005). Therefore, because seedling light-saturated photosynthetic rates ( $A_{\text{sat,norm}}$ ) were not significantly different between treatments (Figure 5a), we conclude that the positive effect of weed exclusion on fast-growing plants is due to improved microclimatic conditions rather than developmental changes in inherent photosynthetic capacity *per se*. Possible effects of improved microclimatic conditions are to bring leaf temperature closer to the temperature optimum of photosynthesis (Sage & Kubien, 2007), a reduced leaf-to-air vapor pressure deficit (due to lower leaf temperature) and hence less stomatal closure (Grossiord et al., 2020), or a combination of the two.

## **Fast-growing species disproportionately benefit from weeds**

The facilitative effect of weeds during periods of intense abiotic stress was driven primarily by a positive effect on fast-growing species (Fig 4c). While fast-growing species are desired from a restoration perspective, they may be more prone to succumbing to drought- or heat-induced stress. In contrast, slow-growing species may be better poised to maintain physiological integrity during periods which severely restrict growth, such as in nutrient and light-deficit conditions (Reich, 2014; Chapin et al., 1986). In this manner, fast-growing species may experience higher mortality rates if under stress, as fast aboveground growth may come at the expense of below-ground resource allocation (Tilman, 1985). Therefore, cover cropping strategies in restoration (Flores & Jurado, 2003) may require incorporating early successional weed forbs that develop substantial canopies early in the growing season and persist sufficiently long to offer shade during periods of high temperature and VPD if they are to benefit fast-growing target species. Alternatively, fast-growing restoration species may themselves act as nurse protégés, providing shade to other seedlings during stressful periods, which could be accomplished in restoration pockets (Ren, Yang, Liu, 2008).

## **Conclusion**

The large-scale implementation of nurse plant theory into ecological restoration practice will thus depend on the development of strategies that account for rapid climate change and the success of previous restoration attempts. Future work should consider the ecological strategies of pioneer weed species to maximize growth in secondary succession by strategically planting clustered, multispecies restoration pockets in harsh environments that serve to ameliorate

microclimatic conditions and minimize the effects of herbivory. With predictions of rapid climate change and temperature increases in drylands up to 4°C by the end of the 21st century, it is important that restoration projects take into account the local species richness of reforestation sites to preserve ecosystem functioning (Maestre et al., 2012). Management strategies must minimize the effects of herbivory in successional restoration projects if nurse-protégé interactions are to be fully realized. Moreover, a holistic assessment of all species interactions and target plant-environment relationships are needed to maximize the benefits of plant-plant facilitation in restoration settings.

#### **Authors' contributions:**

BC, RK, and MA designed the study. MA, SM, and JC collected data. MA and BC analyzed data. MA wrote a first draft with input from BC. All authors reviewed the manuscript prior to submission.

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## CHAPTER III

### CONCLUSIONS AND FUTURE DIRECTIONS

Our study underscores the importance of incorporating a holistic function-based approach to management and restoration practices in the Lower Rio Grande Valley. Whereas efforts have continuously been made to repopulate degraded land with native woody thornscrub species, fundamental challenges associated with mammalian herbivory and the successional trajectory of the non-woody flora remain (Alexander, Moczygemba, & Dick, 2016; Friends of the Wildlife Corridor, 2016). A holistic function-based approach gives equal importance to the non-woody flora and fauna, which, while not the immediate targets of restoration activity, are nonetheless vital functional components of the ecosystem, potentially acting as facilitators and mediators of plant-plant competition, thereby promoting biodiversity. It is common for the herbaceous flora of restoration sites to go through successional stages leading towards dense stands of invasive grasses (Arriaga et al., 2004), to the detriment of the planted woody community, but our study demonstrates a potentially overlooked role for native herbaceous plants. Ecological restoration's objectives are to preserve the ecological integrity of an ecosystem so that it attains long-term sustainability and resilience (Bradshaw, 1997). While reconstruction, referring the restoration of native plant communities in land previously used for agricultural practices (Stanturf, Palik, & Dumroese, 2014), is undeniably critical in reclaiming land lost to agricultural expansion in the LRGV, future restoration strategies in the area should aim to achieve the rehabilitation of the ecoregion.

Rehabilitation as a goal, the restoring of an ecosystem's status to its previous condition, implies a shift of focus from the structure of the ecosystem to the functional processes necessary for it to achieve sustainability (Bradshaw, 1997). In this manner, a focus on the biotic and abiotic factors of plant growth habit, herbivory, and microclimatic stress that are yet unexplored in the LRGV would be beneficial to increase the success of restoration in the area. Our study suggests biotic interactions should not be ignored in forest restoration practices; in particular, there is a possibility to exploit plant-plant facilitation in the area. Management and restoration practices can investigate the planting of mixed species in clusters as a strategy to offset the effects of competition during harsh abiotic conditions with the inherent growth habit responses to stress by target seedlings (Saha et al., 2012; Callaway, 1995; Grime, 1977). Studies following this recommendation should consider the complex responses and interactions between target seedlings and herbaceous vegetation are dependent on not just species identity and growth habits, but their dynamic interactions with the physical environments they occupy. This consideration highlights a need to compare landscape characteristics such as closeness to remnant undisturbed vegetation (Díaz-Rodríguez, Blanco-García, Gómez-Romero, & Lindig-Cisneros, 2012), thus calling for further research in controlled and field settings at varying spatial and temporal scales.

Designs that incorporate mixed species during periods of intense abiotic stress, imitating the agroecological practices of cover-cropping, may also allow for native herbaceous vegetation to mitigate the effects of competition between these and target seedlings while diminishing the need for interventions such as herbicide use (Stanturf, Palik, & Dumroese, 2014; Blay, 2012). Exploring the possibility of cover-cropping as a forest restoration strategy calls for an exploration of species-specific characteristics of native herbaceous vegetation to potentially identify weed species that offset the competitive nature of invasives (Blay, 2012). Of equal

importance is continuous exploration of interactions between native weed species and target seedlings, as our results suggest a continuum of competitive and facilitative interactions. Further emphasis on ecological processes at play between these agents could further clarify if these interactions are exclusively due to contrasting growth rates or if other processes, such as mycorrhizal interactions and nutrient cycling, can aid in identifying suitable cover-crop candidates in the area (Ruiz-Jaén & Aide, 2005).

In the same manner, a need remains to mitigate the effects of mammalian herbivory in restoration practices, and future studies should focus on assessing the effects of species-specific palatability as well as shielding measures to inform future species selection in restoration (Cushman, Lortie, Christian, 2011). Specifically, species selection in reforestation practices would benefit from the identification of species deemed “unpalatable” and able to serve as nurse neighbors to target seedlings, underscoring the potential of mixed species in reforestation as a suitable alternative to costly shielding interventions (Callaway, Kikodze, Chiboshvili, & Khetsuriani, 2005). This possibility requires studies that focus on identifying native species that are not just desired in the target landscapes, but unpalatable to native herbivores, calling for the exploration of herbivore responses to different varied species mixtures.

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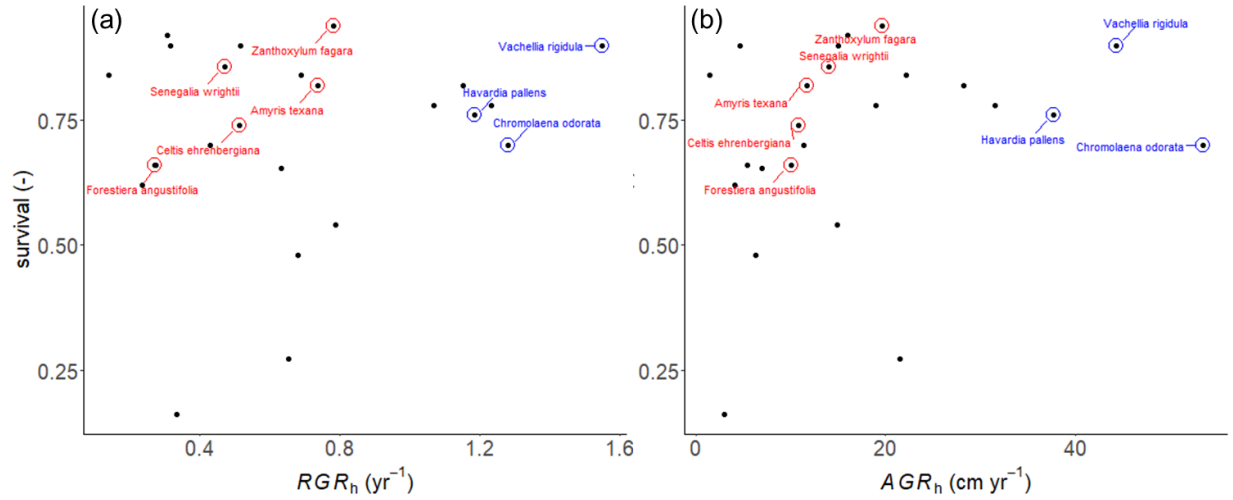
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## APPENDIX A

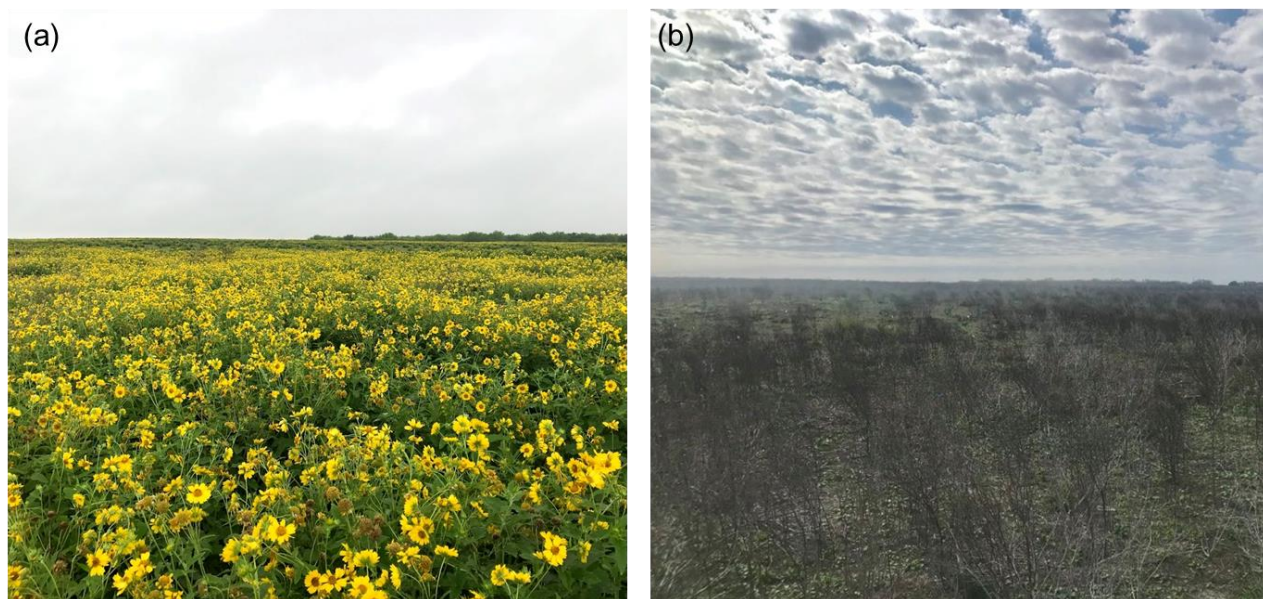
## APPENDIX A



**Figure S1.** Growth-survival relationships for a pool of 24 native seedlings from which the eight focal species in the present study were selected and classified as slow- (red) versus fast- (blue) growing. Survival (fraction of planted individuals still alive after 1 year) versus a) relative height growth rate ( $RGR_h$ ) and b) absolute growth rate ( $AGR_h$ ). Survival estimates based on  $n = 50$  planted individuals per species in October 2018 at the study site; growth estimates are for individuals not affected by significant animal damage ( $n = 4$  to 29 individuals per species; see Methods in main text). Data from Mohsin et al. (in prep).

## APPENDIX B

## APPENDIX B



**Figure S2.** Weed cover of Cowpen daisy (*Verbesina encelioides*), fully developed in March 2019 (a) and fully senesced in January 2020 (b) (photos taken Mylen Arias).



## APPENDIX C

## APPENDIX C

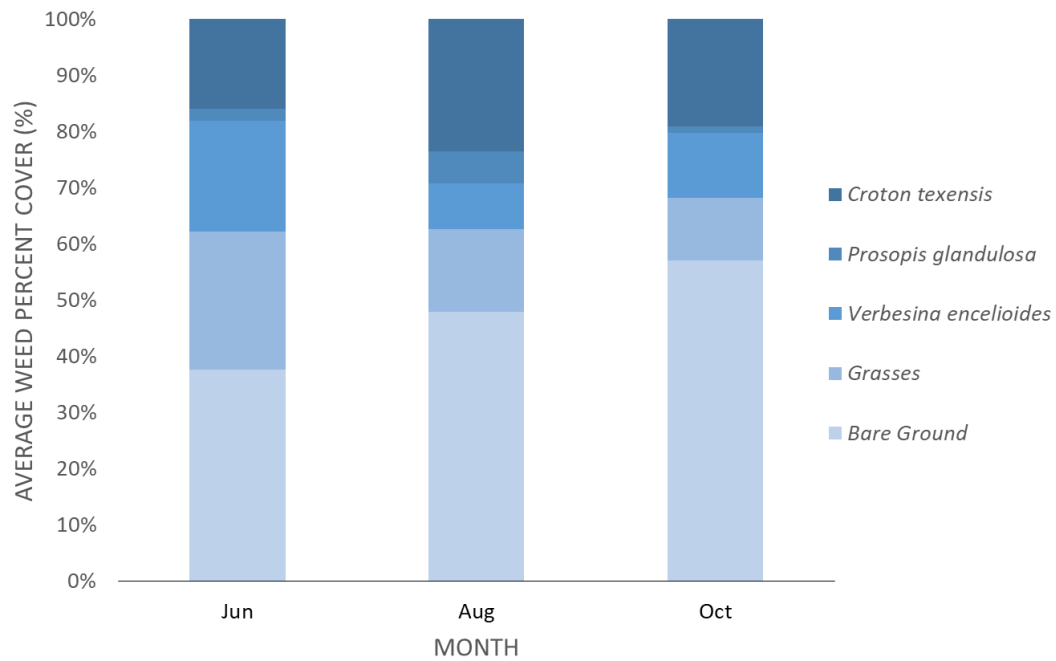
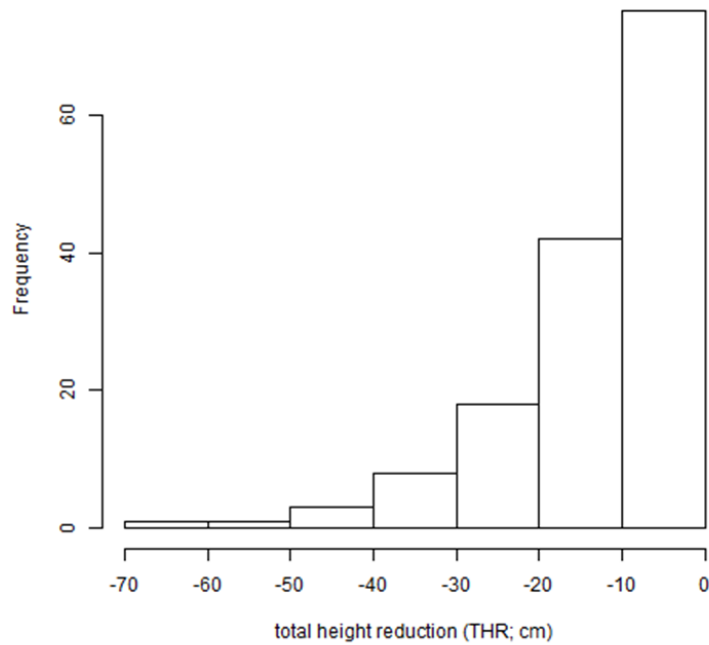


Figure S3. Percent weed cover (n = 25 sample points centered around control seedlings) in 2019. Dominant weed species are Cowpen Daisy (*Verbesina encelioides*), Doveweed (*Croton texensis*), and Honey Mesquite (*Prosopis glandulosa*).

## APPENDIX D

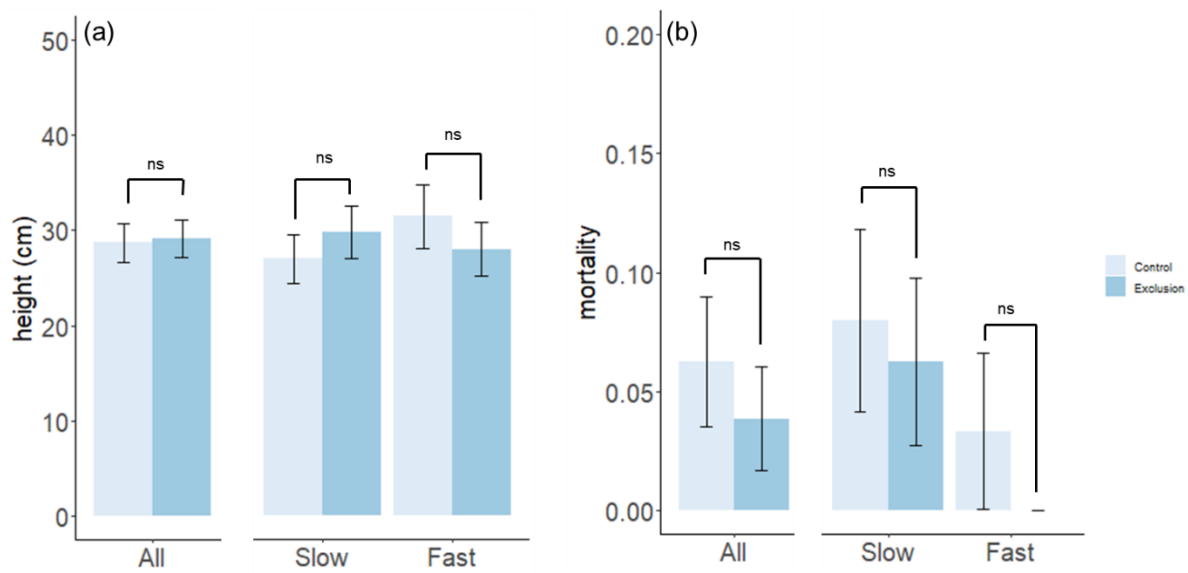
## APPENDIX D



**Figure S4.** Frequency distribution of total height reduction (THR; cm) of 148 individuals (out of 160) that experienced at least one reduction in plant height over the course of the study. THR was estimated as the cumulative sum (across multiple censuses) of height growth which was less than zero (for each individual separately) over the entire study period (Jun 2019 – Jan 2020).

## APPENDIX E

## APPENDIX E



**Figure S5.** Pre-treatment mean seedling height (a) and total mortality (b) +/- sem (error bars) in July 2019. Mortality represents the fraction of new deaths relative to live individuals in June 2019. (n = XX per experimental unit – need to fill this in). Significance codes: ‘\*\*\*’ p < 0.001; ‘\*\*’ p < 0.01; ‘\*’ p < 0.05; ‘ns’ p > 0.05.

## APPENDIX F

## APPENDIX F



**Figure S6.** Photosynthetic rate measurements of colima (*Zanthoxylum fagara*) on site using a Licor 6400 XT infrared gas analyzer with a 6400-22 opaque conifer chamber attachment. Picture taken on June 27<sup>th</sup>, 2019 by Mylen Arias.



## BIOGRAPHICAL SKETCH

Mylen A. Arias attended elementary, middle, and high school in Río Bravo, Tamaulipas, México. She completed her high school studies in Alamo, Texas, to then attend the University of Texas Rio Grande Valley in 2012, where she completed a Bachelor of Arts in Philosophy in 2017. In 2018, she completed a Post-Baccalaureate Bachelor of Science in Biology, to then complete a Master of Science in Biology in 2020. Mylen served as a Writing Consultant for the UTRGV Writing Center for five years, earning the title of Tutor of the Year in 2016. Her interdisciplinary background has inspired her to aid and encourage others to engage in social and political activity through involvement in civic engagement, as well as to explore ways in which habitat conservation and sustainability can be brought to the attention of our communities. With education as her main passion, she is committed to sharing her work with others.

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