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Exploring Plant-Plant Interactions and Nutrient Manipulation as Strategies for Thorn Scrub and Thorn Forest Restoration

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EXPLORING PLANT-PLANT INTERACTIONS AND NUTRIENT MANIPULATION AS STRATEGIES FOR THORN SCRUB AND THORN FOREST RESTORATION

A Thesis

by

Edgar D. Vasquez

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EXPLORING PLANT-PLANT INTERACTIONS AND NUTRIENT MANIPULATION AS STRATEGIES FOR THORN SCRUB AND THORN FOREST RESTORATION

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August 2020

ABSTRACT

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Restoration of thorn scrub and thorn forest communities in South Texas is hindered by harsh environmental conditions and hypercompetitive invasive grasses. The success of thorn scrub/thorn forest restoration efforts depends on increasing seedling survival and growth by reducing stress after transplantation. Four experiments evaluated initial effects of plant-plant interactions and nutrient manipulation on thorn forest restoration. High density planting along with the use of nurse plants look promising; survival was generally higher under these conditions and growth was unaffected or in the case of high-density planting, promoted. Nutrient sequestration by sorghum had little effect on seedling performance and cover of grass and other plants, but localized fertilization benefited the seedlings. Allelopathy is promising; however, field studies must be performed to confirm benefits in the field. Overall positive plant-plant interactions resulted higher survival and growth in this study and should be implemented as thorn scrub and thorn forest restoration strategies.

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TABLE OF CONTENTS

Page

ABSTRACT……………………………………………………………………………iii
ACKNOWLEDGEMENTS……………………………………………………………iv
TABLE OF CONTENTS………………………………………………………………v
LIST OF TABLES……………………………………………………………………vii
LIST OF FIGURES…………………………………………………………………..viii
CHAPTER I. INTRODUCTION ……………………………………………………......1
Restoration of semi-arid habitats……………………………………………………1
Invasive Grasses in the RGV………………………………………………………..3
Invasive Species as Problems in Restoration……………………………………….4
Nutrient Manipulation……………………………………………………………….5
High-Density Planting……………………………………………………………….6
Nurse Plants…………………………………………………………………………7
Allelopathy…………………………………………………………………………..8
CHAPTER II. METHODS…………………………………………………………......10
Study Area……………………………………………………………………………...10
Nutrient Manipulation Experiment…………………………………………………10
Density Experiment…………………………………………………………………12
Nurse Plant Experiment……………………………………………………………13
Allelopathy of *Vachellia schaffneri*……………………………………………………14
Data Management……………………………………………………………………15
Data Analysis…………………………………………………………………………15
CHAPTER III. RESULTS………………………………………………………………...18
Nutrient Manipulation Experiment……………………………………………………...18
Nutrients Sequestered…………………………………………………………….....18
Height………………………………………………………………………………......18

Basal Diameter…………………………………………………………………….....19

Chlorophyll content………..……………………………………………………...19

Plant Cover…….……………………………………………………………..........20

Survival…………………………………………………………………………....21

Density Experiment…………….……………………………………………….......21

Height…..………………………………………………………………….........21

Basal Diameter…….………………………………………………………….......22

Chlorophyll Content…….…………………………………………………….......22

Plant Cover…………………………………………………………………….…..23

Survival……………………………………………………………………….…...24

Nurse Plant Experiment………………………………………………………….….24

Height…….…………………………………………………………………….....24

Basal Diameter…….………………………………………………………….......25

Chlorophyll content…….………………………………………………….….......25

Temperature & Light Intensity...…………………………………………….........26

Survival……………………………………………………………………….......26

Allelopathy Experiment…………...………………………………………………..26

CHAPTER IV. DISCUSSION..………………………………….…………………....28

Nutrient Manipulation………………………………………………………………..28

High-Density Planting…..………………………………………………………….....29

Nurse Plants………….……………………………………………………………......32

Allelopathy of *Vachellia shaffneri*..………………………………………………….34

Recommendations for Restoration..………………………………………………….35

Future Studies……………….....……………………………………………………..36

Conclusions…….………………………………………………………………….....37

REFERENCES………………………………………………………………………...38

APPENDIX.....…………………………………………………………………….......45

BIOGRAPHICAL SKETCH………………………………………………….............65

LIST OF TABLES

Page

Table 1: Common name, scientific name, and growth habit of species selected for the density planting experiment……………………………..……………………..…46

Table 2: Average amount of nutrients sequestered by sorghum in the nutrient manipulation experiment. ………………………..…………………………..….47

Table 3: Parameters of the germination process of *S. bicolor* as affected by various concentrations of plant tissue extracts. VSL = *V. shaffneri* leaf extract, VFL = *V. farnesiana* leaf extract, VSR = *V. shaffneri* root extract, VFR = *V. farnesiana* root extract, MGT = Mean germination time, CVG = coefficient of velocity of germination. Lower case letters show Tukey Kramer post hoc groupings……………………………………………...62

Table 4: Parameters of the germination process of *L. sativa* as affected by aqueous extracts of huisache and huisachillo and their fractions. VSL = *V. shaffneri* leaf extract, VFL = *V. farnesiana* leaf extract, -ether and -DCM refer to aqueous extracts with the diethyl ether and DCM soluble compounds extracted. MGT = Mean germination time, CVG = coefficient of velocity of germination. Lower case letters show Tukey-Kramer post hoc groupings………………………………………..………..64

LIST OF FIGURES

Page

Fig. 1: Mean height of seedlings in cm at each sampling date for *F. angustifolia* (A) and *E. ebano* (B). Seedlings were planted in plots with a pre treatement of either sorghum or control (no sorghum planted). Plots were then divided into subplots where one subplot received a fertilizer application and the other subplot received a water control. Error bars show SEM for each treatment. ……………………………………………………………..…48

Fig. 2: Mean basal diameter of seedlings in mm at each sampling date for *F. angustifolia* (A) and *E. ebano* (B). Seedlings were planted in plots with a pre-treatment of either sorghum or control (no sorghum planted). Plots were then divided into subplots where one subplot received a fertilizer application and the other subplot received a water control. Error bars show SEM for each treatment. ……………………………………………………………….…………49

Fig. 3: Mean chlorophyll content of seedlings at each sampling date for *F. angustifolia* (A) and *E. ebano* (B). Seedlings were planted in plots with a pre treatement of either sorghum or control (no sorghum planted). Plots were then divided into subplots where one subplot received a fertilizer application and the other subplot received a water control. Error bars show SEM for each treatment……………..……………………………………………………….….50

Fig. 4: (A) Annual Cover, (B) Perennial Cover and (C) Total Cover of plants in sorghum treated and control plots. Sorghum treated plots were first seeded with sorghum then sorghum was harvested in order to sequester nutrients from the soil. Error bars show SEM for each treatment……………………………………………..…………………………….51

Fig. 5: (A) Average survival of *F. angustifolia* and (B) *E. ebano* seedlings in each treatment of the nutrient manipulation experiment. Seedlings were planted in plots with a pre treatement of either sorghum or control (no sorghum planted). Plots were then divided into subplots where one subplot received a fertilizer application and the other subplot received a water control. Each subplot contained 12 seedlings. Error bars show SEM for each treatment. n = 4…………………………………………………….…..52

Fig. 6: Mean height in cm at each sampling date for *F. angustifolia* (A) and *E. anacua* (B). Seedlings were planted at low (2,571 seedlings/ha) and high (15,428 seedlings/ha) densities. Seedlings were planted in three different mixes monospecific *F. angustifolia*, monospecific *E. anacua*, and a mix composed of nine foundation species. Error bars show SEM for each treatment. ……………………………………………………….……….53

Fig. 7: Mean basal diameter in mm at each sampling date for *F. angustifolia* (A) and *E. anacua* (B). Seedlings were planted at low (2,571 seedlings/ha) and high (15,428 seedlings/ha) densities. Seedlings were planted in three different mixes monospecific *F. angustifolia*, monospecific *E. anacua*, and a mix composed of nine foundation species. Error bars show SEM for each treatment………………………………………………………………......54

Fig. 8: Mean chlorophyll content at each sampling date for *F. angustifolia* (A) and *E. anacua* (B). Seedlings were planted at low (2,571 seedlings/ha) and high (15,428 seedlings/ha) densities. Seedlings were planted in three different mixes monospecific *F. angustifolia*, monospecific *E. anacua*, and a mix composed of nine foundation species. Error bars show SEM for each treatment………………………………………………………………..…….55

Fig. 9: (A) Annual Cover, (B) Perennial Cover and (C) Total Cover of plants in the density experiment. Seedlings were planted at low (2,571 seedlings/ha) and high (15,428 seedlings/ha) densities. Seedlings were planted in three different mixes monospecific F. angustifolia, monospecific *E. anacua*, and a mix composed of nine foundation species. Post hoc tukey tests were performed for each sampling time separately and results are indicated by lower case letters. Error bars show SEM for each treatment…………………………………..…56

Fig. 10: Total seedling survival per plot type. Seedlings were planted at low (2,571 seedlings/ha) and high (15,428 seedlings/ha) densities. Seedlings were planted in three different mixes monospecific *F. angustifolia*, monospecific *E. anacua*, and a mix composed of nine foundation species. Each high-density plot contained 54 seedlings each low-density plot contained 9 seedlings. Error bars show SEM for each treatment. n = 4…………………………………………………………………..57

Fig. 11: Mean height (A), basal diameter (B), and chlorophyll content (C) of *F. angustifolia* seedlings. Seedlings were planted 20cm to the east and west of a *B. neglecta* nurse plant or in open gaps 20cm to the east and west of a marker. Red asterisks signify orientation was significant green signify nurse plant significance and black asterisks signify nurse plant*orientation significance. Error bars show SEM for each treatment……………………………………..……..58

Fig. 12: Chlorophyll content (Chloro) versus leaf area index (LAI) with regression lines and pearson coefficients for (A) east nurse plant seedlings (n=12) and (B) west nurse plant seedlings (n= 22). ………………………...59

Fig. 13: (A) Air temperature at the soil surface and (B) light intensity under *F. angustifolia* seedlings. Seedlings were planted 20cm to the east and west of nurse shrubs *B. neglecta*. Error bars show SEM for each treatment…………..60

Fig. 14: Survival of *F. angustifolia* seedlings in the nurse plant experiment. Seedlings were planted either with a nurse plant or in a gap (NP or Gap) and either to the east or west of the nurse plant or marker (E or W). n = 10……………………………………………………………………….…61

Fig. 15: Cumulative germination plots of *S. bicolor* seeds when exposed to 100%, 75%, 50%, 25%, and 0% (Control) extracts of (A) V. schaffneri leaves, (B) V. farnesiana roots, (C) V. farnesiana leaves, and (D) V. schaffneri roots……………………………………………...............................61

Fig 16: Cumulative germination plots of *L. sativa* seeds when exposed to complete, diethyl ether extracted, DCM extracted and 0% (Control) extracts of (A) *V. schaffneri* leaves, and (B) *V. farnesiana* leaves…………………………………………………………………..…63

CHAPTER I

INTRODUCTION

Restoration of semi-arid habitats

Restoration of semi-arid habitats has inherent difficulties due to a suite of abiotic and biotic stressors. Scarce rainfall and high evapotranspiration place planted seedlings under great water stress and can be an important impediment to successful restoration (Jurado et al 2006). In South Africa soil management practices that increase soil moisture content facilitate the revegetation of degraded sites (Snyman 2003). This implies that lack of rainfall is an important abiotic stressor for plants in restoring arid and semi-arid regions. Le Houérou (2000) provides further evidence suggesting that reforestation is usually only successful in zones that have more than 200 mm of rain per year. Aronson et al (1993) discuss how prolonged use and degradation of soils and plant communities by humans lead to transformations where the depth and water reserves of a soil are reduced which makes restoration even more difficult. High solar radiation, high temperatures, low water availability, and water loss are all major problems for restoration in the Lower Rio Grande Valley (LRGV) because native plant communities have been removed for human land use and agriculture. More than 91% of native woodlands in Cameron County have been converted to agricultural land (Tremblay et al 2005).

Restoration efforts in the LRGV should be focused on increasing seedling survival. Fulbright et al (1992) showed that seedling survival increased under shade in the LRGV, which may point at the benefits of sheltering from the intense heat and solar radiation seedlings are exposed to. These conditions may exacerbate as climate change proceeds. New approaches for increasing restoration success are needed to reduce the harmful effects of the changing climate (Oliet & Jacobs 2012). Enclosures to protect seedlings from browsing animals is another strategy frequently used in arid and semi-arid environments. Enclosures can facilitate seedling establishment and growth by excluding browsing animals, which can be considered a significant biotic stressor for establishing seedlings (Le Houérou 2000, Yayneshet et al 2009).

In South Texas, abiotic and biotic stressors for seedlings may be severe and include extreme hot weather, dry soil conditions, hypercompetitive invasive grasses and vertebrate herbivory. These stressors must be alleviated for a successful restoration (Le Houérou 2000). Shelter tubes, which protect from herbivory and help to conserve water, increased survival and growth rates for thorn scrub and thorn forest seedlings but only if they are used for a year or longer. Exclosures to protect from vertebrate herbivory also increased growth rate but had no effect on survival (Dick et al 2016). Jurado et al (1998, 2006) report higher seedling emergence and growth under dense thorn scrub when compared to open areas and thorn scrub edge. Although all monitored seedlings died by late spring in that study, dense thorn scrub seedlings survived the longest. Thorn scrub species may thus have evolved to germinate and grow under the protection of nurse plants no longer present in open, deforested sites.

Selection of species for restoration is also a major factor that affects restoration success. Padilla et al (2009) suggest reforesting with mid successional shrubs rather than late successional species. Late successional species typically grow slower and have higher mortality rates when grown in open areas. Padilla et al (2009) suggest using species that maximize resource uptake and limit water loss to be planted first to and allowed to form a canopy that can shelter slower growing species. Overall, intense heat, low moisture, and intense solar radiation appear to be the greatest abiotic impediments to reforestation in the RGV. Highly competitive invasive grasses are one of the main biotic stressors for thorn scrub and thorn forest seedlings in the LRGV.

Invasive Grasses in the RGV

The LRGV is impacted by the abundant presence of highly invasive exotic grasses and much of the native herbaceous flora has been replaced by few exotics (Best 2006). Three prominent exotic grasses in South Texas are guinea grass (*Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs), buffelgrass (*Pennisetum ciliare* (L.) Link), and Kleberg bluestem (*Dicanthium annulatum* (Forssk.) Stapf). These grasses were introduced from Africa in the 20th century and were intended to enhance livestock productivity (Best 2006). *Pennisetum ciliare* is known worldwide as an invasive species and rapidly converts thorn scrub/thorn forest and desert vegetation into monospecific grassland. It is now the dominant herbaceous cover in $10x10^6$ ha of semiarid land throughout the world (Williams & Baruch and references therein 2000). Invasive plant species often alter the carbon, nitrogen, and water cycles of an ecosystem (Ehrenfeld 2003). In Hawaii, the removal of introduced perennial bunchgrasses has been shown to increase native plant size, increase nitrogen in native plant tissues, and increase the number of seedlings found in cleared plots (D'Antonio et al 1998). In Texas, buffelgrass cover causes a reduction in native forb species richness, cover and density (Sands et al 2009). This relationship between exotic grass cover and native species richness and size reduction suggests that the exotics must be controlled if higher biodiversity is desired in restored areas.

Herbicide application can be effective in preventing grass growth (Cione et al 2002, Alexander et al 2016), but frequently only kills above ground growth and repeated applications are needed in order to prevent re-sprouting (Best 2006). Other grass control options need to be explored in the RGV in order to prevent the perpetuation of these exotic grasses if restoration success is to be increased.

Invasive species as problems in restoration

Invasive species are defined as species with self-sustaining populations at a significant distance from the point of introduction to an area, and eventually survive and reproduce at multiple sites across a greater or lesser spectrum of habitats and extent of occurrence (Blackburn et al 2011). Invasive species are becoming more common around the world due to human actions. Species are often introduced into new areas so they can be used to provide new services and resources; like pine tree (*Pinus spp.*) introduction to South Africa and buffelgrass introduction to various parts of the world. Pine trees were introduced to South Africa to provide a source for timber but now are a threat to water sources and biodiversity (Woodford et al 2016). Buffelgrass is commonly introduced because it is a good forage grass with high productivity under water scarcity, however the grass invades native grasslands and savannas, and can alter landscape processes like runoff, erosion and also threatens biodiversity (Belnap et al 2012 and references therein). They can cause rapid ecosystem degradation, homogenization of regional biotas, and are one of the greatest threats to biodiversity where they are established (Wilcove et al 1998, Pyšek & Richardson 2010).

Invasive species can cause changes to natural ecosystem cycles that affect the restoration approaches. In South Africa *Acacia saligna,* a native of Australia, increases plant-available nitrogen in soils that would otherwise be nitrogen deficient. Clearing out the trees causes more nitrogen to be mineralized by soil microbes, which leaves the cleared areas vulnerable to invasion by nitrophilous invasives (Yelenik et al 2004). Land managers must take steps to reduce the impacts of increased nitrogen availability when restoring areas invaded by this acacia. In Ohio an invasive honeysuckle (*Lonicera maackii*) reduces the recruitment of native seedlings under its canopy. Transplanted native seedling survival is reduced in areas in which the honeysuckle is left intact and clearing out the shrub is recommended before seedlings are transplanted (Hartman & McCarthy 2004). A few promising topics for restoration research in the RGV are nutrient manipulation, high-density plantings, nurse plants use and identification of native species which show allelopathic effects on the exotic invaders.

Nutrient Manipulation

Correction of soils' physical and chemical properties may be needed to facilitate the recovery of targeted plant associations. It is important to determine the soil properties of a potential restoration site and implement the necessary corrections to support the desired restored community (Heneghan et al 2008). Invasive plant species typically outcompete native species in high nutrient environments and respond to higher nutrient levels with increased growth relative to natives; survival of invasive species can also be higher than native species in higher nutrient environments (Gurevitch et al 2008, Leishman & Thompson 2005). The restoration of abandoned agricultural land is difficult because of the excess nutrients left over from years of repeated fertilization. High nutrient availability favors growth of invasive plants and can be detrimental to restoration efforts (Heneghan et al and references therein 2008). Manipulation of soil properties must then aim to decrease nutrient availability if invasive species suppression is desired. Carbon addition used to immobilize plant available nitrogen has been used to combat invasive species in enriched soils (Blumenthal et al 2003). Using high nutrient demanding crop plants to extract nutrients before reforestation efforts can be another approach to soil nutrient manipulation. Annual rye has been used to extract nitrogen and phosphorous from soil and dropped the competitiveness from one invasive species to allow for bunchgrass restoration (Herron et al 2001). However, lower nutrient availability could have negative effects on the seedlings' initial growth after planting. The local application of fertilizer could reduce the effects of the reduced nutrient availability after sequestration by carbonaceous amendments or crop harvest. Pre-transplanting fertilization of tomato seedlings with nitrogen and phosphorous has had positive effects on growth and early yield of the crop (Garton & Widders 1990). Localized fertilization of horticultural transplants is a common practice at transplantation.

High-Density Planting

Restoration of plant communities in the RGV is difficult because of the high levels of solar radiation, high summer temperatures, and low rainfall amounts during the summer months. One way to ameliorate these conditions is by taking advantage of positive plant-plant interactions. Shrubs have facilitative effects on growth and survival of their neighbors in semi-arid climates. The facilitative effects of shrubs are especially noticeable in their tree seedling neighbors (Gómez-Aparicio 2009). A method that exploits these facilitative effects of shrubs and trees should be considered in a seasonally hot and dry climate like the one found in the RGV. The Miyawaki method is a reforestation strategy that has been successful in several regions of the world characterized by higher rainfall than the in the LRGV (Miyawaki 1999 and references therein). This method involves high density planting a mix of tree species found in the climax vegetation of the desired plant community. Miyawaki (1999) recommends densities as high as 2 or 3 seedlings per square meter (25,000 seedlings/ha). Schirone et al (2011) show that the method can produce encouraging results in Mediterranean-like climates characterized by hot dry summers and mild wet winters and can be a viable approach for restoration in dry areas.

Invasive grass suppression is an important factor in any reforestation strategy implemented in the RGV. This is because invasive grasses quickly outcompete young thorn scrub and thorn forest seedlings for resources and hinder restoration efforts. Any thorn scrub and thorn forest restoration strategy in the LRGV must include a plan to control invasive grasses present in the area. The invasive grass *M. maximus* has also become a problem in Hawaii where it causes similar problems as in the LRGV. Ammondt & Litton (2012) grew the grass with native plants at three levels of functional diversity. They found that as functional diversity increased the grasses reproductive tiller production was decreased. This suggests that planting a variety of native species together at high densities could reduce invasive grass competition.

Nurse Plants

Abiotic stressors of semi-arid environments make restoration in these climates difficult. The high amounts of solar radiation and high daytime temperatures negatively affect seedling establishment. Fulbright et al (1992) found that survival of five native tree and shrub species was enhanced under artificially created shade when compared to seedlings planted in open areas in the LRGV. Abiotic conditions of this sort may be ameliorated through the use of nurse plants. Nurse plants are mature plants that, when found in close proximity with seedlings, facilitate the growth and/or survival of the seedling. Daytime temperatures can be reduced, and nighttime temperatures can be moderated for seedlings found in association with nurse plants (Groenveveld et al 2007). Nurse plants simultaneously compete with and shelter seedlings around them. When the positive effects of the interaction outweigh the negative effects, the seedling shows increased vigor and survival (Padilla & Pugnaire 2006). Restorations in semi-arid environments are most likely to benefit from these nurse plant effects because the abiotic conditions especially during the hot summers are particularly tough for seedlings. Late successional and shade tolerant species benefit more from nurse plants than early successional species (Padilla & Pugnaire 2006). Gómez-Aparicio (2009) found that shrubs are best suited to be nurse plants and recommend augmenting populations of shrubs for the restoration of woody late successional communities.

Allelopathy

Allelopathy is defined as the suppression of the growth and/or establishment of neighboring plants by chemicals released from a plant or plant parts. Allelochemicals are secondary metabolites that interact with their environment to produce allelopathic effects. The *Novel Weapons Hypothesis* states that invaders produce secondary metabolites that native species have not been exposed to which could by chance have inhibitory effects on growth or emergence (Wardle et al 2011). Allelopathy is most commonly studied in the contexts of plant invasions and much research has focused on how allelopathy enhances the success of invading exotic species (Heisey 1990, Fulbright & Fulbright 1990, Al-Humaid & Warrag 1998, Ridenour & Callaway 2001, De Feo et al 2003, Callaway et al 2008, Csiszár 2009, Wu et al 2009, Wardle et al 2011). On the other hand, if native species produce secondary metabolites that suppress the invasives in their area; then they could be used to reduce the competitiveness of their invaders. A South American legume has been shown to have allelopathic effects on the African grass *Cynodon dactylon* (L.) Pers. (Bermuda grass) (Al-Humaid & Warrag 1998). The invasive African grasses are one of the greatest challenges to overcome for restoration in the RGV and restoration success is largely dependent on their suppression. If a native species is found that has strong allelopathic effects on the invasive grasses, it can be used to reduce impacts of the grasses in affected areas.

In general, critical factors affecting restoration of thorn scrub and thorn forest in the RGV are highly competitive invasive African grasses, and harsh environmental conditions newly transplanted seedlings encounter. The objective of this study is to explore reforestation strategies that will alleviate these conditions. Nutrient manipulation, a modified Miyawaki method, nurse plants, and allelopathy will be explored to determine if they are viable strategies for this region. Four hypothesis are being tested: 1) macronutrient limitation (N and P) followed by localized supplementation, reduce grass competitiveness while enhancing seedling establishment and growth; 2) higher planting densities will allow interspecific and intraspecific positive interactions resulting in better survival, physiological status and growth, 3) Native, early succession shrub species reduce environmental stresses to adjacent seedlings, resulting in better survival, physiological status and growth, and 4) Native species with evolved chemical strategies for competition can reduce germination potential of other plant species. To test these hypotheses, a series of field and laboratory experiments were executed.

CHAPTER II

METHODS

Study Area

The study took place at southmost preserve in Brownsville, Texas. The 1,014-acre preserve contains one of the last Mexican sabal palm/thorn scrub stands left in the United States. The portions of the preserve where this study was situated were previously organic grapefruit orchards. In 2014 the orchard was cleared when it became infested with fruit flies. The land was left fallow until restoration activities commenced (M. Pons, personal communication, August 10th, 2020). Climate is semi-arid and subtropical. Summers are hot and dry; winters are wet and mild. Soils in the study site are 61% silt, 28 % sand, and 11% clay (NRCS Web Soil Survey). Rainfall in the study area was 585.98 and 580.64 mm 2018 and 2019 respectively (NOAA National Climatic Data Center, Station USW00012919). The preserve operates a native plant nursery where as many as 70,000 seedlings/ year of 30+ thorn scrub and thorn forest species are grown for LRGV restoration efforts. Restoration activities at the preserve include invasive grass control and planting of nursery grown seedlings. The seedlings used in this study were produced at that nursery.

Nutrient Manipulation Experiment

The effects of sorghum (*Sorghum bicolor* (L) Moench, (a domesticated African grass) planting in order to extract nutrients out of the soil on native tree seedling survival and growth, as well as its effect on invasive grass cover was examined in this experiment. The experimental plots were covered by lush *M. maximus* and were first disked twice, followed by an herbicide application of 3% Roundup (glyphosate) on weed regrowth. Sorghum was seeded using a manual spreader at a rate of 60 kg of seed per hectare followed by shallow disking to bury the seeds. Sorghum was harvested at flag stage by cutting all plants at ground level and fresh weight was recorded on site. Subsamples were dried (45ºC), weighted to obtain a fresh: dry conversion factor. Four whole plants of average size per plot were dried and combined in a composite sample for tissue nutrient analyses at Texas A&M AgriLife extension laboratory. Plant nitrogen was determined by high temperature combustion. Other plant minerals were determined by ICP analysis of the nitric acid digest of plant material. This information was used to estimate the total amount of nutrients removed by the sorghum harvest. A localized starter fertilizer solution treatment was tested, a water only control was included. The fertilizer solution was prepared with a commercial soluble mix (9-58-8) following the instructions of the maker. A volume of 400 ml per seedling of fertilizer solution or water was applied to the base of seedlings upon transplanting. The experiment was run two times concurrently, once with a leguminous species, Texas ebony (*Ebenopsis ebano* (Berland.) Barneby & J.W. Grimes), and again with a non-leguminous species elbow bush (*Forestiera angustifolia* Torr.). It was expected that potential negative effects of nitrogen removal by sorghum is less prevalent in the leguminous species due to the expected presence of rhizobial nitrogen-fixing bacteria in its roots. This was a factorial experiment in a split-plot design with four replications. Sorghum treatment was applied as the main plot treatment while localized fertilization application was the subplot treatment.

The experiment was established in November of 2017 and was monitored until the spring 2020. Parameters measured were in vivo chlorophyll content of seedling leaves, height and basal diameter of seedlings, total plant cover excluding seedlings, annual plant cover and perennial plant cover, and seedling survival. Chlorophyll content was measured using a chlorophyll content meter (Mod. CCM300, Opti-Sciences, Hudson NH). Seedling height was measured from soil level to the highest living part of the seedling. Basal diameter was measured one cm above soil level using digital calipers. Total plant cover, annual cover, perennial and grass cover was were determined using the line intercept method (Caratti 2006). Herbaceous annual plants were considered for annual cover. Herbaceous and woody non-invasive grass plants were considered for perennial cover. The sum of annual, perennial, and invasive grass cover was reported as total cover. Chlorophyll content, plant cover, basal diameter and height were recorded on eight marked seedlings per subplot every four months. Survival of all seedlings was recorded twice a year.

Density Experiment

Effects of high- and low-density planting on survival and growth of seedlings as well as plant cover was tested. The experimental plots were prepared as described for the nutrient manipulation experiment (above). Two density treatments compared in this experiment were low density planting (2,571 seedlings/ha) and high-density planting (15,428 seedlings/ha). The 2,571 seedlings/ha density aligned with typical planting densities used in restoration in the LRGV. The 15,428 seedlings/ha density was similar to planting densities used in Miyawaki-like restorations. In addition, three different species compositions were tested: 1) elbow bush (*F. angustifolia* potentially allelopathic), 2) anacua (*Ehretia anacua* (Terán & Berland.) I.M. Johnst) (non-allelopathic), and 3) nine thorn scrub/thorn forest species mix (Table 1). Allelopathic effects were assessed by comparing total plant and grass cover among species compositions.

This experiment was established in the winter of 2017 and was monitored until the spring of 2020. Parameters measured were in vivo chlorophyll content of seedling leaves, height and basal diameter growth of seedlings, total, annual and perennial cover, and seedling survival. Chlorophyll, plant cover, basal diameter and height were recorded on eight marked seedlings per subplot every four months. Survival of all seedlings was recorded twice a year.

Nurse Plant Experiment

An experiment using a native fast-growing early succession shrub growing in between two seedlings was established in the fall of 2017. Ten naturally occurring young false willow (*Baccharis neglecta* Britton) shrubs were selected as nurse plants and pruned to 50 cm high in order to equalize sizes. A 1 m^2 plot was cleared around the base of the ten false willow shrubs. Ten control plots (no willow) were also cleared close by the shrub plots. Two elbow bush seedlings were planted 20 cm to the east and west of the false willow shrubs. Seedlings on the east side of the shrubs were protected from afternoon sun and it was hypothesized that they are less stressed. This was assessed indirectly through measurements of chlorophyll content of leaves. Basal diameter, survival, height and chlorophyll content of planted seedlings were recorded every three months.

Height, diameter at breast height, and leaf area index (LAI) were also recorded on the nurse plants in order to determine if there is any relationship between nurse plant size and chlorophyll content of seedlings. LAI was determined with a LAI ceptometer (AccuPAR PAR/LAI ceptometer LP-80). In May 2019 HOBO loggers were placed under seedlings to continuously record light intensity and air temperature at the soil surface.

Allelopathy of *Vachellia schaffneri*

A native tree *Vachellia schaffneri* (S. Watson) Seigler & Ebinger (huisachillo) is suspected of having evolved a chemical strategy to compete against neighboring plants and possibly affecting invasive African grass species. Leaf and root tissue of *V. schaffneri* were collected to determine the effects that extracts would have on germination of of *S. bicolor,* an African grass. *Vachellia farnesiana* (L.) Wight & Arn. (huisache) is likely non-allelopathic and a closely related species, it was used as a control species. Leaf and root tissue were collected from mature specimens of this species as well. Leaf and root tissue from both species were dried in an oven at 40°C for three days. The tissues were ground into a powder and 45 g were placed into 900 ml of deionized water. The tissues were extracted at room temperature with a magnetic stirrer for 28 hours and then filtered through a sieve. Extract concentrations of 100%, 75%, 50%, 25%, and 0% were prepared and tested on sorghum seeds. The 6 ml of each extract were then placed into petri dishes which contained 100 sorghum seeds and paper towels. Seeds were allowed to germinate and counted as such when the radicle was 5mm long.

A separate experiment was performed to determine polarity of allelochemicals. Extract was prepared by stirring 5 g of both huisache and huisachillo leaf powder in 100 ml of DI water separately. Resulting extracts were filtered then brought to 200 ml by adding DI water. 5 ml of this solution were placed into five separate petri dishes which contained 30 *Lactuca sativa* L. seeds. A liquid-liquid extraction was performed on remaining solution with 200 ml of ditheyl ether then 5 ml of aqueous portion were placed into five different petri dishes containing 30 *L. sativa* seeds. A liquid-liquid extraction was performed on remaining aqueous solution with 200 ml of dichloromethane (DCM). 5 ml of the remaining aqueous portion was used to moisten five petri dishes containing 30 *L. sativa* seeds. A DI water control in five petri dishes was used for this experiment.

Data Management

In both germination experiments, total germinability (G), mean germination time (MGT), and coefficient of the velocity of germination (CVG) were calculated using the following formulas.

$$G = \frac{Total\ germinated\ seeds}{Total\ seeds}$$

$$MGT = \frac{\Sigma_{i=1}^{k} n_i t_i}{\Sigma_{i=1}^{k} n_i}$$

where t_i is the time from the start of the experiment to the ith observation in days, n is the number of seeds germinated on the ith time, and k is the last day of germination.

$$CVG = \frac{100(A_1 + A_2 + \cdots A_x)}{(A_1 T_1 + A_2 T_2 + \cdots A_x T_x)}$$

where A is the number of germinated seedlings on each day, and T is the number of days that have passed since the start of the experiment (Ranal et al 2009, Ranal & Garcia de Santana 2006)

Data Analysis

Initial height and basal diameter for the nutrient manipulation experiment were analyzed using ANOVA as a two-way factorial (plot pretreatment, seedling fertilization, and their interaction) as the initial data was collected ten months prior to regular sampling. Changes in seedling height, basal diameter, chlorophyll content, and percentage of invasive grass cover per plot were analyzed using repeated measures (RM) ANOVA as a two-way factorial (fixed effects = plot pretreatment, seedling fertilization, and their interaction). Data were checked for normality and homoscedasticity with Kolmogorov-Smirnov and Levene's tests respectively. Transformations were applied to meet the ANOVA assumptions (*E. ebano* basal diameter = sqrt, grass cover = angular transformation, *E. ebano* chlorophyll content = $\log_{10}$).

For the density experiment, seedling height, basal diameter, chlorophyll content, and percentage plant cover per plot were analyzed using repeated measures (RM) ANOVA as a two-way factorial (fixed effects = seedling density, species planting mix, and their interaction). A two factor ANOVA was performed on February 2020 height data upon inspection of data as it seemed that the RM ANOVA did not detect a large difference in heights among treatments. One high density mixed plot and one high density anaqua plot were removed from the plant cover analysis as they were suspected to be outliers due to uneven herbicide application. Transformations were applied to meet the ANOVA assumptions for the density experiment (height = sqrt, basal diameter = $\log_{10}$, grass cover = angular transformation).

For the nurse plant experiment, seedling height, basal diameter, and chlorophyll content were analyzed using ANOVA as a two way factorial (fixed effects = nurse plant, orientation, and their interaction) separately at each sampling time in an effort to determine if the effect of the nurse plant on the seedlings was changing throughout the course of the experiment. Data were checked for normality and homoscedasticity with Kolmogorov-Smirnov and Levene's tests respectively. Transformations were applied to meet the ANOVA assumptions (height = sqrt, basal diameter = $\log_{10}$). Differences in nurse plant and control (gap) chlorophyll content were taken then Pearson correlation coefficients were calculated between these chlorophyll differences and nurse plant height, diameter at breast height, and LAI.

Data from the germination experiments were analyzed using a two factor ANOVA. One factor was the combination of species and tissue type and the other was concentration. Tissue type had four levels (two species each with roots and leaves), concentration had five levels (0%, 25%, 50%, 75%, and 100%).

E. ebano basal diameter for the nutrient manipulation experiment, and chlorophyll data for the nutrient manipulation experiment failed the Kolmogorov-Smirnov test but were confirmed to be near-normal by analyzing their Q-Q plots. The Satterthwaite adjustment was made to ANOVA's when data was found to be heteroskedastic (Westfall et al 2011). Tukey's post-hoc testing was performed whenever higher order significance was found. Seedling survival is reported as the average per treatment for each experiment. All data were analyzed using SAS University Edition 3.8.

CHAPTER III

RESULTS

Nutrient Manipulation Experiment

Nutrients Sequestered

Average fresh mass of 10 sorghum plants harvested from the eight sorghum plots was equal to 0.547 kg. Average dry mass was 0.168 kg. These values were used to back calculate amount of nutrients extracted per hectare. The four nutrients sequestered by the sorghum pretreatment in the greatest quantities were potassium with 48.09 kg/ha, calcium with 8.87 kg/ha, nitrogen with an average of 24.90 kg/ha, and phosphorus with 8.48 kg/ha removed (Table 2).

Height

Forestiera angustifolia seedlings in sorghum plots were 11% taller than those in control plots in December 2017, two months after planting, (ANOVA $F_{1,123} = 4.94$ $p = 0.0280$) (Fig 1A). Height increased for seedlings over the course of the experiment (RM ANOVA $F_{4,326} = 80.28$ $p = <0.0001$) (Fig 1A). Seedlings which had received starter fertilizer were taller than control seedlings at each sampling time (RM ANOVA $F_{1,117} = 3.98$ $p = 0.0485$).

Ebenopsis ebano seedlings in sorghum plots were 7% taller than those found in control plots during December 2017, two months after planting, (ANOVA $F_{1,123} = 4.26$ $p = 0.0411$). Height increased for all seedlings over the course of the experiment (RM ANOVA $F_{1,124} = 147.52$ $p = <0.0001$) (Fig 1B). The effect seen in December 2017 was lost over the 10 months between the next sampling date. Starting in October 2018 seedlings in all treatments had similar heights and were unaffected by either sorghum or fertilizer treatment (RM ANOVA $F_{1,124} = 0.26$ $p = 0.6139$, $F_{1,124} = 1.92$ $p = 0.1689$ respectively).

Basal Diameter

Forestiera angustifolia seedlings all had similar basal diameters in December 2017, two months after plating, regardless of treatment received (ANOVA $F_{1,122} = 1.84$ $p = 0.1772$ & $F_{1,122} = 1.02$ $p = 0.3139$ for sorghum and fertilizer treatments respectively) (Fig 2A). Basal diameter of *F. angustifolia* seedlings which had received fertilizer were larger than unfertilized seedlings throughout the experiment (RM ANOVA $F_{1,124} = 4.25$ $p = 0.0412$). At the end of the experiment fertilized seedlings had basal diameters 8% larger than unfertilized seedlings. While seedlings in sorghum plots had larger basal diameters than those in control plots, this difference in basal diameter was not significant ($F_{1,124} = 2.28$ $p = 0.1333$).

Ebenopsis ebano seedlings all had similar basal diameters in December 2017, two months after planting, regardless of treatment received (ANOVA $F_{1,123} = 0.50$ $p = 0.4822$ & $F_{1,123} = 0.10$ $p = 0.7498$ for sorghum and fertilizer treatments respectively) (Fig 2B). At the close of the experiment, basal diameter of *E. ebano* seedlings was slightly lower for fertilized seedlings compared to non- fertilized seedlings. This difference however was not significant (RM ANOVA $F_{1,124} = 3.71$ $p = 0.0564$).

Chlorophyll content

Fertilized *F. angustifolia* seedlings had higher chlorophyll content in November 2018 and 2019 (RM ANOVA $F_{1,490} = 3.99$ $p = 0.0464$) (Fig. 3A). In March 2019 unfertilized seedlings in sorghum plots had the highest chlorophyll content confirmed by the significant fertilizer x time interaction term (RM ANOVA $F_{3,490} = 3.37$ $p = 0.0184$) (Fig 9). Seedlings in sorghum plots had lower chlorophyll contents during November 2018 and 2019 but this effect was not significant (RM ANOVA $F_{1,490} = 3.70$ $p = 0.0549$).

Chlorophyll content in *E. ebano* seedlings was not affected by the treatments the seedlings received (RM ANOVA $F_{1,124} = 0.04$ $p = 0.8438$ & $F_{1,124} = 1.03$ $p = 0.3118$ for sorghum and fertilizer treatments respectively). Chlorophyll content in *E. ebano* seedlings varied over time and was highest in November 2018 followed by November 2019 then March and July 2019 (RM ANOVA $F_{3,372} = 2521.53$ $p = <0.0001$) (Fig. 3B).

Plant Cover

Annual cover decreased over time (RM ANOVA $F_{4,120} = 87.20$ $p = <0.0001$) and was not affected by sorghum pretreatment (RM ANOVA $F_{1,30} = 1.57$ $p = 0.2200$) (Fig. 4A). Perennial cover varied throughout the course of the experiment (RM ANOVA $F_{4,120} = 35.15$ $p = <0.0001$).

Perennial cover increased from August 2018 to April 2019 before dropping to starting levels in August 2019 but increasing again in January 2020 (Fig 10B).

Total plant cover increased as time went by (RM ANOVA $F_{4,120} = 223.81$ $p = <0.0001$) (Fig. 4C). Total plant cover was initially higher in control plots and lower in sorghum, but the opposite was true by the end of the experiment (RM ANOVA $F_{4,120} = 3.79$ $p = 0.0100$) (Fig. 4C). There was no difference in total plant cover for sorghum or control plots for each sampling date (RM ANOVA $F_{1,30} = 0.00$ $p = 0.9850$) (Fig. 4D).

Survival

Seedlings of *F. angustifolia* that had received the sorghum pre-treatment had slightly higher initial survival, however overall survival was statistically similar for all treatments (Fig. 5A). *Ebenopsis ebano* survival was unaffected by either sorghum pre-treatment or fertilization treatment (Fig. 5B). Only one individual *E. ebano* seedling died in total which was treated with the sorghum plus fertilizer treatment.

Density Experiment

Height

Forestiera angustifolia seedlings grew taller over time throughout all treatment plots (Fig. 6A) (RM ANOVA $F_{5,325}$= 34.98 p= <0.0001). *Forestiera angustifolia* seedling height was greater in high density plots (Fig. 6A) and was approaching significance (RM ANOVA $F_{1,68}$= 3.22 p= 0.0772). The planting mix did not have a significant effect on *F. angustifolia* height (RM ANOVA $F_{1,68}$= 1.77 p= 0.1878) and *F. angustifolia* seedlings in each treatment plot were similar in height (Fig. 6A). The Density x mix interaction was not significant (RM ANOVA $F_{1,68}$= 0.78 p= 0.3812), however, *F. angustifolia* seedlings in high-density mixed plots were consistently taller than in other treatments (Fig. 6A). Results from the two-way ANOVA on Feb. 2020 were not significant (ANOVA $F_{1,1}$ = 1.98 p = 0.1637 $F_{1,1}$ = 0.02 p = 0.8852 for density and mix respectively).

Ehretia anacua seedling height increased over time throughout all treatment plots (RM ANOVA $F_{1,303}$= 11.70 p= <0.0001). Seedling planting density or mix did not have any significant effects on *E. anacua* seedling height (RM ANOVA $F_{1,63}$= 1.79 p= 0.1855, $F_{1,63}$= 1.04 p= 0.3117 respectively). *Ehretia anacua* seedlings in monospecific low-density plots were consistently the shortest (Fig. 6B) but this difference was not significant (density x species mix RM ANOVA $F_{1,63}$= 1.56 *p*= 0.2161). Results from the two-way ANOVA on Feb. 2020 reveal a density significance for height (ANOVA $F_{1,1}$ = 6.72 *p* = 0.0120 $F_{1,1}$ = 0.70 *p* = 0.4069 for density and mix respectively).

Basal Diameter

Forestiera angustifolia seedling basal diameter also increased over the course of the study (Fig. 2A) (RM ANOVA $F_{5,20.9}$= 53.14 *p*=< 0.0001). Basal diameter of seedlings was significantly greater in high density plots (RM ANOVA $F_{1,18}$ = 8.03 *p* = 0.0110), and greatest in mixed plots (RM ANOVA $F_{1,18}$ = 7.42 *p* = 0.0139), (Fig. 7A).

Ehretia anacua seedling basal diameter increased through each sampling date (Fig. 7B) (RM ANOVA $F_{5,19.4}$= 10.34 *p*= <0.0001). *Ehretia anacua* seedlings in high density plots had larger basal diameters than seedlings in low density plots at each sampling date (Fig. 7B) (RM ANOVA $F_{1,10.3}$= 16.99 *p*= 0.0019). Planting mix did not affect seedling basal diameter in this species (RM ANOVA $F_{1,10.3}$= 0.51 *p*= 0.4914).

Chlorophyll Content

Forestiera angustifolia chlorophyll content varied through sampling dates (Fig. 8A) (RM ANOVA $F_{3,196}$= 42.95 *p*= <0.0001). Chlorophyll content was highest in July 2019 and lowest in March 2019. *F. angustifolia* chlorophyll content did not respond to seedling planting density or to species mix (RM ANOVA $F_{1,68}$= 1.31*p*= 0.2566, RM ANOVA $F_{1,68}$= 1.30 *p*= 0.2580 respectively) (Fig. 8A).

Ehretia anacua chlorophyll content was different through sampling dates (RM ANOVA $F_{5,955}$= 6.15 *p*= 0.0010). Chlorophyll content was highest in November 2018 and lowest in March 2019. *Ehretia anacua* chlorophyll content was consistently higher in high-density plots than in low density plots (Fig. 8B) (RM ANOVA $F_{1,62}$= 12.25 *p*= 0.0009. The species mix and density x mix interaction were not significant (RM ANOVA $F_{1,62}$= 1.95 *p*= 0.1680, RM ANOVA $F_{5,955}$= 1.89 *p*= 0.1747 respectively) indicating that chlorophyll content in seedlings is not responding to species mix or the density x mix interaction.

Plant Cover

Annual cover was different at each sampling date and generally decreased with the exception of April 2019 (RM ANOVA $F_{5, 80}$ = 35.63 *p* = <0.0001). Planting mix did have a positive effect on annual cover with *E. anacua* planted plots having a higher annual cover specifically in April 2019 (RM ANOVA $F_{2,16}$ = 4.12 *p* = 0.0361) (Fig. 9A). High density plots of the same type all had higher annual cover than their low-density counterparts, but the effect was not enough to be significant (RM ANOVA $F_{1,16}$ = 3.55 *p* = 0.0779).

Perennial cover was low throughout the experiment but generally increased as time went on (RM ANOVA $F_{5,80}$ = 19.66 *p* = <0.0001). Cover in planting mix was slightly higher in high density plots throughout the experiment (RM ANOVA $F_{1,16}$ = 4.50 *p* = 0.0500) with the exception of low-density *F. angustifolia* plots in June 2020, which had a higher cover than the high-density *F. angustifolia* plots (Fig. 9B).

Total Cover increased over the course of the study (RM ANOVA $F_{(5, 80)}$ = 81.84 *p* = <0.0001) (Fig. 9C). Total cover was lower in high density plots (RM ANOVA $F_{(1, 16)}$ = 7.55 *p* = 0.0143). The interaction between density and planting mix was significant (RM ANOVA $F_{(2, 16)}$

= 4.10 $p = 0.0365$) and by the end of the study high-density mixed plots had the lowest total cover while low-density mixed plots had the highest cover.

Survival

Forestiera angustifolia seedlings had a higher survival rate than *E. anacua* seedlings throughout the study in both high- and low-density plots (Fig. 10). Overall seedling survival was highest in low-density monospecific *F. angustifolia* plots with a survival rate of 91.2% and lowest in low-density monospecific *E. anacua* plots with a survival rate of 72.2% (Fig. 10). High-density mixed plots had the second lowest survival rate at 77.7%.

Nurse Plant Experiment

Height

Seedling height increased throughout the study in all treatments. Seedlings on the east side of nurse plants tended to be higher, statistically significant effects were detected in June, September, and December 2019 when the nurse plant x orientation interaction became significant (ANOVA $F_{(1,23)} = 6.13$ $p = 0.0208$, $F_{(1,23)} = 5.75$ $p = 0.0250$, $F_{(1,23)} = 4.52$ $p = 0.0445$ respectively). In June 2019 nurse plant seedlings in the east orientation were 28% taller than seedlings in gaps in the east orientation while seedlings under nurse plants in the west orientation were 32% shorter than seedlings in gaps in the west orientation. In September the east nurse plant seedlings were 25% taller than their gap counterparts and west nurse plant seedlings were 25% shorter than their gap counterparts (Fig. 11A).

Basal Diameter

Seedling basal diameter increased throughout the study in all treatments. Basal diameter of seedlings on the west sides of nurse plants was reduced compared to seedlings in other treatments throughout the experiment. The differences in basal diameter were not significant until September 2019 when the control seedlings were girthier than the nurse plant seedlings (ANOVA $F_{1,23} = 6.75$ $p = 0.0164$). In December basal diameter of nurse plant seedlings were 9% and 44% smaller for the east and west orientations respectively (Fig. 11B) (ANOVA $F_{(1,22)} = 4.41$ $p = 0.0475$). East side seedlings were also girthier than their west counter parts at this time (ANOVA $F_{(1,22)} = 4.79$ $p = 0.0395$).

Chlorophyll Content

Seedling chlorophyll content varied throughout the study with the highest values recorded in December 2019. Seedling chlorophyll content was affected by nurse plant presence only in September and June 2019 when nurse plant seedlings had less chlorophyll than control seedlings (ANOVA $F_{1,23} = 14.29$ $p = 0.0010$, $F_{1,23} = 5.43$ $p = 0.0289$ for September and June respectively) (Fig. 11C). In June 2020 all seedlings in the west orientation had a higher chlorophyll content than those in the east orientation ($F_{(1,23)} = 10.33$ $p = 0.0038$). The differential in chlorophyll content between east seedlings under nurse plants and east seedlings in control plots was slightly positively correlated with the leaf area index (LAI) of the nurse shrub (Pearson correlation coefficient = 0.55586 p = 0.0606 N=12) (Fig. 12A) This correlation was weaker in seedlings in the west orientation (Pearson correlation coefficient = 0.36670 p = 0.0932 N = 22) (Fig. 12B).

Temperature & Light Intensity

In general, air temperature at the soil surface was highest during the month of August and lowest in December and January. Temperature was consistently higher under seedlings planted in gaps when compared to soil temperature under seedlings planted under nurse plants except for the months November 2019 through March 2020 (RM ANOVA $F_{1,26} = 9.06$ $p = 0.0057$) (Fig. 13A). Temperatures for seedlings on the east and west sides of nurse plants were statistically similar throughout the experiment (RM ANOVA $F_{1,26} = 0.36$ $p = 0.5511$) but seedlings on the west side of the nurse plants had slightly higher temperatures (Fig. 13A).

Nurse plants reduced the intensity of light reaching seedlings under their canopies ($F_{1,26} = 5.81$ $p = 0.0233$). This effect was most prevalent during the summer and spring months and disappeared through the winter and fall months (nurse plant*time $F_{11,208} = 3.10$ $p = 0.0007$). Light intensity was lower for seedlings on the east side of the nurse plant (Fig. 13B).

Survival

Survival of *F. angustifolia* seedlings was greatly enhanced for seedlings planted close to the nurse plants compared to seedlings planted in gaps (Fig. 14). Survival of seedlings in gaps dropped to 60% while survival close nurse plants was maintained at 87.5% and 100% for the east and west orientations respectively.

Allelopathy Experiment

Leaf extract from *V. schaffneri* leaves was the only extract to have a significant effect on *S. bicolor* germination, mean germination time, and coefficient of velocity of germination. Germinability in the 100% extract was only 88% while germinability with all other extracts were

95-96% which were consistent with controls. Mean germination time increases slightly when any extract is used in place of pure water but *V. schaffneri* leaf extract is the only extract in which the control is placed in a different Tukey grouping than the higher concentrations (Fig 15, Table 3). A similar trend was detected for the coefficient of velocity of germination (Table 3).

Results from the allelochemical polarity experiment suggest that the allelochemical affecting the germination process is only soluble in water and not soluble in the DCM or the diethyl ether solvents as both of these have similar cumulative germination to the complete extract (Fig. 16). Germinability, MGT, and CVG are similar for all *V. schaffneri* extracts except for the ether fractioned extract (Table 4).

CHAPTER IV

DISCUSSION

Nutrient Manipulation

Invasive plants, including invasive grasses, are known to compete and perform better under conditions with high soil nutrients (Rickey & Anderson 2004, Gurevitch et al 2008, and Leishman & Thomson 2005). Total plant cover included invasive grass cover in great proportions and was not affected by sorghum pretreatment in this study. Other studies which have used nutrient sequestration as a tool for invasive species management report much higher rates of nutrient removal (Török et al 2000, Blumenthal et al 2003). Total plant cover (comprised of a large proportion of invasive grass cover) was not affected by sorghum treatment which points to the possibility that not enough nutrients were sequestered. Other studies have used much higher seeding densities (3000 seeds/m^2 compared to 600 seeds/m^2 used in this study) to remove nutrients from the soil (Herron et al 2001) or added carbon to sequester (i.e. immobilize) soil nutrients (Blumenthal et al 2003, Morghan & Seastedt 1999). It would be worthwhile to conduct specific pot or lab experiments to further understand the relationship between problem invasive grasses in the LRGV and soil nutrients availability to acertain if nutrient sequestration is a viable restoration technique. *Ebenopsis ebano* (a leguminous plant) did not respond to either the sorghum or fertilizer treatment which is to be expected as it is a leguminous plant able to obtain nitrogen from symbiotic bacteria and rely less on soil nitrogen reserves. *Forestiera angustifolia* did respond to fertilizer addition resulting in taller seedlings with larger basal diameters and increased chlorophyll content. Marler et al (2001) show increased growth, higher numbers of stems, more leaves, and root:shoot ratios with fertilization of non-leguminous woody species in the Salicaceae family. This is similar to the increased growth of the non-leguminous *F. angustifolia* seedlings (Oleaceae family) used in this study. *F. angustifolia* seedlings in sorghum plots were slightly larger in size than seedlings in control plots which could suggest that they were better able to compete with neighboring plants, likely due in part to the localized fertilizer application and a slightly lower plant cover. This difference was not significant, however, which could be due to the fact that only one series of pretreatment nutrient-sequestering plants (sorghum) was used at a low seeding rate. In Herron et al (2001) nutrient removal with annual rye (*Secale cereale*) (seeded at high density) shifted the competitive balance away from spotted knapweed (*Centaurea maculosa*), an invasive plant, towards the native bluebunch wheatgrass (*Pseudoroegneria spicatum*). In the future either a higher seeding density or two seeding series of sorghum should be tested to determine effects of greater nutrient removal on *M. maximus, D. annulatum,* and *C. ciliaris* in the LRGV. Survival was similar in all treatments for both *E. ebano* and *F. angustifolia* seedlings, but sorghum treated *F. angustifolia* seedlings had slightly higher survival. The use of one sorghum pretreatment did not have negative effects on the seedlings and resulted in taller seedlings only on the first sampling date which further supports the hypothesis that more nutrients need to be extracted for more marked positive effects.

High-Density Planting

High-density planting has been used in various restoration sites across the world with forest systems being rehabilitated in as little as 20 – 30 years with high success rate when natural succession would have taken much longer (Miyawaki 1999). The method has been shown to be successful in several climates and for different vegetation types (Fujiwara et al 1993, Miyawaki & Golley 1993, Miyawaki 1999 and references therein, Schirone et al 2011), plant biodiversity is higher and evolving plant communities are sustained without further operative support after planting. In this study, seedling survival and growth, as well as plant stress (with chlorophyll content as a proxy) and colonizing herbaceous plant cover, were the main response variables following seedling transplantation. Seedling growth is typically rapid in Miyawaki-type restorations and forests resembling natural forests can be reconstructed in as little as 20 years (Miyawaki & Golley 1993, Miyawaki 1999). In this study *E. anacua* and *F. angustifolia* seedlings showed different responses to the high density and planting mix treatments. Height of both *E. anacua* and *F. angustifolia* seedlings were generally unaffected by planting density alone but *E. anacua* seedlings in low density monospecific plots were much shorter than seedlings in any other treatment. Also, *E. anacua* seedlings planted at high density in a mix of species grew consistently taller. A closer look at the last data set also revealed that *E. anacua* seedlings in high density plantings grew taller than their low-density counterparts. Basal diameters of for both E. anacua and F. angustifolia seedlings were greater in high density plots. These observations suggest that high-density planting was beneficial to both of these species, similar to what has been reported when the Miyawaki method has been applied with high growth under high-density conditions (Miyawaki 1999 and references therein). Callaway (1997) discusses how positive plant interactions are common and become more important as abiotic stress increases. Both *F. angustifolia* and *E. anacua* seem to perform better in terms of growth when placed in high-density mixed plantings where interspecific interactions can occur. Chlorophyll content for *E. anacua* seedlings was much greater in high density plots which suggests that seedlings in high density plots were less stressed when compared to seedlings in low density plots. There are many studies showing that interspecific positive plant interactions are common and become increasingly important as abiotic conditions become more severe (Choler et al 2001, Maestre et al 2001, Tewksbury & Lloyd 2001, Flores & Jurado 2003, & Callaway et al 2002) which could be an important interaction in the harsh LRGV climate. Interestingly the chlorophyll content for *F. angustifolia* was higher for the high-density treatment but only when planted in mixed plots. This supports our hypothesis that positive interactions between different species can be an important factor in thorn scrub and thorn forest restoration. Positive plant interactions are common and Atala et al (2019) also show a more suitable physiological state of native plants under *Deschampsia antarctica* (a native in Antarctica). Monospecific stands of *F. angustifolia* seedlings showed lower chlorophyll contents and were presumably more stressed as stressed plants have lower chlorophyll contents (Taïbi et al 2016). This could be due to allelopathy and/orcompetition. *F. angustifolia* was chosen for this experiment as a suspected allelopathic plant. Allelopathic plants have been shown to have negative intraspecific effects on growth of seedlings in their vicinity (Groner 1974). It could be possible that high density planting of *F. angustifolia* is causing an accumulation of allelochemicals in the soil which have a negative effect on seedling physiology. Another possibility is that *F. angustifolia* seedlings are competing for resources with adjacent individuals of the same species. Total cover (a large portion of which was comprised of invasive grasses) was lowest in high density mixed and *F. angustifolia* monospecific plots. Because *M. maximus* responds to increasing functional diversity with lower reproductive tiller formation (Ammondt & Litton 2012), we expected invasive grass cover to be lowest in mixed plots. Total cover was lowest in high density mixed plots suggesting that species and functional diversity do negatively affect invasive grass cover. *Forestiera angustifolia* was chosen for this experiment specifically for its apparent allelopathic activity. *Forestiera* *angustifolia* plots were the 2nd lowest in total cover supporting this hypothesis. Perennial cover did not vary much throughout the treatments but looking at the last sampling date, it seems as if *E. anacua* plots will have more perennial cover in the near future compared to plots which received other treatments. Survival was high, above 70% by the end of the study in all plots. High density *E. anacua*, *F. angustifolia*, and low-density *F. angustifolia* plots all had similar survival rates close to 90% in January 2020. High-density mixed plots had lower survival which was mostly due to poor survival of *Celtis laevigata* Willd. and *Randia rhagocarpa* Standl.. Low density *E. anacua* plots had the lowest survival at 72%. Survival of thorn scrub and thorn forest seedlings used in the LRGV vary between 60%-75% without any planting treatments like tubes or shading (Alexander et al 2016, Fulbright et al 1992). These results are promising for application in the LRGV as the lowest survival in a high-density treatment is at 80% and growth parameters are improved under these planting conditions.

Nurse plants

In the past, restoration efforts in areas characterized by low rainfall and harsh abiotic conditions have involved removing shrubs as they have been seen as competitors to seedlings being planted (Mesón and Montoya 1993, García-Salmerón 1995, Savill et al. 1997). Recent research has focused on how shrubs and other types of plants can be used as nurse plants which improve survival and growth characteristics of seedlings (Gómez-Aparicio et al 2004, Valiente-Banuet & Ezcurra 1991, Groenveld et al 2007, Yelenik et al 2015). In this study use of the nurse shrub *B.* neglecta resulted in higher survival and higher chlorophyll content of *F. angustifolia* seedlings (with increasing LAI), indicative of its facilitative effects. While height of seedlings on the east side of nurse plants was greater through the study, the height was not much greater than seedlings in control groups. In addition to this, seedlings on the west side of nurse plants were shorter than any other treatment group. Similar trends are seen in the basal diameter of seedlings with seedlings on the west sides of nurse plants showing the lowest basal diameters while the rest of the treatment groups had similar basal diameters. Chlorophyll content was consistently lower for seedlings under nurse plant canopies however there was a positive trend with increasing LAI of nurse plants. Findings from Franco and Nobel (1989) along with findings from this study suggest that the use of nurse plants facilitate survival but may reduce growth rates of seedlings. Gómez-Aparicio (2009) state that neighboring plants tend to have negative to neutral effects on growth and positive effects on survival of their neighboring plants. In addition, shrubs more often had positive effects on neighboring plants when compared to trees or herbs. Nurse plants can offer facilitative effects due to a variety of reasons buffering of extreme temperatures and shading from solar radiation (Ren et al 2008). In this study air temperatures at the soil level and light intensity were greater in gaps than under nurse plant canopies especially during August, the warmest month of the year. This could contribute to the facilitative effects *B. neglecta* had on *F. angustifolia* seedlings, increasing their survival. Although *B. neglecta* had a facilitative effect on survival and physiological status of nurse seedlings associated with the nurse plants, competitive effects seem to account for the reduction in growth parameters. Future studies should focus on the distance between nurse plant and nurse seedling to find the optimal distance where the balance between facilitation and competition is most favorable for the seedlings. Other native species of shrubs but also of large annual and perennial herbaceous are worth investigating to generate options applicable to various conditions throughout south Texas. Nurse plants have been used to ameliorate stressful conditions for seedlings in forest restoration in dry sunny areas

(Gómez-Aparicio et al 2004, Gómez-Aparicio 2009, Groeneveld et al 2007) and may be a useful tool in south Texas for restoration of thorn scrub and thorn forests.

Allelopathy of *Vachellia schaffneri*

Extracts made from leaves of *V. schaffneri* clearly show inhibitive effects on the germination process of the domesticated African grass *S. bicolor*. Total germination is reduced only with the leaf extract of *V. schaffneri* and not with its root extract or with the leaf or root extract of *Vachellia farnesiana*, a closely related tree. *Mikania micrantha* Kunth is a vine which has also been to have allelopathic properties in its leaves (Wu et al 2009). *Mikania micrantha* allelopathy increased with extract concentration and also showed higher levels of allelopathy in certain tissues. With *V. shaffneri,* germination, mean germination time (MGT), and coefficient of velocity of germination of *S. bicolor* were all reduced with increasing concentration of extracts. This would result in fewer plants able to germinate and establish quickly after sporadic rain events. The ether extract had lower MGT and higher CVG, however this difference was not noted in the DCM fractioned extract. The DCM fraction was removed after the ether fraction so it is unlikely that this difference was due to chemicals removed by the ether and may be an effect of residual ether in the assay. The allelochemical was shown to only be soluble in the aqueous extract and not soluble in DCM or diethyl ether. The allelochemical must then be a compound with relatively high polarity. Legumes produce a suite of secondary metabolites which can be affect their neighboring plants (Yoneyama et al 2010). Many phenolic compounds are soluble in water and are known allelochemicals (Yoneyama et al 2010). It is possible that the allelochemical can be a phenolic compound which are soluble in water. Further testing must be performed to determine exact chemical responsible for the interaction. Germination was lower for diethyl ether extracted *V. farnesiana* extracts but this is likely an error in preparation of plates or residual diethyl ether as a lower germination would mean presence of more allelochemical which is not possible through the liquid-liquid extraction performed. Although bioassays are insightful, field tests and assays which test allelopathy and viability of allelochemicals in the field are necessary for the study of allelopathic plants (Inderjit & Nilson 2003). If further studies are conducted and *V. schaffneri* or other native species are found to be allelopathic then the "Homeland security hypothesis" can be tested to see if invasive grass cover decreases with increasing density of native allelopathic plants used in restoration (Cummings et al 2012). If native plants are used to subdue invasive species, then natural community dynamics which lead to succession of native species could be restored in disturbed areas.

Recommendations for Restoration

Invasive grasses presence is a major obstacle to thorn scrub and thorn forest restoration in the LRGV; successful restoration efforts must include measures to reduce presence of invasive grasses and problems associated with their presence. High-density planting of multiple thorn scrub and thorn forest species should be considered to reduce impacts from invasive grasses as cover of invasive grasses is reduced by this planting method. In addition to the benefits of decreased invasive grass cover, high-density planting resulted in increased growth of the two studied thorn scrub and thorn forest species in this study. Very large restoration plots with high-density seedling planting may quickly become expensive, but smaller irregularly spaced tracts for high-density planting may be more cost effective. Planting small patches of land with high seedling density could result in a more successful restoration with higher survival and growth of seedlings. These small pockets of vegetation could result in the recovery of forest function much faster than low-density planting of seedlings. Nurse shrubs increase seedling survival and should be used in restoration in the LRGV. “Weedy”, early succession native trees and shrubs may improve micro-climatic conditions and should not be removed prior to revegetation with seedlings. Fertilization with a starter fertilizer should be considered at least for non-leguminous seedlings as it may improve growth, physiological status, and survival of transplanted seedlings. A combined approach of localized starter fertilization of non-leguminous seedlings, along with high-density planting has the potential to increase restoration success in the LRGV.

Future Studies

Effects of nutrient sequestration at greater rates than reported in this study should be determined on invasive grasses. Methods for reducing nutrient loads in invasive infested soils may be a higher seeding density of nutrient-sequestering plants, or addition of carbon to increase microbial activity and lower available nitrogen. Long-term monitoring of high-density plots should be performed so that the effects of high-density planting can be evaluated on a long-term basis. We expect effects of high-density planting on invasive grass cover to intensify as the canopies of the seedlings begin to overlap. As canopy cover of seedlings closes and excludes invasive grasses, natural successional processes may be restored. Different nurse shrubs/trees should be identified and optimal distances from these nurse plants should be determined. Fertilization of different woody species used for restoration in the LRGV should be explored and the effect of fertilization on growth and survival of multiple thorn scrub and thorn forest species should be quantified.

Conclusions

Several factors influence the outcome of restoration efforts in the LRGV including abiotic factors such as low rainfall, high solar radiation, and high temperatures, as well as biotic factors like invasive species. High density planting is a promising approach which could lead to restored plant communities reaching climax much sooner than currents reforestation protocols and natural succession could allow. In this short-term monitoring of high-density plantings, plant growth of *F. angustifolia* and *E. anacua* is increased relative to standard or low-density plantings and survival is also increased. Nurse plants can be used to increase survival of thorn scrub and thorn forest seedlings, and more experimentation should be conducted to identify more species of suitable nurse plants along with suitable species to be used in restoration in association with nurse plants. Growth and plant stress of *F. angustifolia* are only slightly inhibited by *B. neglecta* and were neutral for most parts of the year. The use of *S. bicolor* as a means of reducing soil nutrients had the did not have any effect on total cover in this study and should be attempted again either at higher density or with more than one addition of sorghum. Nutrient sequestration did not have any effect on *E. ebano* seedlings but did result in taller *F. angustifolia* seedlings only for the first sampling date. Fertilization of seedlings upon planting caused an increase in the basal diameter, height, and resulted in higher chlorophyll content levels in the non-leguminous *F. angustifolia.* This points to the conclusion that localized fertilization of non-leguminous seedlings could result in a more successful restoration. It seems apparent that *V. schaffneri* produces allelochemicals which negatively impact germination of *S. bicolor*, an African grass. More germination assays and field studies should be conducted to determine if these allelopathic chemicals affect the invasive grasses present in the RGV and if this plant and other native plants should be used in future restoration efforts.

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APPENDIX

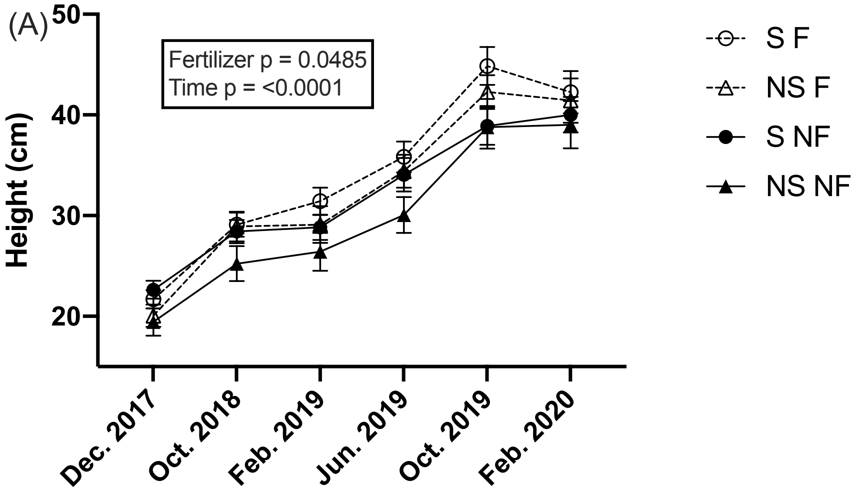
APPENDIX

Table 1. Common name, scientific name, and growth habit of species selected for the density planting experiment.

Common name	Scientific Name	Growth habit
Elbow bush	*Forestiera angustifolia*	Shrub
Anacua	*Ehretia anacua*	Tree
Sabal palm	*Sabal mexicana*	Tree
Texas ebony	*Ebenopsis ebano*	Tree
Crucillo	*Randia rhagocarpa*	Shrub
Palo blanco	*Celtis laevigata*	Tree
Huisache	*Vachellia farnesiana*	Shrub/small tree
Tenaza	*Havardia pallens*	Shrub/ small tree
Tepehuaje	*Luecaena pulverulenta*	Tree

Table 2. Average amount of nutrients sequestered by sorghum in the nutrient manipulation experiment.

Element	g Removed Per Plot	Kg Removed Per Ha
Nitrogen	56.02	14.0
Phosphorus	19.08	4.76
Potassium	108.20	27.05
Calcium	19.95	4.98
Magnesium	9.67	2.42
Sodium	0.27	0.07
Zinc	0.20	0.05
Iron	0.91	0.23
Copper	0.04	0.01
Manganese	0.14	0.04
Sulfur	6.15	1.54
Boron	0.04	0.01



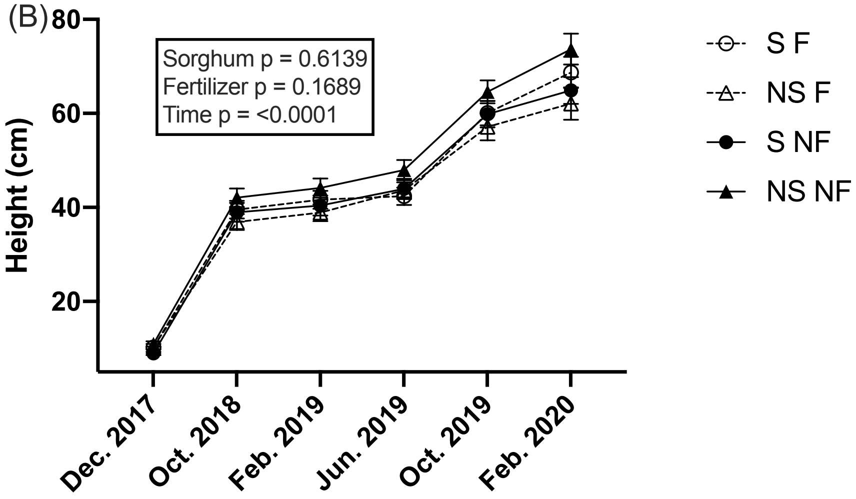


Fig. 1 Mean height of seedlings in cm at each sampling date for *F. angustifolia* (A) and *E. ebano* (B). Seedlings were planted in plots with a pre treatement of either sorghum or control (no sorghum planted). Plots were then divided into subplots where one subplot received a fertilizer application and the other subplot received a water control. Error bars show SEM for each treatment.

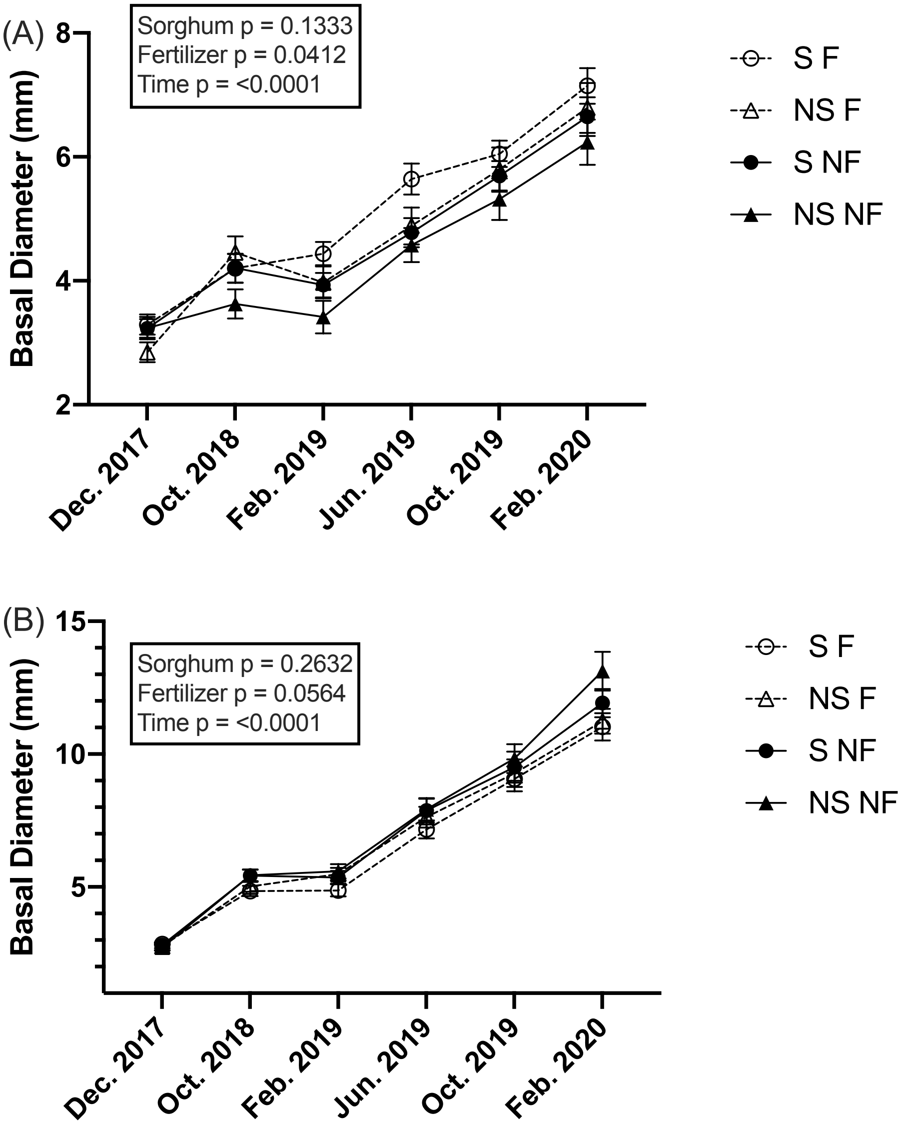


Fig. 2 Mean basal diameter of seedlings in mm at each sampling date for *F. angustifolia* (A) and *E. ebano* (B). Seedlings were planted in plots with a pre treatement of either sorghum or control (no sorghum planted). Plots were then divided into subplots where one subplot received a fertilizer application and the other subplot received a water control. Error bars show SEM for each treatment.

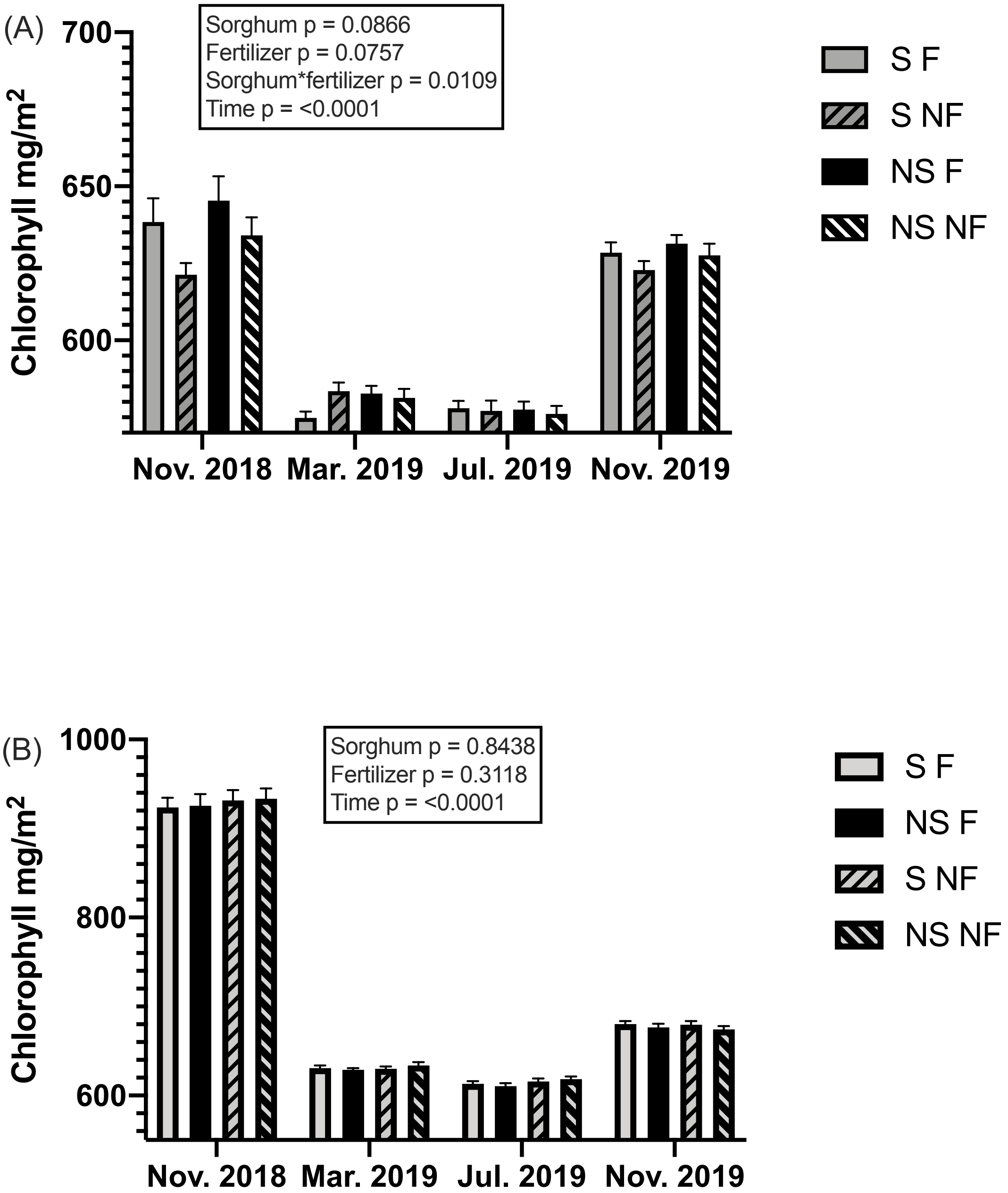


Fig. 3 Mean chlorophyll content of seedlings at each sampling date for *F. angustifolia* (A) and *E. ebano* (B). Seedlings were planted in plots with a pre treatement of either sorghum or control (no sorghum planted). Plots were then divided into subplots where one subplot received a fertilizer application and the other subplot received a water control. Error bars show SEM for each treatment.

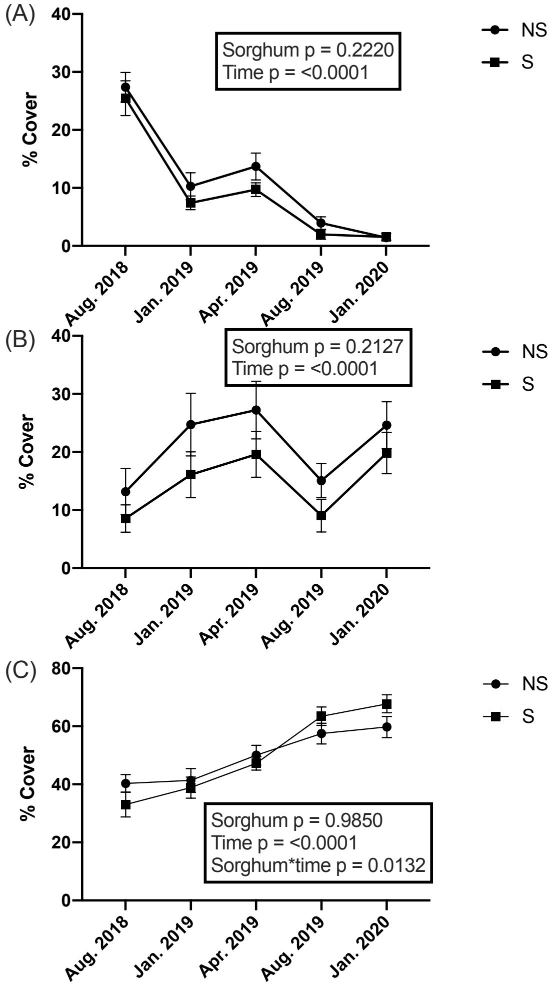


Fig 4 (A) Annual Cover, (B) Perennial Cover and (C) Total Cover of plants in sorghum treated and control plots. Sorghum treated plots were first seeded with sorghum then sorghum was harvested in order to sequester nutrients from the soil. Error bars show SEM for each treatment.

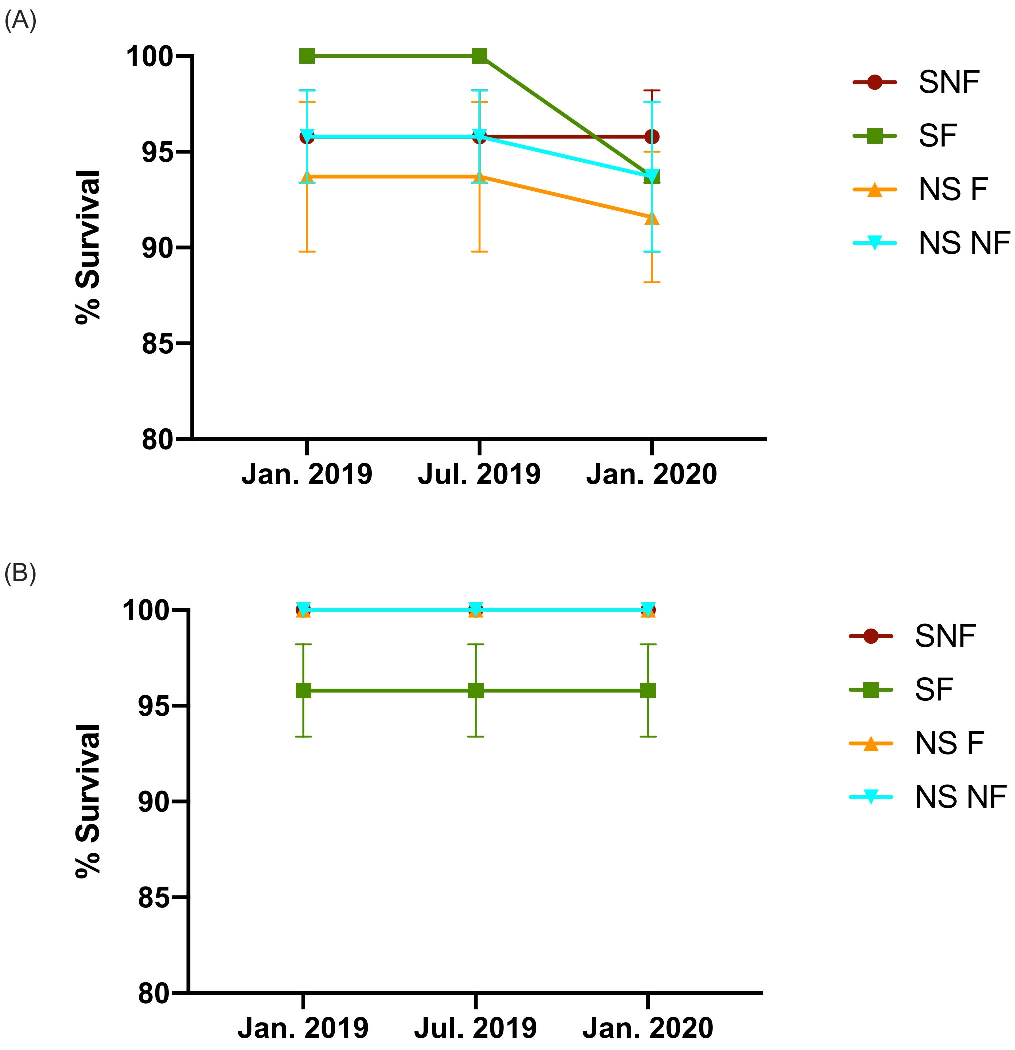


Fig 5 (A) Average survival of *F. angustifolia* and (B) *E. ebano* seedlings in each treatment of the nutrient manipulation experiment. Seedlings were planted in plots with a pre treatement of either sorghum or control (no sorghum planted). Plots were then divided into subplots where one subplot received a fertilizer application and the other subplot received a water control. Each subplot contained 12 seedlings. Error bars show SEM for each treatment. n = 4

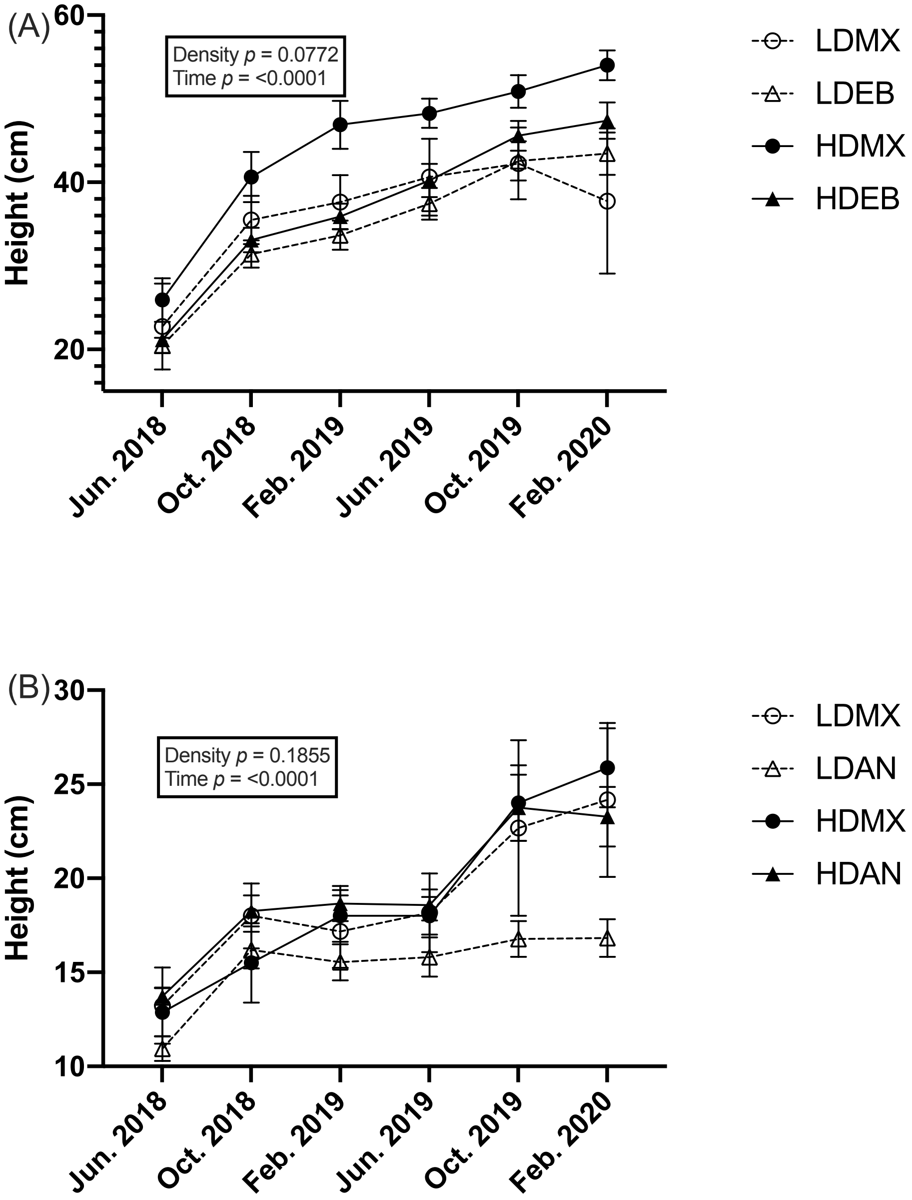


Fig. 6 Mean height in cm at each sampling date for *F. angustifolia* (A) and *E. anacua* (B). Seedlings were planted at low (2,571 seedlings/ha) and high (15,428 seedlings/ha) densities. Seedlings were planted in three different mixes monospecific *F. angustifolia*, monospecific *E. anacua*, and a mix composed of nine foundation species. Error bars show SEM for each treatment.

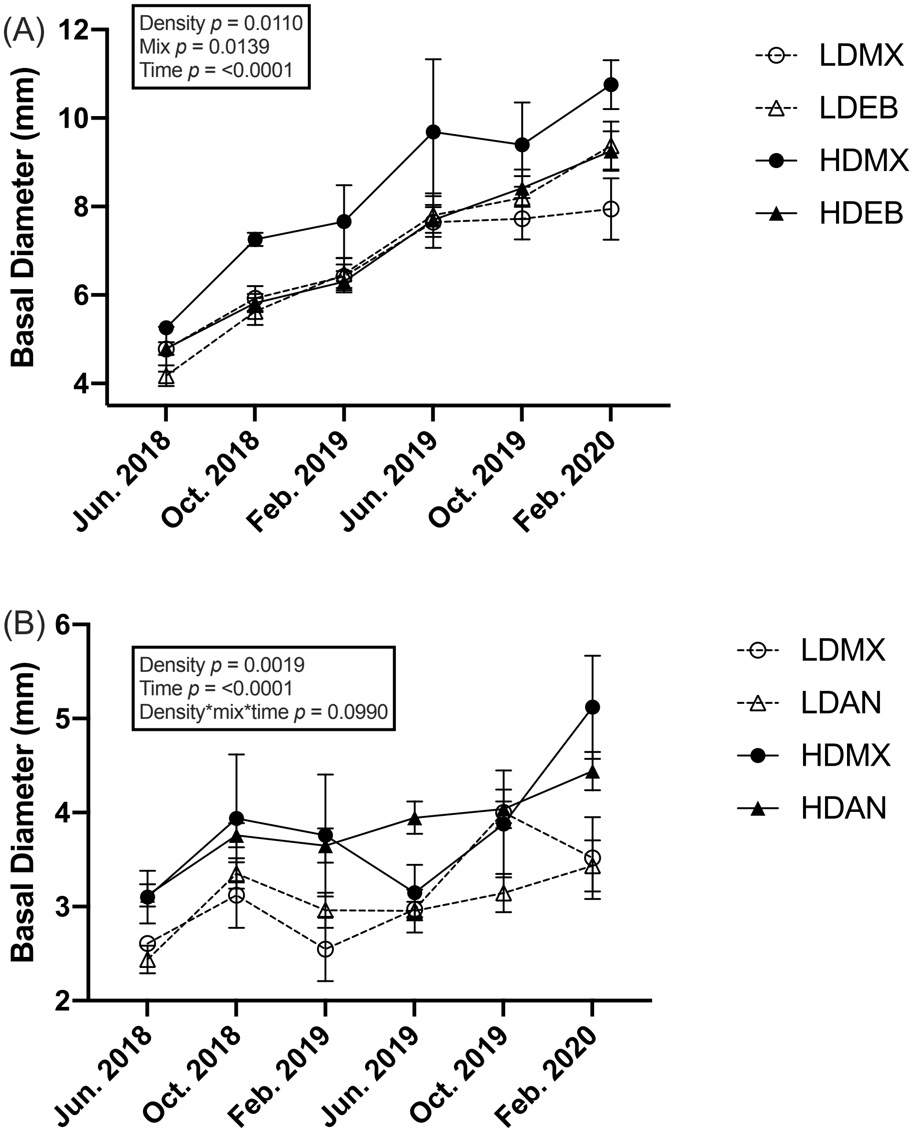


Fig. 7 Mean basal diameter in mm at each sampling date for *F. angustifolia* (A) and *E. anacua* (B). Seedlings were planted at low (2,571 seedlings/ha) and high (15,428 seedlings/ha) densities. Seedlings were planted in three different mixes monospecific *F. angustifolia*, monospecific *E. anacua*, and a mix composed of nine foundation species. Error bars show SEM for each treatment.

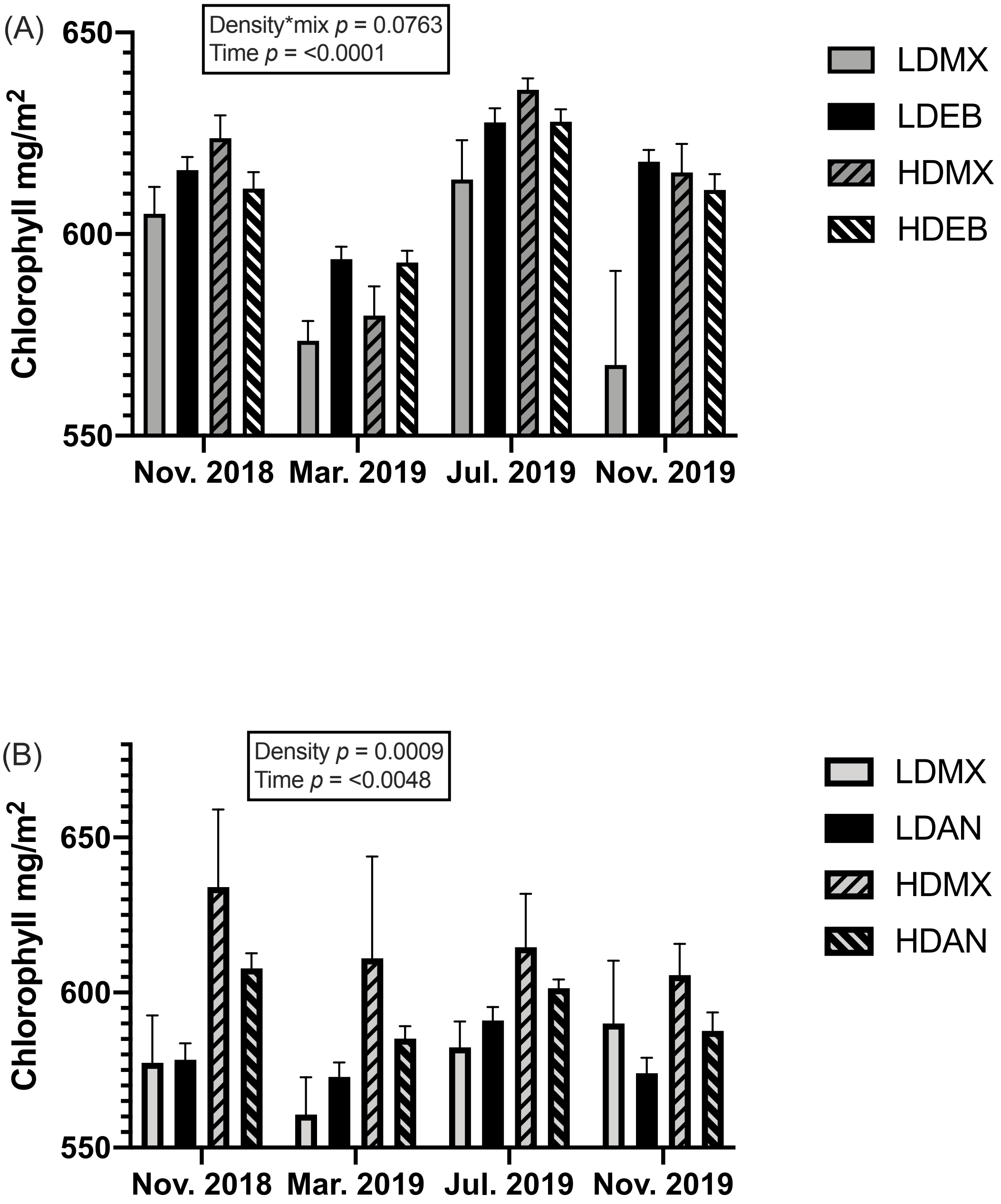


Fig. 8 Mean chlorophyll content at each sampling date for *F. angustifolia* (A) and *E. anacua* (B). Seedlings were planted at low (2,571 seedlings/ha) and high (15,428 seedlings/ha) densities. Seedlings were planted in three different mixes monospecific *F. angustifolia*, monospecific *E. anacua*, and a mix composed of nine foundation species. Error bars show SEM for each treatment.

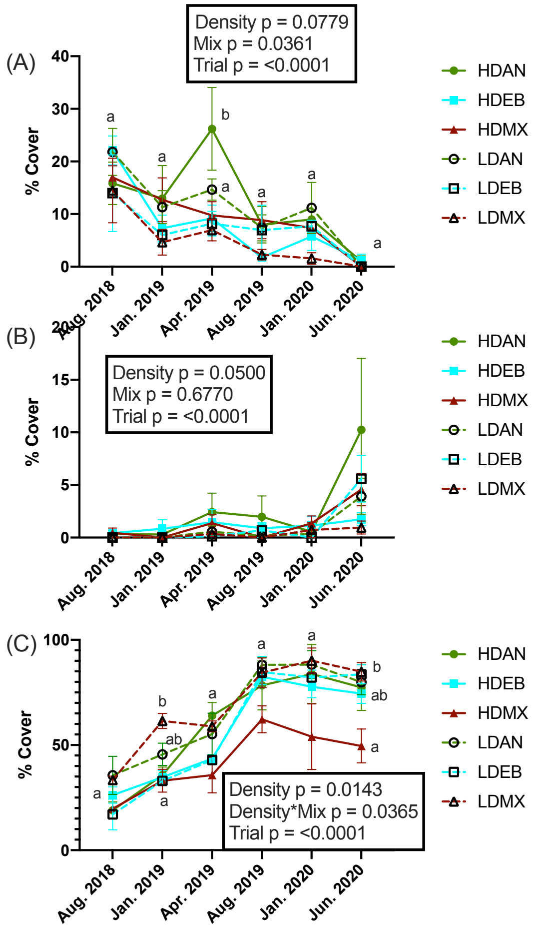


Fig 9 (A) Annual Cover, (B) Perennial Cover and (C) Total Cover of plants in the density experiment. Seedlings were planted at low (2,571 seedlings/ha) and high (15,428 seedlings/ha) densities. Seedlings were planted in three different mixes monospecific *F. angustifolia*, monospecific *E. anacua*, and a mix composed of nine foundation species. Post hoc tukey tests were performed for each sampling time separately and results are indicated by lower case letters. Error bars show SEM for each treatment.

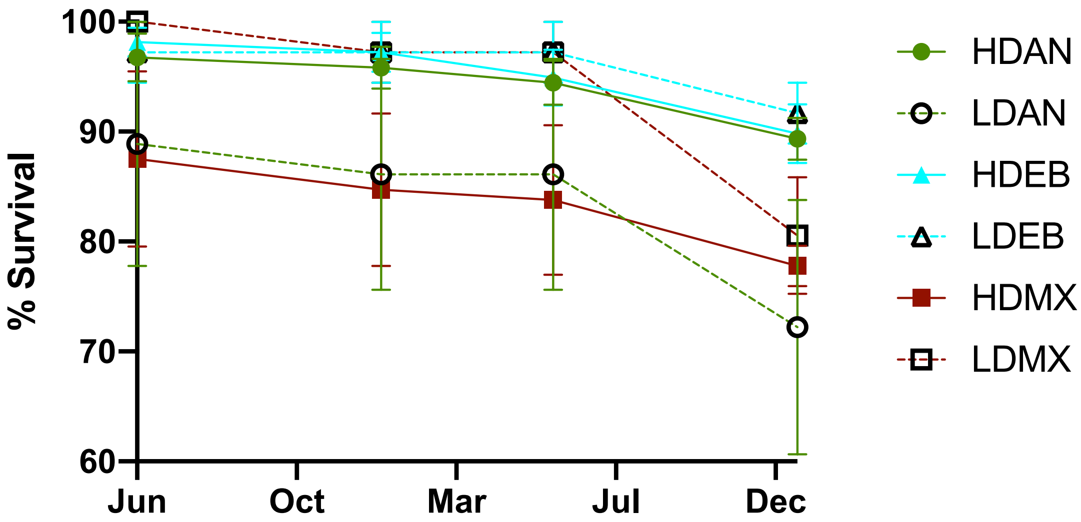


Fig. 10 Total seedling survival per plot type. Seedlings were planted at low (2,571 seedlings/ha) and high (15,428 seedlings/ha) densities. Seedlings were planted in three different mixes monospecific *F. angustifolia*, monospecific *E. anacua*, and a mix composed of nine foundation species. Each high-density plot contained 54 seedlings each low-density plot contained 9 seedlings. Error bars show SEM for each treatment. n = 4

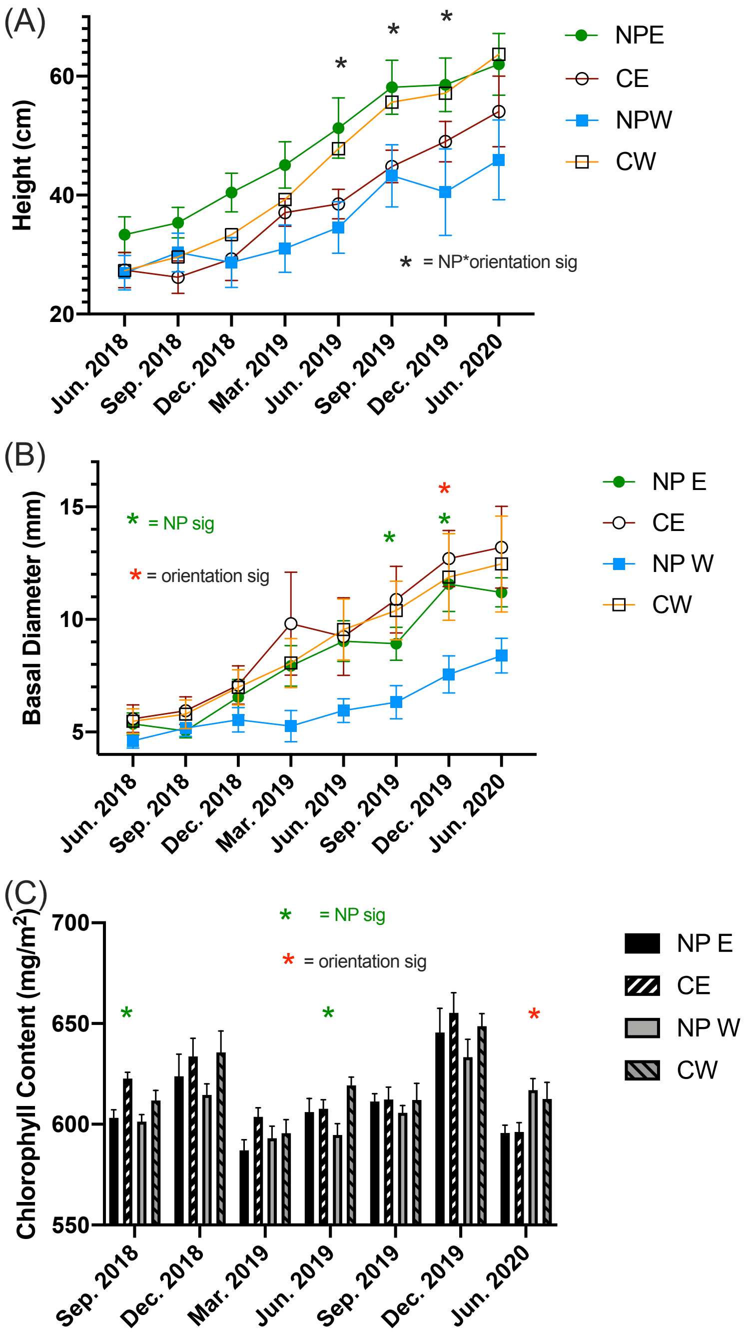


Fig. 11 Mean height (A), basal diameter (B), and chlorophyll content (C) of *F. angustifolia* seedlings. Seedlings were planted 20cm to the east and west of a *B. neglecta* nurse plant or in open gaps 20cm to the east and west of a marker. Red asterisks signify orientation was significant green signify nurse plant significance and black asterisks signify nurse plant*orientation significance. Error bars show SEM for each treatment.

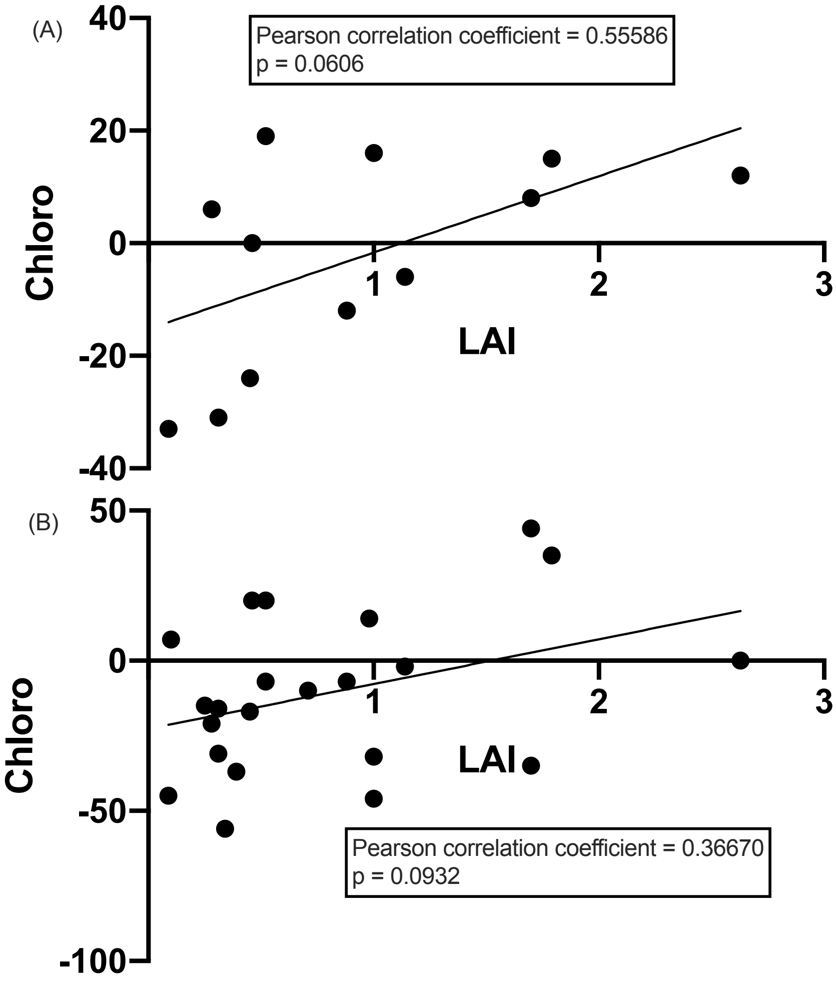


Fig 12 Chlorophyll content (Chloro) versus leaf area index (LAI) with regression lines and pearson coefficients for (A) east nurse plant seedlings (n=12) and (B) west nurse plant seedlings (n= 22).

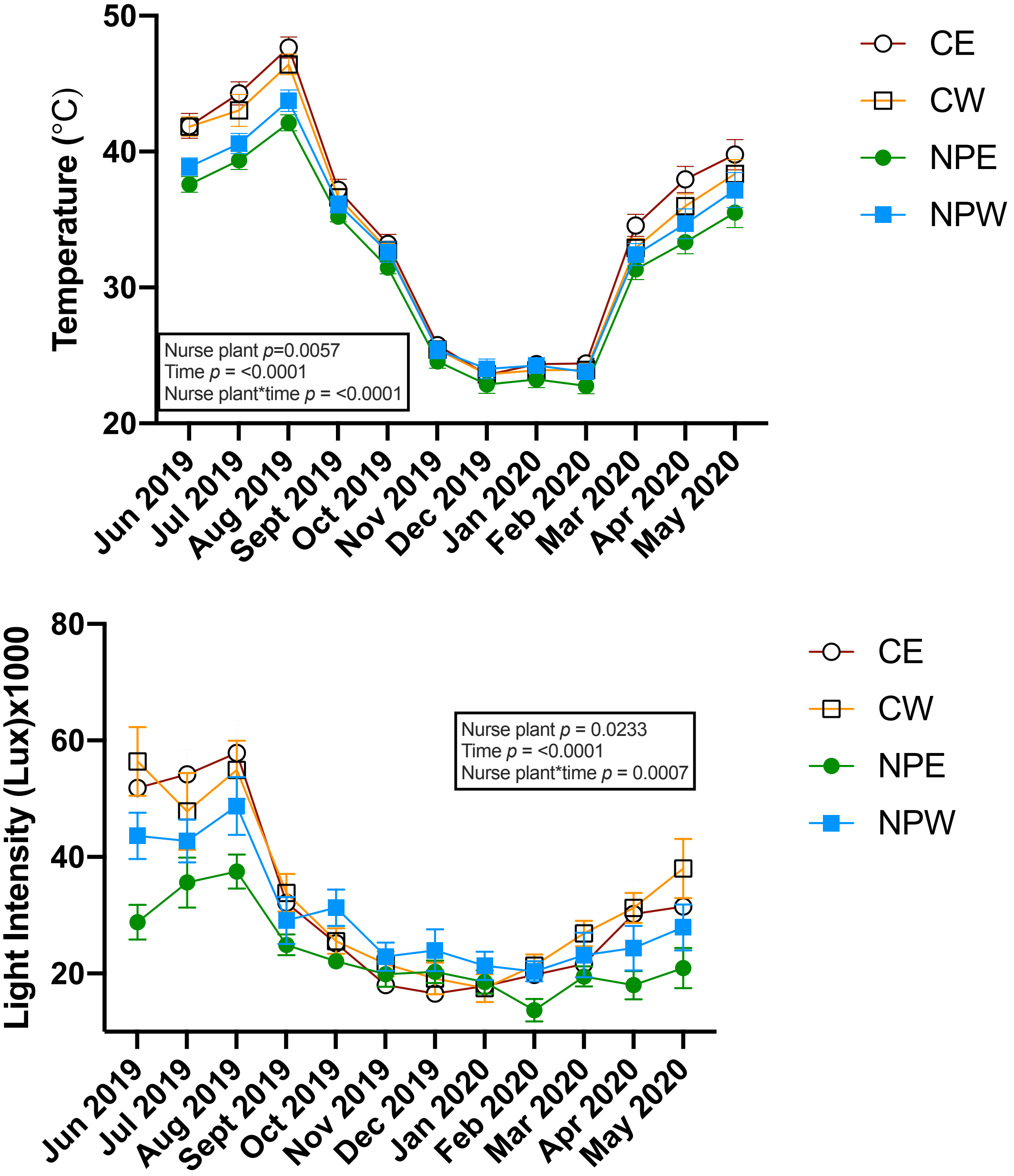


Fig 13 (A) Air temperature at the soil surface and (B) light intensity under *F. angustifolia* seedlings. Seedlings were planted 20cm to the east and west of nurse shrubs *B. neglecta*. Error bars show SEM for each treatment.

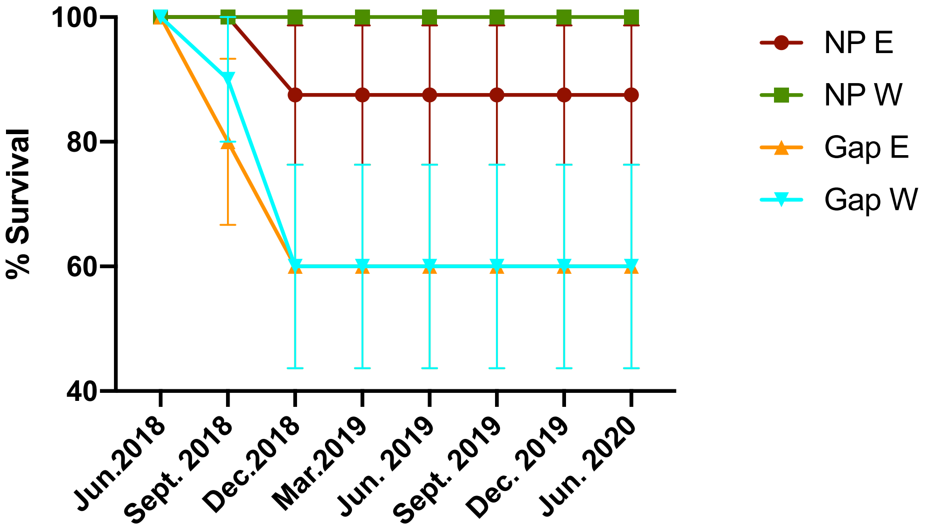


Fig 14 Survival of *F. angustifolia* seedlings in the nurse plant experiment. Seedlings were planted either with a nurse plant or in a gap (NP or Gap) and either to the east or west of the nurse plant or marker (E or W). n = 10

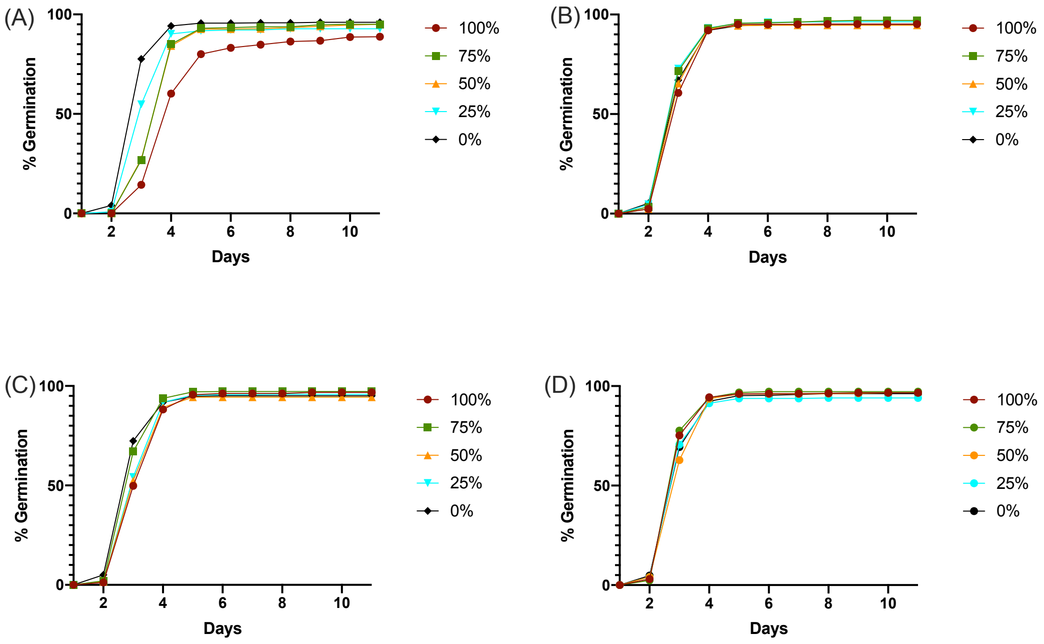


Fig 15 Cumulative germination plots of *S. bicolor* seeds when exposed to 100%, 75%, 50%, 25%, and 0% (Control) extracts of (A) *V. schaffneri* leaves, (B) *V. farnesiana* roots, (C) *V. farnesiana* leaves, and (D) *V. schaffneri roots.*

Table 3. Parameters of the germination process of *S. bicolor* as affected by various concentrations of plant tissue extracts. VSL = *V. shaffneri* leaf extract, VFL = *V. farnesiana* leaf extract, VSR = *V. shaffneri* root extract, VFR = *V. farnesiana* root extract, MGT = Mean germination time, CVG = coefficient of velocity of germination. Lower case letters show Tukey-Kramer post hoc groupings.

Extract	Concentration	Germinability^{b}	MGT^{a}	CVG^{b}
VSL	100%	88.8^{b}	4.45^{a}	23.03^{c}
	75%	$95.0^{a,b}$	$3.89^{a,b,c}$	$25.77^{b,c}$
	50%	$95.0^{a,b}$	$3.94^{b,a}$	$25.44^{b,c}$
	25%	$92.8^{a,b}$	$3.45^{b,c,d}$	$29.12^{a,b}$
	0%	$96.0^{a,b}$	3.18^{d}	31.44^{a}
		Germinability^{a,b}	MGT^{b}	CVG^{a}
VFL	100%	96.4^{a}	$3.60^{b,c,d}$	$27.88^{a,b}$
	75%	96.8^{a}	$3.33^{c,d}$	30.09^{a}
	50%	$94.4^{a,b}$	$3.48^{b,c,d}$	$28.76^{a,b}$
	25%	$95.8^{a,b}$	$3.47^{b,c,d}$	$28.86^{a,b}$
	0%	$95.0^{a,b}$	3.22^{d}	31.09^{a}
		Germinability^{a}	MGT^{b}	CVG^{a}
VSR	100%	96.6^{a}	3.23^{d}	31.04^{a}
	75%	97.2^{a}	3.21^{d}	31.13^{a}
	50%	96.6^{a}	$3.37^{c,d}$	29.80^{a}
	25%	$94.0^{a,b}$	3.24^{d}	30.88^{a}
	0%	$96.2^{a,b}$	3.29^{d}	30.45^{a}
		Germinability^{a,b}	MGT^{b}	CVG^{a}
VFR	100%	$95.2^{a,b}$	$3.38^{b,c,d}$	29.66^{a}
	75%	97.0^{a}	3.30^{d}	30.42^{a}
	50%	$94.6^{a,b}$	3.31^{d}	30.31^{a}
	25%	$96.2^{a,b}$	3.24^{d}	30.89^{a}
	0%	$93.6^{a,b}$	3.28^{d}	30.59^{a}

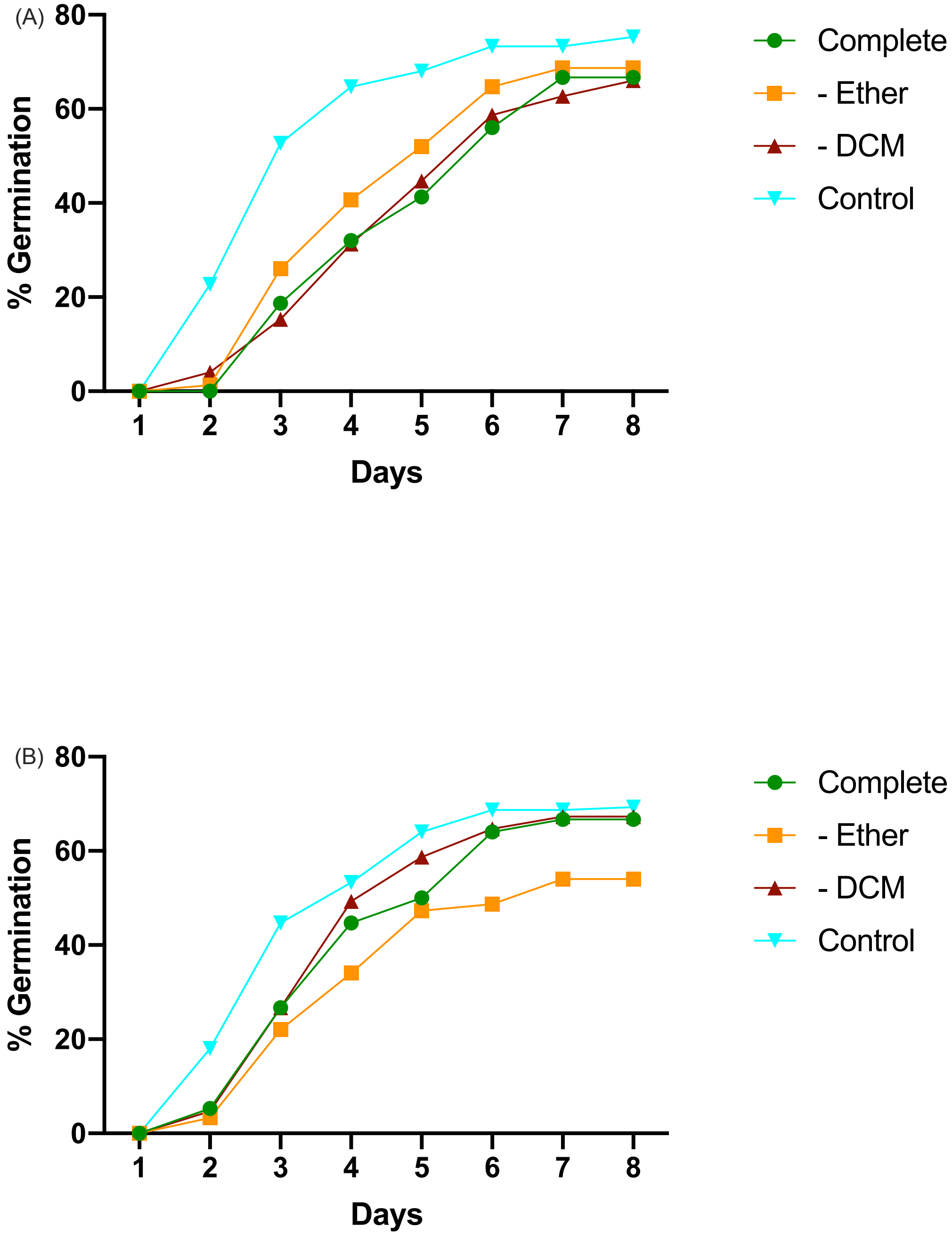


Fig 16 Cumulative germination plots of *L. sativa* seeds when exposed to complete, diethyl ether extracted, DCM extracted and 0% (Control) extracts of (A) *V. schaffneri* leaves, and (B) *V. farnesiana* leaves.

Table 4. Parameters of the germination process of *L. sativa* as affected by aqueous extracts of huisache and huisachillo and their fractions. VSL = *V. shaffneri* leaf extract, VFL = *V. farnesiana* leaf extract, -ether and -DCM refer to aqueous extracts with the diethyl ether and DCM soluble compounds extracted. MGT = Mean germination time, CVG = coefficient of velocity of germination. Lower case letters show Tukey-Kramer post hoc groupings.

Species	Extract	Germinability	MGT	CVG
VSL	Complete	66.6 ^{a,b}	4.78^{a}	20.92^{d}
	-Ether	68.6 ^{a,b}	4.30^{b,c}	23.25^{c,d}
	-DCM	66.0 ^{a,b}	4.69^{a}	21.37^{d}
	Control	75.3^{a}	3.27^{d}	30.59^{a,b}
		Germinability	MGT	CVG
VFL	Complete	66.6 ^{a,b}	4.16^{a,b,c}	24.07^{c,d}
	-Ether	55.3 ^{b}	4.17^{a,b,c}	24.27^{c,d}
	-DCM	67.3^{a,b}	3.88^{b,c,d}	25.92^{b,c}
	Control	70.0^{a,b}	3.49^{c,d}	29.01^{a,b}

BIOGRAPHICAL SKETCH

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