The Relative Importance of Abiotic and Biotic Factors for Seedling Establishment in the Colorado Desert, CA

DISSERTATION

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By

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Abstract

Drought combined with warmer temperatures has caused extensive mortality in US southwest desert ecosystems. Disproportional mortality among long-lived shrub species can have dire consequences for community regeneration if novel recruitment is dependent on facilitated establishment. However, regeneration niches can be under biotic or abiotic control.

My dissertation research examines mechanisms that control juvenile recruitment in the Colorado Desert, CA. Specifically I examine, seed and seedling responses to resources by partitioning those resources that are under direct biotic control based on the production and consumption patterns of standing vegetation, and those that are under abiotic control due to the effects of temperature and precipitation. By examining the relative importance of abiotic and biotic factors that affect plant recruitment, I can anticipate community stability in the face of natural or anthropogenic disturbance and if necessary offer strategies to mitigate species loss by identification of relevant variables to promote recruitment of target species.

In chapter 1 I characterize the spatial and temporal distribution of nitrogen for three years at a long-term study site in the Colorado Desert. In chapter 2, I examine the capacity for dominant shrubs to serve as plant facilitators for seed establishment and use a greenhouse experiment to identify the distinct roles of light and nitrogen on seed
germination. In a third experiment, I examine the roles of neighboring species identity, frequency and density on seedling performance when water and light are limiting.

I found that each focal species distinctly influenced nitrogen availability, which is generally higher near plant canopies. I also found higher levels of nitrogen under *Ambrosia dumosa* compared to the other focal species and the interspace. These results were highly dependent upon season and year (Chapter 1). Adult shrubs did not uniquely impact germination but beneficiary species identity was the main factor for germination. In the greenhouse, differences in percent germination were strongest at full light, and canopy light and nitrogen levels did not affect beneficiary germination (Chapter 2).

Using average rainfall, temperature and nitrogen concentrations from canopy microhabitats of *Ambrosia dumosa* from 2011-2012, I found that competition influenced early performance of *A. dumosa*, and *Eriogonum fasciculatum*, but not *Larrea tridentata*. Reduced growth of *L. tridentata* seedlings was not attributable to competition (Chapter 3).

Given these results, seed germination is not strongly regulated by abiotic or biotic factors; however, shrub canopies may be important for seedling growth and survival. If seedling persistence responds to nitrogen resources, *A. dumosa* may be instrumental in facilitating the establishment of perennial species. Nevertheless, all facilitators are important for providing increased heterogeneity in the landscape, which may increase regeneration niches for species not examined in my research. Nitrogen levels under shrub control would diminish without shrubs and subsequently, regeneration niches
would also decrease. However, relative competitive abilities can influence future community composition at the study site.
This document is dedicated to my brother, Lawrence C. Woods, my grandmothers, Mary L. Terry and Edna Mae Yates, and my brother-in-law Anthony Hardy who are no longer here to see me complete this goal.
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INTRODUCTION

Since 2002 US southwestern deserts have undergone dramatic losses in biodiversity due to extensive drought mortality of native shrub species (Bowers, 2005; Hamerlynck and McAuliffe, 2008; Miriti et al., 2007). While droughts lasting ten years or more are common in the US southwest over geological time scales (Ingram and Malamud-Roam, 2013), warmer temperatures due to climate change has exacerbated the effects of drought on vegetation (Breshears et al., 2005). In arid and semiarid environments water is scarce and the drivers of primary production, rainfall and nutrients, vary in spatial and temporal distribution (Noy-Meir, 1979, 1973). Many seedlings that establish in desert ecosystems are near their physiological threshold for survival and may depend on facilitation from adult shrubs for survival (Bruno et al., 2003). Plant facilitation increases the range of survival for a plant through stress amelioration, access to resources, and niche partitioning of vital resources (Brooker et al., 2008; McIntire and Fajardo, 2014). Because of the interdependence among plant species for survival, the loss of long-lived shrubs can have important consequences for community structure (McAuliffe and Hamerlynck, 2010; Tiedemann and Klemmedson, 1986).

In addition to extensive losses in standing vegetation, multidecadal drought events can cause even greater changes to communities because when well-established plants die, resources that were once held in place can become displaced (Tiedemann and
Klemmedson, 1986). Of particular concern is the displacement of nitrogen, second only to water in limiting primary production in desert ecosystems. Through positive feedback processes, litter trapping and soil organic matter build up under plant canopies, shrubs typically have higher concentrations of nutrients and microbes than interspaces (Herman et al., 1995; Titus et al., 2002). Other nutrients do not become limiting unless nitrogen becomes abundant, which is occurring in many US southwest ecosystems due to nitrogen deposition from nearby urban areas (Allen et al., 2009; Fenn et al., 2003). Because plant aggregations establish in high nutrient patches, changes in resource availability are often tied to changes in community structure.

Two of the most challenging questions facing ecologists today are how plant communities will respond to and recover from anthropogenic and climate induced disturbances. It is widely acknowledged in ecological theory that plant facilitators have an important role in structuring plant communities in high stress environments (Bruno et al., 2003; He et al., 2013; McIntire and Fajardo, 2014). However, with such interdependence of plant communities, the loss of facilitators may have negative consequences for community recovery unless abiotic heterogeneity overrides the effects of plants. Shrubs are the primary life form of facilitators (Filazzola and Lortie, 2014). Facilitators modify the environment, which may benefit species that respond favorably to modified microhabitats and can and tolerate conditions posed by the shrub. Facilitators partition resources allowing species coexistence (Schöb et al., 2012); however, shrubs can also reduce light levels for seedlings below their capacity for photosynthesis (Forseth et al., 2001; Holmgren et al., 2012, 1997). Although the relationship between facilitator
and beneficiary is complex, shrubs play a vital role in maintaining community patterns and processes in desert ecosystems (Butterfield and Briggs, 2011).

Plant interactions are dynamic and sensitive to environmental changes. Facilitators may compete with beneficiaries during favorable conditions and facilitate when environmental stress increases (Callaway, 2007; Maestre et al., 2009); however, if facilitators are stressed beyond their capacity, biotic interactions become irrelevant (Michalet et al., 2006). Local extinctions or high canopy dieback of facilitators can cause seedlings to be more vulnerable to changes in climate (Huggins et al., 2010). The seedling stage of development is the most vulnerable stage in the life of a plant, and seedling recruitment is frequently dependent upon the conservation or creation of regeneration niches, which can be under biotic or abiotic control.

Facilitators may differentially impact seedling establishment through their architecture, which defines how available moisture is collected, the amount of litter trapped and the amount of light that is available for photosynthesis. Shrubs also have distinct rates of mineralization and nitrification (Titus et al., 2002) and through their root systems can acquire nutrients from great distances from the plant canopy (Smith et al., 1997). Conversely, shrubs may deter neighbors through the secretion of allelopathic chemicals which deters root elongation of competitor species (Mahall and Callaway, 1992). Interactions with facilitators and beneficiaries can be species-specific, for example *Larrea tridentata* will deter conspecific roots and the roots of *Ambrosia dumosa* but facilitates some annual species (Fonteyn and Mahall, 1981; Schafer et al., 2012). The relative abundance of facilitators changes across communities (Butterfield and Briggs,
2011), thus determining which species are facilitators can greatly impact the assessment of community mortality and potential for recovery. Tantamount to identity of facilitators is response of beneficiaries. Determining which species are in need of species-specific interactions is important to identify because loss of adult shrubs could preclude the establishment of beneficiaries (McAuliffe and Hamerlynck, 2010).

Recently the role of shrubs in creating soil nitrogen heterogeneity above background heterogeneity has been challenged (Allington and Valone, 2014) as well as the role of shrubs in creating favorable microhabitats for establishment (Forseth et al., 2001; Walker et al., 2015, 2001). Examining factors that enhance or prevent seedling regeneration are crucial to community recovery. Recruitment is of particular interest in the Colorado Desert, CA because extensive drought caused high mortality among adults and juveniles in a long-term study plot, with little to no subsequent recruitment of new individuals. In addition, studies in neighboring regions show longer consecutive dry days during the summer (Petrie et al., 2014) and high levels of anthropogenic nitrogen (Fenn et al., 2003), which favors certain invasive species that can further slow drought recovery (Brooks, 2003).

My dissertation research is separated into three lines of investigation: 1) *What is the relative importance of shrub versus edaphic controls on the distribution of nitrogen?* 2) *To what extent does variation among species influence patterns of seed germination?* 3) *How does microhabitat variation influence seedling survival?* Ongoing research at the study site shows nonrandom species associations that suggest facilitation strongly structures species distributions, but the extent to which shrubs control soil nitrogen
distributions has not been examined. Species-specific changes in spatial and temporal distributions of nitrogen can influence recruitment patterns and future community composition. In chapter 1, I measure the effects of facilitator species on soil nitrogen availability along a gradient from shrub center to an open area 45 cm from shrub canopies. Additionally, I measure edaphic variation by assessing non-plant sites ≥1.5 m from shrub canopies. This approach allows me to examine how nitrogen changes among shrubs and with respect to the open. Additionally, I can assess within and among season nitrogen dynamics. Since microhabitat heterogeneity can influence where species establish, in chapter 2, I conduct a germination experiment with seeds of focal species buried along a gradient from canopy center to the open. This experiment was designed to examine species-specific interactions that may influence where germination occurs. Within shrub microhabitats seedlings may compete for resources; therefore, I conducted a response surface experiment in chapter 3, in which I varied the density and frequency of interacting species to determine which factors allowed them to coexist or competitively exclude one another. Relative competitive abilities among species may influence future community composition.

Because global climate change can lead to reduced opportunities for facilitated establishment via anticipated increases in drought frequency, drought related changes in resource availability, and mortality induced changes in species composition, these results contribute to the comprehensive assessment of the impact of plant facilitators in arid ecosystems. The results of my dissertation experiments are interpreted in light of patterns
of seedling recruitment and mortality observed for over 20 years at the long term study site (Miriti et al. 1998, 2007; Wright and Howe, 1987).
Chapter 1: *Shrub influences on nitrogen distributions in the Colorado Desert*

INTRODUCTION

Heterogeneity in soil resources is an important factor structuring plant communities (McAuliffe, 1994; Robertson et al., 1988; Williams and Houseman, 2013). Plant distributions respond to and contribute to soil resource availability with measurable effects on resource distribution (Finzi et al., 1998; Serrano-Vázquez et al., 2013) and community composition along successional (Wiegand et al., 1998) or disturbance gradients (García-Palacios et al., 2012). However, plant distributions are also influenced by interactions among neighboring individuals (e.g. Pacala et al., 2015; Toft and Fraizer, 2003), which can subsequently modify local soil resource distributions and the character of plant interactions. Such biotic-abiotic exchanges can be particularly important when plant interactions drive species establishment and community composition. This dynamic exchange between individual species and soil resources may limit community recovery after disturbance in systems where both plant interactions and resource heterogeneity strongly influence species distributions, particularly if mortality among dominant species is disproportional.

In desert systems, facilitation is an important structuring interaction (Armas and Pugnaire, 2005; Brooker et al., 2008; Butterfield and Briggs, 2011; Holmgren et al., 2012; Pugnaire et al., 1996). An important component of facilitation in desert systems is
plant improvements to soil fertility (De Soyza et al., 1997; Sala et al., 1989; Schlesinger et al., 1996), the quality of which can be species-specific (Titus et al., 2002). Further, stress-related reductions in resource availability can reduce the relative importance of facilitation (Maestre et al., 2009; Soliveres et al., 2013), resulting in changes in patterns of plant distribution, regeneration, and species relative abundance. Studies of plant distributions and recruitment patterns indicate the prevalence of facilitation in desert plant communities at large spatial scales (Flores and Jurado, 2003; Miriti, 2007; Miriti et al., 1998), but the strength and sign of plant interactions is not independent of soil conditions (Schenk et al., 2003). Plant distributions provide important controls over community composition because resources accumulate with plant density and create heterogeneity in soil resources.

Deserts are regions in which water limits plant growth. The lack of water results in a corresponding lack of water-soluble mineral nutrients such as nitrogen, which is the most limiting nutrient for primary production (Austin et al., 2004). Water and nitrogen, therefore, often co-limit productivity in arid systems (Hamerlynck et al., 2004). Because rainfall is infrequent in arid systems and organic matter is low, resources are pulsed in a manner that uniquely regulates production in these systems (Chesson et al., 2004; Goldberg and Novoplansky, 1997). Because of the importance of nitrogen to plant distributions, the relative importance of abiotic and biotic factors on nitrogen distributions may explain a large proportion of the influence of soil heterogeneity on plant distributions.
Taken together, these studies suggest that after a disturbance, community recovery in deserts will likely be driven by 1) the underlying distribution of nitrogen (Allen et al., 2009; Brooks, 2003) and 2) the relative abundance of species that can facilitate juvenile establishment (Huggins et al., 2010; McAuliffe and Hamerlynck, 2010). Examination of the current spatial heterogeneity in nitrogen resources may be predictive of favorable future sites of regeneration.

In this study, we examined spatial and temporal changes in nitrogen distributions in mapped plots located in the Colorado Desert. This region sustained extensive mortality after severe drought that affected much of the US Southwest (Breshears et al., 2005a; Miriti, 2007). Although it has been speculated that regeneration events should follow extensive adult mortality in arid communities (Wiegand et al., 2004), there has only been negligible recruitment identified in mapped plots in over a decade. Three shrub species represent (89) % of adults: *Ambrosia dumosa, Larrea tridentata,* and *Tetracoccus hallii*. *Ambrosia dumosa* suffered higher mortality than the other two species. Longitudinal spatial analyses suggest that facilitation by *Ambrosia dumosa* is an important dynamic structuring this community (Miriti, 2007; Miriti et al., 1998), and heavy adult mortality of this and other dominant species may be limiting recruitment. However, positive spatial associations do not eliminate the possibility that the facilitator and beneficiary may be responding to the same edaphic resource. Although limited juvenile associations with other dominant species suggest poor ability to facilitate juvenile recruitment, this result may also occur even if the canopies of these species promote favorable soil characteristics, but dispersal to these species is limited. As
dominants, all three species have the greatest impact on nitrogen spatial heterogeneity in this community and their population dynamics may play a critical role in future plant aggregations, thus influencing the productivity of this ecosystem.

The objectives of this study were to distinguish biotic and abiotic controls over spatial and temporal variation in soil nitrogen. We present data from a three-year study designed to 1) determine spatio-temporal species-specific influences on nitrogen levels among three co-dominants occurring at distinct spatial locations and in distinct species assemblages; 2) determine spatial variation in nitrogen levels independent of species; and 3) determine if species effects on nitrogen distributions are detectable in light of edaphic heterogeneity. Our results were used to assess the impact of high shrub mortality. If the availability of nitrogen is determined by living shrubs, then the loss of shrubs will have a negative impact on community regeneration. However, if there are a priori areas of enhanced nitrogen fertility, the community could regenerate in light of shrub mortality.

METHODS

Study site

The study site is located in Joshua Tree National Park, CA, within 10 km of the transition zone between the Mojave and Colorado Deserts (33°44′50″ N, 115°48′38″ W; elevation 1006 m), on a bajada that slopes slightly to the southwest of Eagle Mountain. Elevations range from 628 to 1028 m among all sites included in this study. Generally, the largest pulses of rain typically occur between September and March, but summer
monsoonal precipitation sometimes occurs starting in mid-July through mid-August. During the study period rainfall averaged 7 cm per year and the mean winter and summer temperatures were 16°C and 28°C, respectively (National Climatic Data Center, Twentynine Palms Station). The soil is classified as an aridisol, with 62.4% sand, 29.8% gravel, 7.8% clay (Wright and Howe, 1987).

This three-year study uses plots in which all perennials have been mapped and monitored beginning in 1984 (Miriti et al., 2007, 1998; Wright and Howe, 1987). These consist of a one hectare plot delineated into four 50 x 50 m subplots (designated H1, H2, H3 and H4) and two 35 x 35 m plots (designated satellite A and satellite B) (Figure 1). Satellite plots A and B were mapped in 1999, and 2000. The elevations of these plots are 1003 m (H1), 1002 m (H2), 1003 m (H3), 992m (H4), 1022 m (satellite A) and 629 m (satellite B). Satellite A is 520 m southeast and satellite B is 7.4 km southeast from the one hectare plot. The locations of these plots cover an area approximately 2 km x 7 km.
Figure 1: Plot Locations. The study site is located in Joshua Tree National Park near the Cottonwood Springs station. Plot locations are indicated by colored squares in the top right map. Symbols for the hectare subplots and satellite A overlap due to the small distance between them. There is little topographical variation among these plots. The elevations are 1003 m (H1), 1002 m (H2), 1003 m (H3), 992 m (H4), 1022 m (Satellite A) and 629 m (Satellite B). Satellite A is 520 m southeast of the hectare subplots and satellite B is 7.4 km southeast of the hectare subplots. With the exception of Satellite B, in which *T. hallii* does not occur, all plots contain 7 individuals of each focal species and 5 open microsites. Symbols may overlap when plants are located in close proximity to each other.
**Focal Species**

*Ambrosia dumosa* (A. Gray), Payne (bursage), hereafter *Ambrosia*, is a drought-deciduous shrub that reaches a maximum height of <1 m. It has a low-lying hemispherical canopy that traps seeds and litter. *Ambrosia* serves as a facilitator for high densities of annual species (Schenk et al., 2003), and at the study site many juveniles are positively spatially associated with it (Miriti, 2007).

*Larrea tridentata* (DC) Coville, hereafter *Larrea*, is the largest woody species at the study site. Adults typically exceed 1.5 m in height. It has an obconic morphology which concentrates water at the base of its canopy through stemflow (Whitford et al., 1997). *Larrea* has a large open canopy that supports many annual species, but it is also documented to have allelopathetic effects (Mahall and Callaway, 1992). *Larrea* may be facilitative to some species while inhibiting other species potentially through alleopathecic effects (Schafer et al., 2012). Deep and lateral tap roots allow *Larrea* to extend its influence on soil nutrients beyond its canopy. Deep tap roots allow for uplifting of water from deep soil horizons and lateral roots allow for access of nutrients and water from neighboring intersapces in relatively shallow soils (Smith et al., 1997).

*Tetracoccus hallii* Brandegee, hereafter *Tetracoccus*, is highly branched with an open understory. Little is known about nutrient uptake or inputs by *Tetracoccus*. Long-term observations do not show significant spatial associations between *Tetracoccus* and co-occurring perennials (Miriti, 2007).

The study plots were generally similar in species composition with a few exceptions. The hectare plots and satellite plot A contain all of the focal species. The
relative abundance of *Ambrosia* in these plots ranges from 65 to 52%, *Larrea*, 3 to 2.8% and *Tetracoccus* 14 to 11%. Satellite B does not include *Tetracoccus*. The relative abundance of *Ambrosa* and *Larrea* in satellite B are 55% and 3%, respectively.

*Sampling Design*

Sampling was designed to quantify underlying nitrogen heterogeneity among each of the plots and to evaluate variation in the nitrogen environment attributable to the focal species. Because plant effects on nitrogen may extend beyond the canopy, we sampled nitrogen along a horizontal gradient at three collection locations defined by each plant canopy: at the canopy center, canopy edge, and 45 cm from the canopy edge (open site) of each focal plant to capture the extent of plant influences on soil nitrogen. To assess underlying variation in edaphic nitrogen availability at the plot level, interspace microhabitats, positioned at least 1.5 m from any plant canopy, were included starting in the summer of 2012. We also were interested in temporal heterogeneity in nitrogen levels. To assess seasonal variation in nitrogen, which is expected to vary with water availability, we sampled nitrogen accumulation during the wet and dry seasons. Finally, we monitored nitrogen for three years to assess the consistency of our treatment and response variables.

In summary, our treatment variables were species, canopy gradient, year, season and plot identity, and our response variable was nitrogen level, separated into $\text{NH}_4^+$ and $\text{NO}_3^-$ availability. In five of the six plots presented above, seven of each focal species and five interspace microhabitats were randomly selected. The same design was used for satellite B, only satellite B does not contain *Tetracoccus*. To include seasonal variation,
nitrogen levels were collected during the time of the greatest rainfall for this region (mid-August through mid-March) and during the dry season (mid-March through mid-August) from August 2010-August 2013 (Figure 2).
Figure 2: Precipitation and resin burial period. Resin bags were buried 7.5 cm in the ground during the winter (August-March) and summer (March-August). Arrows indicate months when resin bags were placed in the soil. Total rainfall for each time interval is indicated in the box at the top right. Precipitation data are the mean ± SE of 9 nearby weather stations, Blythe Airport (71.38 km), Blythe (71.38 km), Eagle Mountain (0 km), Hayfield Pumping Plant (21.34 km) Indio Fire Station (71.74 km), Iron Mountain (21.34 km), Mecca Fire Station (64.85 km), Palm Springs (77.6 km), Twentynine Palms (64.29 km).
Nitrogen extraction

We used mixed bed ion-exchange resins (Sigma Aldrich, Dowex Marathon MR-3) to capture $\text{NH}_4^+$ and $\text{NO}_3^-$ nitrogen at our experimental locations. We were interested in both forms of nitrogen because plants use each form differently, and shrubs present distinct rates of mineralization and nitrification (Titus et al., 2002). Ion-exchange resins simulate plant root activity by capturing and retaining nitrogen when water is available (Skogley and Dobermann, 1996). Therefore, this technique accumulates nutrients over time, thereby allowing us to measure nitrogen availability in the rooting zone of seedlings over the duration of each of our winter and summer sampling intervals.

Resin bags were constructed by weighing 24 g of mixed bed ion exchange resin into a small nylon bag. Following Nave et al. (2011) bags were soaked in 10% HCl for two hours and rinsed six times in deionized. Bags were air dried before being placed at sampling locations. Resin bags were held in place by PVC pipes at a depth of 7.5 cm. The depth is reflective of the depth of seedling roots in arid environments (Whitford et al., 1997). After retrieval from the field, resin bags were stored at 20°C. Nitrogen was extracted in 2 M KCl and extracts were analyzed for $\text{NH}_4^+$ and $\text{NO}_3^-$ concentrations using a Bio Tek ELx800 Universal Microplate Reader. Nitrogen analysis was performed as described by Sims et al. (1995).

Statistical Analysis

Plot effects: Variation in nitrogen among plots over each season and year was assessed using the 5 interspace locations in each of the six plots. Standard least-squares methods were conducted using JMP v11. Data were square-root transformed to meet
homoscedasticity requirements. Tukey’s HSD post-hoc analyses ($\alpha=0.05$), or Student’s t-test when only two factors were compared, were used to examine differences in means within individual treatments.

*Species effects:* Standard least-squares methods were used to determine the main effects of focal species, canopy gradient, season, and year on NH$_4^+$ and NO$_3^-$ availability. For these analyses, plot is included as a random variable. We used a full-factorial analysis, and because of our mixed-model, restricted maximum-likelihood was used to estimate treatment effects. Data were transformed to homoscedasticity requirements. NH$_4^+$ data were square-root transformed, and NO$_3^-$ data were log transformed. Tukey’s HSD post-hoc analyses ($\alpha=0.05$) were used to examine differences in means within individual treatments.

RESULTS

*Plot effects*

There was significant temporal and spatial variability in nitrogen availability among plots in areas not under immediate plant control (Tables 1 and 2). For both forms of nitrogen, levels were significantly higher in the winter compared to the summer, and in 2012 compared to 2013 (Student’s t, $\alpha=0.05$) (Figure 3). Higher concentrations of NH$_4^+$ versus NO$_3^-$ differed by plot. NO$_3^-$ nitrogen was highest in plots H3 and H4, and lowest in plots H1 and Satellite B (Tukey’s HSD, $\alpha=0.05$). In contrast, NH$_4^+$ nitrogen was highest in Satellite B, and lowest in H1 (Tukey’s HSD, $\alpha=0.05$). Year and season and year and plot for NH$_4^+$ nitrogen (Table 1) show significant spatial and temporal
variation in nitrogen availability (Figure 3). Similarly, there was a three-way interaction among season plot and year for NO$_3^-$ (Table 2) (Figure 4).

Table 1: Effect of season, year and plot on the spatial distribution of NH$_4^+$. Six plots were examined during 2012 and 2013. There was an effect of season, year and plot and significant interactions between season*year and year*plot.

<table>
<thead>
<tr>
<th>Effect</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Season</td>
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<td>&lt;0.001</td>
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<tr>
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<tr>
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<td>0.1593</td>
</tr>
<tr>
<td>Year*Plot</td>
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<td>3.53</td>
<td>0.0066</td>
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<td>Season<em>Year</em>Plot</td>
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<td>1.80</td>
<td>0.1247</td>
</tr>
</tbody>
</table>

Table 2: Effect of season, year and plot on the spatial distribution of NO$_3^-$. Six plots were examined during 2012 and 2013. There was an effect of season, year and plot and significant interactions between season*plot, year*plot and a significant three way interaction between season*plot*year.

<table>
<thead>
<tr>
<th>Effect</th>
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</thead>
<tbody>
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<td>154.52</td>
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</tr>
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</tr>
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<td>Year*Plot</td>
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<td>Season<em>Year</em>Plot</td>
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<td>5.09</td>
<td>0.0005</td>
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</table>
Figure 3: Effect of year on plot (B) NH$_4^+$ concentrations were averaged by plot for 2012 and 2013. Panel A shows that ammonium was more available during the winter than the summer and panel B shows that there was significantly greater ammonium available by plot during 2012 than 2013. The asterisk represents a nitrogen mean of zero. Values represent the mean log (±SE).
Variation in nitrogen availability among focal species

There was significant spatiotemporal variation in nitrogen availability among the focal species. NH$_4^+$ and NO$_3^-$ responses to treatment variables were unique and are presented separately. All reported responses include plot level heterogeneity, which was included as a random variable.
Main effects: There were significant differences in NH$_4^+$ among species (p=0.0003), canopy gradient (p<0.0001), season (p<0.0001) and year (p<0.0001) (Table 3). Among species, *Ambrosia* had higher NH$_4^+$ than the other two focal species, and winter showed higher NH$_4^+$ levels than summer. The canopy gradient was apparent, with significant differences among the ranking canopy>edge> open, and over the three years of observation, significant differences were detected among the ranking 2012>2011>2013.

Table 3: Effect of species, season, gradient, and year on NH$_4^+$ availability in plant microsites. There were significant effects of species, season, gradient and year and significant interactions between species*season, season*gradient, species*year, season*year and gradient*year on NH$_4^+$ availability.

<table>
<thead>
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<th>Effect</th>
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<tbody>
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<td>Season*Gradient</td>
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<tr>
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</tr>
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<td>Species<em>Season</em>Year</td>
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</tr>
<tr>
<td>Gradient*Year</td>
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<td>6.33</td>
<td>&lt;0.0001</td>
</tr>
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<td>1.27</td>
<td>0.2558</td>
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<td>Species<em>Season</em>Gradient*Year</td>
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<td>0.33</td>
<td>0.9534</td>
</tr>
</tbody>
</table>

Interactions: Significant interactions were observed between species and season (p=0.0103) (Figure 5A), species and year (p<0.0001) (Figure 5B), season and canopy gradient (p=0.0015) (Figure 5C), season and year (p<0.0001) (Figure 5D), and canopy
gradient and year (p<0.0001) (Figure 5E). No significant three- or four-way interactions were observed. Considering temporal variables, seasonal interactions consistently showed higher NH$_4^+$ concentrations during the winter than the summer. An exception was that NH$_4^+$ was higher in summer 2012, which included unusually high precipitation following a wet winter (Figure 2). Similarly, the interactions between canopy location and year followed the rankings established with the main effects.
Figure 5: Significant interactions were shown for NH$_4^+$ availability due to the effects of year and season. Significant interactions were between species*season (A), species* year (B), season*canopy gradient (C), season* year (D), and canopy gradient* year (E) for NH$_4^+$ availability. Values represent the log average of the mean (±SE). Overall, NH$_4^+$ was higher during the winter for species and canopy gradient. NH$_4^+$ was higher during 2012 than the other years for species season and canopy gradient.
Interactions focusing on species and canopy gradient were more nuanced. As with the species main effect, \( \text{NH}_4^+ \) was higher under \textit{Ambrosia} during the winter, but this difference was not apparent during the summer (Figure 5). Over years, this ranking was not consistent, with \textit{Ambrosia} and \textit{Larrea} showing greater \( \text{NH}_4^+ \) than \textit{Tetracoccus} in 2012, \textit{Ambrosia} and \textit{Tetracoccus} generating more \( \text{NH}_4^+ \) than \textit{Larrea} in 2011, and no measurable differences among species in 2013. The canopy gradient persisted with seasons with greater \( \text{NH}_4^+ \) at canopy and edge locations compared to the open in both winter and summer, and with winter values being greater than summer values. The interaction between canopy gradient and year showed a significant canopy gradient only in 2012. For 2011 and 2013, yearly variation dissipated the canopy gradient with 2011 values being greater than those from 2013.

\textbf{NO}_3^- \text{nitrogen}

Although there were significant species effects on \( \text{NO}_3^- \) levels (p=0.003), \( \text{NO}_3^- \) levels responded more strongly to the temporal variables season (p<0.0001) and year (p<0.0001). There was no measurable canopy gradient in \( \text{NO}_3^- \) availability (Table 4).

\textit{Main effects:} In contrast to \( \text{NH}_4^+ \) levels, \( \text{NO}_3^- \) levels were greatest under \textit{Tetracoccus} and indistinguishable under \textit{Ambrosia} and \textit{Larrea}. Consistent with \( \text{NH}_4^+ \), \( \text{NO}_3^- \) was higher in the winter than in the summer, and significant differences were measurable among years with 2012>2011>2013.
Table 4: Effect of species, season, gradient, and year on NO$_3^-$ availability in plant microsites. There were significant effects of species, season and year and significant interactions between season*year and among species*season* year on NO$_3^-$ availability.

<table>
<thead>
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<th>Effect</th>
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<td>1.02</td>
<td>0.4710</td>
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</table>

**Interactions:** Significant interactions were detected between season and year (p<0.0001) and species and year (p=0.0065). High levels of nitrate were similarly high during summer 2012, winter 2012, and winter 2011 (Figure 6). Remaining season by year differences were ranked winter 2012>summer 2011>summer 2013. The interaction between species in year showed the established trend of higher NO$_3^-$ levels in 2012>2011>2013, but the species rankings did not persist. There was a clear separation.
of higher NO$_3^-$ under *Tetracoccus* than *Larrea* during 2012, but otherwise, there was no clear separation of species control over NO$_3^-$. 

A three-way interaction occurred among species, season and year (p<0.0001) (Figure 6). Similar to the two-way interactions, these responses are notable in the significantly lower NO$_3^-$ levels for all species during the summer of 2013.

Figure 6: Effect of year on species during the winter (A). Effect of year of species during the summer (B). Values represent the log average of the mean (±SE). The effect of year on NO$_3^-$ variability was variable among species. The effect of year on season showed that NO$_3^-$ levels were higher for all species during 2012. Asterisks represent mean NO$_3^-$ levels below zero.
DISCUSSION

Our results demonstrate that abiotic and biotic factors influence heterogeneity in soil resources at our study site. Further, biotic influences are species-specific. *Ambrosia, Larrea* and *Tetracoccus* generate distinct influences on nitrogen availability compared to open microsites. NH$_4^+$ and NO$_3^-$ concentrations show unique seasonal variation and species*season interactions. NH$_4^+$ decrease along a distance gradient from shrub canopies but this gradient is absent during the summer. Shrub influences on nitrogen are detectable at the local scales that are associated with juvenile recruitment, suggesting that plant controls on soil resources play a crucial role in determining microsites that favor seedling establishment and persistence. These results are consistent with spatial patterns of juvenile and adult distributions previously quantified at this study site.

Observed patterns of nutrient availability support patterns of juvenile recruitment at the study site demonstrating species have differential abilities to enhance juvenile establishment. In general, *Ambrosia* has the strongest influence on NH$_4^+$ levels while *Tetracoccus* has stronger influences on NO$_3^-$*. In support of juvenile associations with *Ambrosia*, Sala et al. (2012) showed that NH$_4^+$ is the dominant form of nitrogen in shallow soil layers where most seedling roots are accumulated and competition for nutrients is more likely to occur.

Neutral associations of juveniles with *Tetracoccus* may suggest an unimportant role of NO$_3^-$ for juvenile recruitment or limited dispersal to these canopies. While *Larrea* did not show distinct levels of NH$_4^+$ or NO$_3^-$ compared to the other species, NH$_4^+$ levels
can be highly variable across studies (Titus et al. 2002; Brooks, 1998 and Mudrak et al. 2014). Nutrient heterogeneity attributable to Larrea may be influenced by the distribution of fine roots, which are at higher densities away from the canopy than below the canopy (Hartle et al., 2006; Wilcox et al., 2004), reducing canopy effects of Larrea. Canopy architecture may be important for determining species differences in nitrogen availability. De Soyza et al. (1997) attributed spatial heterogeneity of soil nutrients to the effect of canopy architecture on trapping litter for decomposition. Hemispherical canopies, such as is presented by Ambrosia, show higher nitrogen levels.

Nitrogen levels varied considerably over the three years of this study and between seasons. This temporal variation provides further context for pulsed recruitment in arid systems. Linkages between water availability and nitrogen availability are established such that when water availability is low, nitrogen availability is low (Chesson et al., 2004; Goldberg and Novoplansky, 1997). Winter precipitation was notably high for 2011 relative to the other years of the study. Nevertheless, accumulation of NH$_4^+$ was higher during winter 2012 than winter 2011. This result is consistent with Reynolds and colleagues’ (1999) finding that high rainfall does not always equate higher nitrogen availability. A notable difference during this time is that 2011 precipitation was marked by months with high input followed by months with zero rainfall (Figure 2). NH$_4^+$ activity may be more strongly influenced by more moderate, but consistent levels of precipitation rather than isolated, but large amounts of precipitation. NH$_4^+$ and NO$_3^-$ levels responded as expected to the high summer pulses of precipitation. This sensitivity to precipitation consistency during the winter may influence recruitment of woody
perennials, which typically occurs during the winter. This recruitment may be sensitive to \( \text{NH}_4^+ \) concentrations.

It should not be ruled out that temporal differences in nitrogen availability may be influenced by patterns of plant metabolic activity. \textit{Ambrosia} and \textit{Tetracoccus} become dormant during low resource periods, but \textit{Larrea} does not. This study suggests that when shrubs go dormant or decrease metabolic activity during the summer, nitrogen availability decreases. Although \textit{Larrea} can shift its metabolic activity to endure periods of drought or low nitrogen availability (Cunningham et al., 1979; Reynolds et al., 1999), it is still drawing down resources during periods of low availability, which may account for some of the heterogeneity observed among seasons. Temporal variability in climate and shrub adaptations may interact to create heterogeneity in soil resources.

We observed significant site level variation in nitrogen. Such heterogeneity may be due to deposition increases in \( \text{NO}_3^- \) levels, as in other arid regions (Fenn et al., 2003) or geological patterns of soil formation (McAuliffe 1994). Our study site is relatively clean of N-deposition (Rao et al., 2009). Although \( \text{NO}_3^- \) levels were high during summer 2012, this was not likely due to deposition because if \( \text{NO}_3^- \) originated from N deposition, these levels would be consistently higher at all locations, and higher in the summer than in the winter (Padgett and Allen, 1999). Large scale heterogeneity in nitrogen may have greater influence over species relative abundance (e.g. McAuliffe 1994) than on patterns of juvenile establishment.
Implications for drought recovery

Our findings suggest that extensive drought mortality can lead to reductions in available nitrogen at scales relevant to patterns of juvenile recruitment. These reductions, however, may not be immediate. Mahall and Callaway (1991) suggest that nitrogen concentrations do not readily dissipate, allowing plants to aggregate along underlying heterogeneity left behind after the death of a shrub. The fate of resource islands after mortality may be dependent upon species identity and life form (Butterfield and Briggs, 2009; Tiedemann and Klemmedson, 1986). Nevertheless, although there may be some legacy of soil nitrogen after plants die, nitrogen is subject to diminish over time without biotic input.

Our results demonstrate that nitrogen is a dynamic resource that is variable from year to year, which may partially explain episodic recruitment in deserts. Abiotic factors such as rain and temperature must sync with biotic conditions such as reproduction and nitrogen mineralization. As a result, the trajectory of desert plant communities in the U.S. southwest may shift due to both abiotic and biotic factors. Abiotic conditions are forecasted to change leaving desert communities near the study site becoming hotter and drier (Overpeck and Udall, 2010), and N-deposition from urban communities increasing with population growth (Fenn et al., 2003).

There are many reasons why regeneration has not been observed such as persistent drought with only intermittent years of above average rainfall, low seed viability, seed predation, high mortality among seedlings, seed dormancy, to name a few,
the primary focus of this study was to identify the influence of shrubs on nitrogen distributions spatially and temporally. The severe mortality of *Ambrosia* adults at this site may reduce local diversity if the nitrogen levels associated with the co-dominants are unable to facilitate common perennials. High mortality of all species at this site and of *Ambrosia* in particular may have direct consequences on the ability of this site to recover pre-drought biomass or pre-drought species composition.
Chapter 2: Ubiquitous germination among common perennial species in response to facilitated and unfacilitated microhabitats

INTRODUCTION

Chronic drought in the US Southwest has caused high mortality of woody species in many arid and semiarid plant communities (Bowers, 2005; Hamerlynck and McAuliffe, 2008b; Miriti et al., 2007). One consequence of this mortality is a reduction in the number of facilitators, which are plants that provide safe-sites for early establishment. Facilitators provide increased moisture and nutrients, (Flores and Jurado, 2003; Schlesinger and Pilmanis, 1998; Schlesinger et al., 1996) and decreased light intensity (Franco and Nobel, 1988) that enhance early survival and growth of beneficiary species. Interactions between facilitators and beneficiaries are dynamic and influenced by environmental conditions (Luzuriaga et al., 2012; Seifan et al., 2010; Yu et al., 2009). Facilitators may compete with seedlings for below-ground resources (e.g. Schenk and Mahall, 2002; Walker et al., 2001) or reduce light availability for establishing seedlings (Seifan et al., 2010). The relationship between environmental severity and frequency or relevance of facilitation is an active, but as yet unresolved area of ecological research (see Soliveres et al., 2015 for a recent review). For arid species that rely on facilitated establishment (Flores and Jurado, 2003), facilitator loss may threaten or preclude novel recruitment (McAuliffe and Hamerlynck, 2010; Tiedemann and Klemmedson, 1986).
This threat is exacerbated if mortality is biased towards species that facilitate a large number of beneficiary species.

Facilitator species identity can influence patterns of recruitment among beneficiary species. Species-level variation influences the reliance of beneficiaries on facilitators (Castro and Gómez, 2002; Padilla and Pugnaire, 2009), and the patterns of mortality among beneficiary species (Callaway, 1998; de la Cruz et al., 2008; Miriti et al., 2007, 1998; Padilla and Pugnaire, 2009). The effectiveness of facilitation can vary with the canopy architecture of facilitators, which affects microhabitats through litter trapping and moisture directed to the understory through stem flow (De Soyza et al., 1997; Tromble, 1988), or heterogeneity of soil resources under facilitator canopies, which determines small-scale distributions of beneficiary establishment (Lopez-Pintor et al., 2003) and survival (Castro and Gómez, 2002; Walker et al., 2001). The relative importance of species-specific variation becomes apparent when multiple facilitator species are examined.

In deserts, where adaptations for long-range or directed dispersal are rare (Ellner and Shmida, 1981), the spatial distribution of facilitators can limit the efficacy of facilitation as a driver of biodiversity. Seeds must arrive at suitable host sites suggesting that species abundance and distribution affects the likelihood that a given species plays a facilitator role (Yoshihara et al., 2010). Similarly, because the responses of seeds and seedlings to individual adult species influence seedling performance, the distribution and abundance of facilitators can determine the rate of recovery and species composition of a community after a disturbance. Studies that examine facilitator roles are commonly
targeted to species that have significant and apparently positive associations with juveniles of one or many species (e.g. Schenk and Mahall, 2002; Schlesinger et al., 1996). It is unclear if species that are not recognized as facilitators lack sufficient resources to support germination and early survival, or if dispersal to those canopies is limited. Drought mortality imposes changes in the distribution of potential facilitators and increases the importance of understanding variation in the ability of different species to facilitate juvenile establishment. Also, the quality of facilitation among survivors may be reduced in response to reduced canopy volume and litter inputs. Disparities among common facilitator species will influence species composition during drought recovery.

We measure species variation in facilitation in a post-drought community in the Colorado Desert in southeastern California using field and greenhouse experiments. Between 1999-2004, this region experienced exceptional drought conditions (Breshears et al., 2005), resulting in 64% adult shrub mortality (Miriti, 2007). Subsequent reestablishment of species has been slow to nonexistent such that no seedlings have been documented since 2004 (personal observation, Miriti lab) even after years of above average precipitation (2005, 2010-2011 http://www.epa.gov/climatechange). Although one common species, *Ambrosia dumosa*, is a strong candidate to be a generalist facilitator due to persistent positive associations with species common to the site (see Miriti, 2007; Miriti et al., 1998) at this site, species-specific variation in the ability to provide or benefit from facilitation has not been experimentally examined. If common species are not equally able to facilitate establishment, such unprecedented mortality may disrupt facilitation networks (Verdú and Valiente-Banuet, 2008) and compromise community
recovery to pre-drought composition. Drought conditions are projected to increase (Overpeck and Udall, 2010), which could have adverse impacts on community regeneration. Although precipitation is a driving factor of species dynamics in arid systems, dense or intermediate shading by facilitators can ameliorate the effects of drought for establishing seedlings (Carvajal et al., 2014), which increases the importance of facilitators during drought intervals.

To anticipate recovery of this shrub community, we compare the abilities of the three most abundant species, *Ambrosia dumosa*, *Larrea tridentata*, and *Tetracoccus hallii*, to facilitate seed germination compared to unvegetated sites. Although patterns of seed germination are not a common emphasis for facilitation studies, we focus on this stage because it is most sensitive to levels of environmental gradients (Parish and Bazzaz, 1985). Further, persistence of long-lived plants in arid communities is considered to be less dependent on novel germination and largely dependent on persistence of reproductives that represent “storage”, thereby reducing the relative importance of recruitment to maintain extant populations (Wiegand et al., 2004). As such, the storage component of seed banks is not well-studied for perennials (but see Cao et al., 2013). As a result, it is important to consider that a transient perennial seed bank may make perennial species more susceptible to local extinction after disturbance that causes extensive adult mortality. Taken together, examination of the pattern of seed germination in response to facilitation can enhance understanding of the mechanism of persistence in arid perennial communities.
Four species once common to this site prior to the extended drought, *A. dumosa*, *L. tridentata*, *Sphaeralcea ambigua* and *Eriogonum fasciculatum*, are used as beneficiary species. These species experienced 2% to 100% local mortality during the drought (Miriti et al., 2007). The aims of the current study were 1) to determine species-specific effects of facilitators on germination, and 2) to determine the influences of light and nitrogen levels associated with the focal facilitator species on germination. Shade and nitrogen are consistent mechanisms of facilitation (see Bonanomi et al., 2011 for a recent review). The results of these experiments are interpreted in light of patterns of species abundance and spatial associations accumulated from more than 20 years of census data collected at the field site (Miriti et al., 2007, 1998; Wright and Howe, 1987). Facilitators that significantly improve early establishment can be managed to enhance recovery of this disturbed plant community.

METHODS

Study site

The study site is located in Joshua Tree National Park, CA, near the transition zone between the Mojave and Sonoran Deserts (33°44′50″ N, 115°48′38″ W; elevation 955 m), approximately 1km west of a 1 hectare plot that has been monitored in five year intervals for over 20 years (Miriti et al., 2007, 1998; Wright and Howe, 1987). Plants characteristic of this desert scrub community include *A. dumosa*, *E. fasciculatum*, *L. tridentata*, *Simmondsia chinensis*, *S. ambigua* and *T. hallii* along with cacti, *Opuntia echinocarpa* and *Opuntia ramosissima* and intermittent distributions of the succulent
plant, *Yucca schidigera* (Miriti et al, 1998). Soils at this site are characterized as 62.4% sand, 29.8% gravel, 7.8% clay (Wright and Howe, 1987).

At the Twentynine Palms weather station, located 103 km from the study site, the average annual temperature was 21°C and average annual precipitation totaled 62.3 mm from 2009-2012 (National Climatic Data Center). In this region, the largest pulse of rain occurs during the winter, although summer monsoonal rains may occur during July-August. Perennial germination typically occurs in the spring following winter precipitation.

*Facilitator species*

Facilitator species were chosen based on their abundance and broad local distribution. *Larrea tridentata* (D.C.) Coville (creosotebush) and *Ambrosia dumosa* (A. Gray) Payne (white bursage) are common among low elevation plant communities throughout US southwestern deserts. In contrast, *Tetracoccus hallii* Brandegee (Hall’s shrubby-spurge) is endemic and rarely found outside of California. The relative abundance of these species at the study site is *A. dumosa* 63%, *T. hallii* 13% and *L. tridentata* 4% (hereafter *Ambrosia*, *Tetracoccus* and *Larrea*). *Ambrosia*, *Tetracoccus* and *Larrea* adults experienced 68%, 58%, and 2% drought mortality, respectively (Miriti et al., 2007). Each of these species presents contrasting canopy morphology and associations with juveniles at the study site.

*Ambrosia* is drought-deciduous with a low-lying canopy, and reaches an average height approximately 90 cm. Several native perennial species show significant positive spatial association with *Ambrosia* at the long-term study site (Miriti, 2007) although it is
unclear if the mechanisms promoting these relationships are due to facilitative interactions or shared microhabitat preferences. *Ambrosia* germinates well in interspace microhabitats and does not require facilitated establishment (McAuliffe, 1988; Walker et al., 2001) even though most individuals occur close to conspecifics (Miriti, 2007; Wright and Howe, 1987).

*Larrea* is a drought tolerant evergreen shrub that can achieve heights exceeding 200 cm at the study site. Adults have an obconic canopy morphology that redirects water to its understory through stemflow (De Soyza et al., 1997) although Walker and colleagues (2001) found soil moisture levels to be lower than that under other candidate facilitators. Although *Larrea* promotes establishment of annuals (e.g. Schlesinger et al. 1996; De Soyza et al., 1997), it may deter the establishment of some perennial species through root-mediated allelopathy (Mahall and Callaway, 1992); its role in facilitating perennials is not well-examined (but see Walker et al., 2001).

*Tetracoccus* is drought tolerant and reaches heights of 150 cm at the study site with a conical shaped morphology. Due to its limited distribution, the California Native Plant Society classifies *Tetracoccus* as an endangered species. Ongoing monitoring shows that juveniles of common species have no spatial association with *Tetracoccus* (Miriti, 2007); its potential as a facilitator is otherwise unexamined.

**Beneficiary species**

Four beneficiary species are used in these experiments. These include the dominants *Ambrosia* and *Larrea*. *Tetracoccus* is not included due to low seed production. The other two beneficiary species are *Sphaeralcea ambigua* (A. Gray), La
Duke (globemallow) and *Eriogonum fasciculatum* (Torrey & A. Gray), (California buckwheat), which are relatively short-lived, drought tolerant shrubs that were once fairly abundant at the study site, but experienced 100% and 96% drought-induced mortality, respectively (Miriti et al., 2007). *Sphaeralcea ambiguа* (hereafter *Sphaeralcea*) grows best in full sunlight and well-drained soil (Selland, 2003). *Eriogonum fasciculatum* (hereafter *Eriogonum*) favors full sunlight (DeSimone and Zedler, 2001), and survives in a wide range of moisture levels. Miriti and colleagues (2007; 1998) show enhanced establishment and survival of these two species near *Ambrosia* but not *Larrea* or *Tetracoccus*.

Seeds of the four beneficiary species were collected from multiple adults near the field site. Seeds were cleaned and processed at The Ohio State University Germplasm Center to maximize the proportion of viable seeds. Random samples of each species were x-rayed after processing to determine the proportion that contained embryos. For each species, 1000 seeds were x-rayed in groups of 100, and the proportion containing embryos in each sample was averaged. The percent of seeds that contained embryos were *Eriogonum* 86%, *Larrea* 96%, *Sphaeralcea* 92% and *Ambrosia* 96%. Species-specific stratification requirements to break dormancy were pre-determined in the lab. Seeds were stratified by soaking them in water for up to 8 hours. A radicle length of at least 2 mm was used to determine germination. Seed preparation was identical for the field and greenhouse experiments.
Variation in soil nitrogen

Nitrogen availability was obtained from an ongoing study at this site. Mixed-bed ion exchange resin (Sigma, Dowex Marathon MR-3) bags were used to measure ammonium and nitrate levels. Resin bags (24g) were buried 7.5 cm in the soil under the canopy of randomly selected facilitator species and in interspace microhabitats. The burial period for ion exchange resin bags was from 09 September 2010-17 March 2011 for facilitated sites and from 09 September-2011-16 March 2012 for the interspace sites. Nitrogen was extracted from ion exchange resin bags with 2M KCl. Extracts were analyzed for ammonium and nitrate using a microplate reader (Bio Tek ELx800 Universal) as described by Sims (1995). Mean ammonium and nitrate levels accumulated over this interval (reported as $\mu$g kg$^{-1}$ day$^{-1}$) were used to calibrate nitrogen levels used in the greenhouse experiment. Differences among species and the interspace were measured using ANOVA.

Canopy light levels

Canopy openness was quantified to determine understory light availability for Ambrosia, Larrea and Tetracoccus. Hemispherical photographs were taken on 11 April 2012 with a Nikon CoolPix 950 digital camera using a fish-eye lens as described by Rich (1990) through the canopy and canopy edge of seven similar sized shrubs of each facilitator. The mean light availability was the value assigned for the canopy openness of the facilitator species. The canopy edge was defined as the location at the drip line of the shrub. Percent canopy openness and photosynthetic photon flux density (PPFD, mol/m$^2$day) were estimated from hemispherical photographs using WinScanopy software
WinScanopy software analyzes a digital image by classifying pixels as canopy or sky based on the light intensity values of each pixel. Briefly, this software uses latitudinal and longitudinal coordinates to determine the position of the sun track, permitting PPFD to be averaged over a 24 hr period at a specific location. Further details of these methods are described in WinScanopy (2003b). Differences in light availability among species and the interspace were evaluated using ANOVA.

Field Experimental Design

Our field experiment manipulated three factors: facilitator species, beneficiary species and microhabitat. Facilitator and beneficiary species were as described above. Microhabitats were three locations defined relative to facilitator canopies: 1) canopy center, 2) canopy edge, identified as the drip line of the focal plant canopy, and 3) an interspace site located 1.5 m from any plant canopy (Figure 7), hereafter referred to as canopy, edge and interspace.

A single replicate included the microhabitats associated with one isolated individual of each facilitator species and an interspace area (Figure 7). To minimize effects of habitat heterogeneity, shrubs within a replicate were selected in close proximity to each other. The maximum distance between facilitators within a replicate was 4.6 m and the maximum distance between replicates was \( \leq 7.5 \) m. The distance among individuals within a replicate never exceeded the distance between distinct replicates, and the experiment included 10 replicates.
Seeds of each beneficiary species were placed in the experimental sites as follows. Seeds were packaged into species-specific nylon bags to separate experimental seeds from seeds in the soil bank and to protect from insect granivores. Bag dimensions were 1.5 x 1 cm for the smaller seeded, *Eriogonum* and *Sphaeralcea*, and 3 cm x 2 cm the larger seeded *Ambrosia* and *Larrea*.

Each bag contained 100 seeds, and one randomly selected bag/beneficiary species was sown at 1 cm depth at each microhabitat within a hardware cloth enclosure (6 x 5 x 4 cm) to protect from mammalian granivores. To be consistent in microclimate and reduce desiccation, enclosures were positioned toward the north because it is the shadier side of the shrub in the northern hemisphere. Seed packets were watered twice daily, once in the morning and once in the evening. We note that by watering seeds, we did not consider direct drought effects in this study. This experiment was initiated on 23 March 2012 and ran through 6 April 2012. Seed bags were frozen within 24 hours after excavation to halt metabolism until germination could be quantified.
Figure 7: Schematic field experimental design and subcanopy images with canopy microhabitat descriptions. A single replicate contained one of each facilitator species and an interspace. The maximum distance between shrubs within the same replicate was ≤4.6 m; the maximum distance from shrub canopies to the interspace microhabitat was 1.5 m, and the maximum distance between replicates was ≤7.5 m. Microhabitats were characterized by canopy openness (%), PPFD (mol/m² day) and nitrogen availability (µg kg⁻¹ day⁻¹). To simplify the schematic, edge microhabitats are not shown, but the details for these microhabitats are included in Table 5.

The effects of facilitator, microhabitat and beneficiary species on germination were analyzed using a generalized linear model. Germination was recorded as percent germination of seeds per bag and analyzed assuming a binomial error distribution. Multiple comparisons were assessed using orthogonal contrasts to detect significant
differences within treatments. Statistical analysis was completed using JMP (Version 11).

**Greenhouse Experiment**

The greenhouse experiment examined the effects of facilitator nitrogen and light environments on beneficiary seed germination. Nitrogen and light levels characteristic of field microhabitats, as described above, were used to determine experimental levels for the greenhouse experiment. Four of the five nitrogen treatments in the greenhouse mirrored the NH$_4^+$, NO$_3^-$ ratios measured at the canopy center of each focal species and the interspace in the field. These ratios were NH$_4^+$: NO$_3^-$ concentrations of 45: 23 µg kg$^{-1}$ day$^{-1}$ associated with *Ambrosia*, 32: 33 µg kg$^{-1}$ day$^{-1}$ for *Larrea*, 32: 18 µg kg$^{-1}$ day$^{-1}$ for *Tetracoccus*, and 25: 48 µg kg$^{-1}$ day$^{-1}$ for the interspace. Since treatments were delivered in solution, a fifth treatment consisting solely of deionized water was added as a procedural control. Because there was no statistical difference in the canopy openness of *Larrea* and *Tetracoccus* (Table 5), three light levels were used, to represent 1) *Ambrosia*, 2) *Larrea* and *Tetracoccus*, and 3) the interspace.

Shade cloth was used to reduce light proportional to the canopy reductions measured in the field (Table 5). For reference, photosynthetic photon flux density (PPFD) measured in the greenhouse was one-third of that measured in the field, which is sufficient for photosynthesis. Light was reduced by 70% to simulate the effect of *Ambrosia* canopies, and by 50% to simulate *Larrea* and *Tetracoccus* canopies. The interspace treatment was represented by ambient light levels in the greenhouse. Photoperiod was controlled to reflect springtime day lengths, and the average temperature
was 20°C. Light and nitrogen levels associated with edge microhabitats were not included in the greenhouse study.

Within each light level, 25 seeds each of *Ambrosia, Larrea, Sphaeralcea,* and *Eriogonum* were randomly placed in five rows and five columns with equal distance between each seed on moistened blotter paper in a germination box. The blotter paper was moistened with 20 ml of one of the five nitrogen treatments. The experiment was replicated four times. The duration of this experiment was four weeks from 18 October 2012 through 15 November 2012. The effect of nitrogen on percent germination of seeds for each species at each light level was analyzed using a generalized linear model with a binomial error distribution. Germination box was included as a random variable. Orthogonal contrasts were used to detect within treatment differences.

**RESULTS**

*Species differences in light and nitrogen*

Microhabitats of the facilitator species were characterized by canopy openness, photosynthetic photon flux density (PPFD) and nitrogen availability (Figure 8). Canopy openness differed among species (p<0.001, Table 5), while PPFD was significantly greater in the interspace than at the canopy of all facilitators (p<0.001). The greatest contrast among facilitators was observed between *Ambrosia* and the microhabitats of *Larrea* and *Tetracoccus,* with *Ambrosia* providing more shade than *Larrea* (Tukey HSD, p=0.006) or *Tetracoccus* (Tukey HSD, p=0.028). *Ambrosia* canopies on average allowed 30% of ambient light to reach the understory while *Larrea* and *Tetracoccus* allowed
nearly 50%. Shading decreased from canopy to edge for all species but these differences were insignificant. For this reason, we limit our comparisons to canopy and interspace microsites.

PPFD levels were inversely proportional to canopy openness. *Ambrosia*, which provided the most shade under the canopy, had significantly lower PPFD compared to *Larrea* and *Tetracoccus* (p<0.0001). For *Larrea* and *Tetracoccus*, PPFD did not significantly increase from canopy to edge. Further, PPFD at the edges of *Larrea* and *Tetracoccus* were not different from interspace measurements.
Table 5: Microhabitats were characterized by canopy openness, PPFD, and nitrogen availability. Within each microhabitat, comparisons (mean ± SE) were made for the above measures among facilitators. The interspace measurements were compared to the canopy microhabitats only. For each measurement, superscripts represent significant differences among species in each microhabitat as determined by ANOVA.

<table>
<thead>
<tr>
<th>Microhabitat</th>
<th>Facilitator</th>
<th>$\text{NH}_4^+$ ($\mu$g kg$^{-1}$day$^{-1}$)</th>
<th>$\text{NO}_3^-$ ($\mu$g kg$^{-1}$day$^{-1}$)</th>
<th>Canopy Openness (%)</th>
<th>PPFD (mol/m$^2$ day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canopy</td>
<td>Ambrosia</td>
<td>$45^{a} \pm 2.75$</td>
<td>$23^{ab} \pm 4.34$</td>
<td>$31.4^{a} \pm 2.11$</td>
<td>$11.1^{a} \pm 1.40$</td>
</tr>
<tr>
<td></td>
<td>Larrea</td>
<td>$32^{b} \pm 1.38$</td>
<td>$33^{b} \pm 3.39$</td>
<td>$51.4^{b} \pm 2.75$</td>
<td>$20.9^{b} \pm 1.32$</td>
</tr>
<tr>
<td></td>
<td>Tetracoccus</td>
<td>$32^{b} \pm 2.28$</td>
<td>$18^{a} \pm 2.65$</td>
<td>$48.6^{b} \pm 1.95$</td>
<td>$19.5^{b} \pm 2.78$</td>
</tr>
<tr>
<td>Edge</td>
<td>Ambrosia</td>
<td>$43^{a} \pm 3.12$</td>
<td>$16^{a} \pm 2.65$</td>
<td>$40.3^{a} \pm 3.14$</td>
<td>$16.5^{a} \pm 1.64$</td>
</tr>
<tr>
<td></td>
<td>Larrea</td>
<td>$32^{b} \pm 1.75$</td>
<td>$20^{a} \pm 2.38$</td>
<td>$61.6^{b} \pm 4.73$</td>
<td>$24.1^{b} \pm 1.03$</td>
</tr>
<tr>
<td></td>
<td>Tetracoccus</td>
<td>$35^{b} \pm 2.11$</td>
<td>$11^{a} \pm 1.48$</td>
<td>$58.2^{b} \pm 4.77$</td>
<td>$22.8^{b} \pm 1.91$</td>
</tr>
<tr>
<td>Interspace</td>
<td>None</td>
<td>$25^{c} \pm 1.27$</td>
<td>$48^{c} \pm 2.22$</td>
<td>$100^{c}$</td>
<td>$28.04^{c}$</td>
</tr>
</tbody>
</table>
Nitrogen availability also varied among species and was dependent on the form of nitrogen. The greatest contrast in NH$_4^+$ availability was found between *Ambrosia* and the other two facilitators. NH$_4^+$ was significantly higher under *Ambrosia* than *Larrea* (p=0.001) and *Tetracoccus* (p<0.001, Table 5), and there was no gradient in NH$_4^+$ from canopy to edge microhabitats (Table 5). *Larrea* contained significantly greater NO$_3^-$ than *Tetracoccus*, but NO$_3^-$ levels were not different from the canopy of *Ambrosia* or the interspace (Table 5). *Larrea* was the only facilitator that significant differences in NO$_3^-$ levels from canopy to edge (p=0.04). Although there were differences in the form of available nitrogen under the canopies of facilitators, total nitrogen availability was similar among facilitators. Facilitator microhabitats were distinguished most strongly by light availability with *Ambrosia* providing the most unique microhabitat among the facilitators in this study.
Figure 8: Percent germination (± S.E.) of beneficiary species at facilitator microhabitats (canopy and edge) and the interspace microhabitat. No beneficiary species showed a preference for microhabitat. Asterisks indicate that *Eriogonum* germination was significantly greater than all other beneficiary species.

**Field Experiment**

Germination did not respond to facilitator or microhabitat, but differences in percent germination among beneficiaries was significant (Figure 8, p<0.001). *Eriogonum* showed the highest percent germination (58% ± 2.63) followed by *Larrea* (16% ± 1.09), *Sphaeralcea* (14% ± 1.65), and *Ambrosia* (9% ± 0.91). Contrast analysis showed that
Eriogonum germination was greater than Ambrosia \((p<0.001)\), Larrea \((p<0.001)\), and Sphaeralcea \((p<0.001)\).

**Greenhouse experiment**

Overall percent germination in the greenhouse did not differ from percent germination in the field \((p=0.45)\). Similar to field results, beneficiary species identity explained differences in average germination in the greenhouse at all light Eriogonum, 29\% ± 1.56 for Larrea, 14\% ± 0.77 for Sphaeralcea, and 6\% ± 0.67 for Ambrosia across treatments.

![Figure 9: Percent germination (± S.E.) of beneficiary species in the greenhouse at three canopy openness levels observed in the field.](image)
At 50% light, significant species differences (p=0.006) were such that *Ambrosia* germination was significantly lower than that of *Eriogonum* (p=0.002) and *Larrea* (p=0.008) (Figure 9). Germination of *Eriogonum* and *Larrea* was comparable, but *Eriogonum* germination was greater than *Sphaeralcea* (p=0.02). At 30% light, species differences (p=0.01) showed *Eriogonum* germination to be greater than *Ambrosia* (p=0.002), and *Sphaeralcea* (p=0.02). This ranking persisted at full light (p=0.008) with *Eriogonum* germination being significantly greater than *Ambrosia* (p<0.001) and *Sphaeralcea* (p=0.009), and marginally greater than *Larrea* (p=0.06).

All species were insensitive to differences in nitrogen availability within the three manipulated light environments. When the response to nitrogen is pooled across light levels, Figure 10 suggests that for *Eriogonum*, low nitrogen may reduce germination success, but our methods do not permit this assessment.

**DISCUSSION**

Facilitation, a key dynamic for juvenile recruitment in arid plant communities, is frequently examined in the context of the unique traits of facilitator species and their consequences on the performance of beneficiary species. The current study examined the relative abilities of three abundant shrubs, *Ambrosia, Tetracoccus* and *Larrea*, to facilitate germination of common species in a desert community that suffered major drought mortality without significant recruitment after more than ten years, despite years with high precipitation. Although historic seedling distribution patterns at the site suggest a dominant facilitative role for *Ambrosia*, our results suggest that facilitation does
not enhance germination. Each facilitator presents unique combinations of light and
$\text{NH}_4^+:\text{NO}_3^-$ concentration ratios, but greenhouse manipulations of light and nitrogen
levels associated with the facilitator species confirm that differences in germination are
more strongly explained by beneficiary species differences than by light or nitrogen
levels. Water availability was not manipulated, and cannot be ruled out as a driver of
germination patterns. Overall, our results suggest that germination can be ubiquitous and
that high mortality among potential facilitators does not explain the lack of juvenile
recruitment at the study site.

Figure 10: Percent germination (± S.E.) of beneficiary species in the greenhouse under
experimental $\text{NH}_4^+:\text{NO}_3^-$ ratios, and pooled over all light levels.
Species-specific patterns of germination

Previous patterns of the spatial distribution of *Eriogonum* suggest a dependence on facilitation (Miriti, 2007; Miriti et al., 1998). Surprisingly, germination of *Eriogonum*, a species that has gone from fairly common to near extinction at the study site (Miriti et al., 2007), surpassed that of the two, more common, facilitator species included as beneficiaries. Germination was markedly lower without nitrogen addition, suggesting that an interaction between light and nitrogen for *Eriogonum* germination, which was not explicitly addressed in this study, may reveal facilitation requirements for this species.

For *Ambrosia*, the lack of facilitator dependence is consistent with long-term observations of seedling establishment in interspace and facilitated locations. Seedling fate in response to germination site is size-dependent (Miriti, 2006) and the benefits of facilitated establishment of *Ambrosia* may not persist more than two years (Schenk and Mahall, 2002). Although adult *Ambrosia* are consistently found with and without near neighbors, long-term population dynamics of this species rely more strongly on plants that established without facilitated establishment (Miriti et al., 2001). This broad tolerance of habitat conditions may explain the high abundance of *Ambrosia* in this and other arid communities of the US Southwest, and reflect its potential as a generalist facilitator.

Our results suggest that germination of *Larrea* can occur in facilitated or interspace microhabitats. Although germination of this species under field conditions is not well-documented (but see Bowers et al., 2004; McAuliffe, 1988), soil moisture is
suggested to strongly limit early survival of *Larrea* (Bowers et al., 2004; Goldberg and Novoplansky, 1997; Woods et al., 2010) and that facilitation may enhance juvenile persistence (McAuliffe, 1988). Seedling survival is high if individuals survive the first year. Nevertheless, germination of naturally occurring *Larrea* is known to be rare or episodic (Goldberg and Turner, 1986) and has never been documented at the study site. Long-lived species such as *Larrea* do not need to recruit very often to maintain their populations (Wiegand et al., 2004). Further, *Larrea* can produce extremely long-lived clones (e.g., Vasek, 1980) which ensure persistence, but clouds the relative importance of germination microhabitat.

*Sphaeralcea*, which experienced complete mortality in the long-term study plot, successfully germinated in facilitated sites and interspace sites. This is in stark contrast to seedling distribution patterns at the site showing that facilitation improves early juvenile survival (Miriti et al., 1998). Experimental and observational studies taken together suggest that the significance of facilitation increases for *Sphaeralcea* at older life stages.

Our findings contextualize the role of facilitation in generating vegetation patterns. With the exception of *Larrea*, all of the focal species present aggregated patches at the study site (Miriti, 2007; Miriti et al., 1998), but our results suggest that these patterns are not the result of germination responses. Studies of effects on germination are rare, and biased towards germination of annuals (Mayor et al., 2007; Thompson and Huenneke, 1996). These studies show that patterns of facilitator effects on germination vary among beneficiary species (Sotomayor et al., 2014; Tielboerger and
Kadmon, 1995), and can show temporal variation in the relative importance of microsite variables (Yu et al., 2009). Our findings are most consistent with those of Sotomayor and colleagues (2014), which show that germination in facilitated and unfacilitated sites does not always vary, even for stress-intolerant desert annuals. Relative competitive ability among germinants (Thompson and Huenneke, 1996) or seedlings (Tielboerger and Kadmon, 1995) modifies patterns established by germination.

We do not discount the importance of water limitation on the relative importance of facilitation. Seedling emergence in semi-arid communities increases with rainfall (Yu et al., 2009) and when examined hierarchically, water stress has been shown to be the primary filter before facilitation in determining community composition (Luzuriaga et al., 2012). We do note, however, that seed germination and subsequent seedling establishment have not been observed at our study site, even during wet years.

*Implications for post-drought recovery*

Long-lived desert plant communities can remain in stasis for decades or longer. The unprecedented mortality recorded in US southwestern deserts affords us a unique opportunity to refine the role of facilitators in the recovery of arid plant communities. Species recovery may depend on several factors including, but not limited to, the reliability of adults to produce viable seed, dispersal of seed into favorable microhabitats, adequate water and nutrients to allow seedlings to reach a critical size before seasonal drought, and the capacity to withstand competition from cohort seedlings and the adult shrub. Results of the current study suggest that the seeds of woody perennial species are
viable and facilitator variation should not limit germination as a first step toward community recovery.
Chapter 3: Frequency- and density-dependence influence competitive outcomes among seedlings

INTRODUCTION

Facilitation of beneficiaries by adult plants has been shown to be an important determinant of community composition in arid shrublands (Verdú and Valiente-Banuet, 2008). However, competitive interactions for space and resources among seedlings co-occurring in facilitated microhabitats can be intense (Mcintire and Fajardo, 2014) and impede the establishment of less competitive species (Davies et al., 2010). Such interactions increase in importance when severe disturbances alter the frequency and distribution of benefactor and beneficiary species in desert communities.

Severe drought has caused extensive mortality in the US Southwest with as yet undetermined consequences on the ability of affected communities to return to pre-drought composition. In desert shrublands that depend on facilitation, the distribution of facilitators and other resources that promote novel recruitment is an important indicator of community recovery potential. Because juvenile densities may be high in these locations, understanding competitive hierarchies among juveniles can refine the ability to forecast community trajectories. Limited dispersal ability may lead to dense aggregations of conspecifics with similar resource requirements, resulting in strong frequency dependent limitations among conspecifics under a parent canopy (HilleRisLambers et al., 2011), and favor heterospecific recruitment. In contrast, common species may occur with
higher frequency due to stronger competitive ability under facilitators independent of
species identity. Biotic interactions may promote coexistence or lead to local extinction.

In the current experiment, we examine the competitive ability of three species of
woody seedlings common to the Colorado Desert in California. The study site
experienced 100% mortality of all seedlings in addition to high adult mortality among
common shrub species during 1999-2004 (Miriti et al. 2007), similar to other mortality
events reported throughout US southwestern deserts (Bowers, 2005a; McAuliffe and
dumosa*, *Larrea tridentata*, and *Eriogonum fasciculatum* (hereafter *Ambrosia*, *Larrea* and
*Eriogonum*) have each been documented to show enhanced juvenile growth and survival
near *Ambrosia* canopies (Miriti et al. 1998; McAuliffe 1984), but the relative competitive
abilities of these species has yet to be examined. *Ambrosia* and *Larrea* co-occur
throughout U.S. southwestern deserts and make up 70% of the Mojave Desert plant
associations (Shreve, 1942). Although *Ambrosia* can facilitate *Larrea* establishment
(McAuliffe 1984), as adults interspecific competition is the dominant interaction between
*Ambrosia* and *Larrea* (Fonteyn and Mahall, 1981). Developmental stage, density, and
frequency may impact this dynamic. *Eriogonum* may change the extent to which
*Ambrosia* and *Larrea* interactions occur. Indirect relationships may occur when multiple
species co-occur, promoting coexistence of neighboring species (Allesina and Levine,
2011). Improved understanding of seedling competition can anticipate changes in
community composition and structure in response to drought.
We designed a response surface experiment in the greenhouse to examine changes in density (number of individuals) and frequency (relative abundance of individuals per species) of *Ambrosia, Larrea* and *Eriogonum*. Our response variables were final biomass and change in height. Our objectives were to determine the relative competitive ability among these common species that likely co-occur in facilitated microsites. Final biomass was used as a proxy for competitive response to resources and changes in height was used as a proxy for growth rate. Because *Ambrosia* is the most common species at our study site that is documented to facilitate perennial establishment, we used nitrogen resources associated with canopy conditions of this species (Chapter 1). Determining which species is competitively dominant in these conditions can forecast patterns of novel juvenile establishment. When seedlings are put into competition, we expect each species to limit conspecific growth but we expect heterospecific competition to be a stronger factor for establishment. As a null hypothesis, we expect competitive hierarchies among juveniles to reflect species relative abundance at the study site.

**METHODS**

We conducted two response surface experiments to examine 1) competitive interactions among the more abundant species *Ambrosia* and *Larrea* and 2) three-way competitive interactions among *Ambrosia, Larrea* and *Eriogonum*.

*Experimental Design*

Response surface experiments are used to evaluate intra- and interspecific interactions when established at different relative abundances at a given density.
(Connolly et al., 2001; Gibson et al., 1999; Vanclay, 2006). The two-species and three-species response surface experiments lasted three months and were conducted in the Biological Sciences Greenhouse at The Ohio State University during September 2013. Each experiment was established at three seedling densities with the two-species experiment including four relative abundances and the three-species experiment including six relative abundances. *Ambrosia* and *Larrea* seedlings were planted in 12 different treatment combinations in the two-species experiment (Table 6), and *Ambrosia*, *Larrea* and *Eriogonum* seedlings were planted in 54 different treatment combinations for the three-species experiment (Table 7). Each treatment combination was replicated four times.

Seeds for each experiment were collected in the field and processed as described in Chapter 2. To increase germination rates, seeds were sown in a 3:1:1 mixture of peat moss, sphagnum and perlite in individual cells (1 x 1 cm) in potting trays. Four to 5 week old seedlings were used for the response surface experiments. Seedlings were transplanted in 3.79 L containers that were 16 cm in diameter using a 3:1 mixture of sand and perlite. The soil mixture was fertilized with 0.3 g/pot of slow release Osmocote® 14/14/14 at the beginning of the experiment. The level of Osmocote® was reflective of average winter season *Ambrosia* nitrogen levels at the field site from 2011 and 2012. We chose nitrogen associated with *Ambrosia* because this is the most common adult canopy at the study site. No additional nitrogen was added for the duration of the experiment. Seedlings were randomly assigned to treatment pots and to a position on a 1 x 1 cm grid within each pot (Figure 11). To compensate for mortality due to transplant shock,
seedlings that did not survive the first week were replaced with a new transplant.

Temperature and water were controlled to simulate the average conditions in the field during the winter season of 2011 and 2012. I used precipitation and temperature data from the Twentynine Palms weather station (National Climatic Data Center). At the end of the experiment, seedlings were harvested from each treatment. Seedlings were dried for two days at 70°C and weighed for final biomass. Initial and final heights were recorded for each seedling.

Statistical Analysis

Linear regression models were used to analyze the relationship between densities of the focal species and the response variables, final biomass and change in height. For the two species experiment, we used the following linear regression model:

$$\ln(Y_1) = \beta_0 + \beta_1 D_1 + \beta_2 D_2 + \beta_3 D_1 D_2 + \epsilon$$

The predictor variables for biomass (or change in height when relevant) of the focal species were $\beta_1 D_1$ (density of species 1) + $\beta_2 D_2$ (density of species 2) + $\epsilon$, which represents error.

Similarly, the resulting linear regression model for the three species experiment was the following:

$$\ln(Y_1) = \beta_0 + \beta_1 D_1 + \beta_2 D_2 + \beta_3 D_3 + \beta_4 D_1 D_2 + \beta_5 D_1 D_3 + \beta_6 D_2 D_3 + \beta_7 D_1 D_2 D_3 + \epsilon$$

The predictor variables for biomass (or change in height when relevant) of the focal species were $\beta_1 D_1$ (density of species 1) + $\beta_2 D_2$ (density of species 2) + $\beta_3 D_3$ (density of species 3) + $\epsilon$, which represents error. We used a two-way ANOVA to compare the magnitude of the response variables of focal species among treatment combinations to
analyze the effects of changes in total density and frequency of interacting species.

Tukey’s simultaneous comparisons were used to conduct multiple comparisons within treatments. Response variables were log transformed to fulfill normality and homoscedasticity assumptions. All analyses were conducted using SPSS (IBM Corporation, 2012 Version 21.0, Armonk, NY).

Table 6: *Ambrosia* and *Larrea* seedlings in a response surface experiment consisted of 12 different treatment combinations with 4 replicates. Treatments were randomly assigned to 48 pots. Each cell represents a single treatment. FS=Focal Species C1=Competitor 1.
Figure 11: Schematic of how seedlings were positioned within a pot. Seedlings were randomly assigned to a 1 x 1 cm grid within each pot. Fifty cells were partitioned to ensure maximum use of space in each pot. One gallon pots contained a minimum of 8 seedlings and a maximum of 32.

A. Soil in pots was sectioned off in a 1x 1 cm grid (squares are not drawn to scale).

B. Random numbers were identified for the placement of seedlings. Black squares indicate random seedling placement.
Table 7: *Ambrosia*, *Larrea* and *Eriogonum* seedlings in a response surface experiment had 54 different treatment combinations with 4 replicates. Each cell represents a single treatment. Treatments were randomly assigned to 216 pots. FS=Focal Species C1=Competitor 1  C2=Competitor 2.

<table>
<thead>
<tr>
<th>% of Focal Species (FS)</th>
<th>Low Density (8 seedlings)</th>
<th>Medium Density (16 seedlings)</th>
<th>High Density (32 seedlings)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FS</td>
<td>C1</td>
<td>C2</td>
</tr>
<tr>
<td>25a%</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>25b%</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>50%</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>75a%</td>
<td>6</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>75b%</td>
<td>6</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>100%</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

RESULTS

*Two Species Experiment: Competition between Ambrosia and Larrea*

*Ambrosia responses*

The linear regression model showed that average final biomass of *Ambrosia* decreased with intraspecific competition (p=0.001, Table 8). The density of *Larrea* did not reduce the average height growth of *Ambrosia* seedlings. *Ambrosia* increased in biomass when the competitor was *Larrea* (p=0.001, Table 8).

The two-way ANOVA showed that total seedling density had a significant effect on the final biomass of *Ambrosia* (p=0.012, Table 9, Figure 12 A). A similar result was also shown with average height growth (p=0.014, Table 9, Figure 12 B). Comparably, the frequency of *Ambrosia* had a significant effect on average final biomass and growth.
of conspecifics. When *Ambrosia* was at a frequency of 25% of all seedlings, *Ambrosia* grew larger than at all other frequencies of *Ambrosia* (Figure 12 A). This was the case for all densities, but was only significantly different when *Ambrosia* planted at a frequency of 25% was compared to *Ambrosia* at a frequency of 100% (p=0.025, Figure 12 B).

Table 8: Regression analysis for growth variables measured during the two-species response surface experiment. Variables indicate final biomass and height growth over the three month time period. Each variable was regressed against the density of *Ambrosia* and the density of *Larrea*. The R² value is listed and p-value for each factor is in parenthesis under the competition coefficient. All response variables were log-transformed to meet assumptions of normality. Asterisks represent a p-value of <0.01.

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>N</th>
<th>R²</th>
<th>Density <em>Ambrosia</em></th>
<th>Density <em>Larrea</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Final Biomass (g)</td>
<td>48</td>
<td>21.5</td>
<td>-0.034*</td>
<td>-0.007ns</td>
</tr>
<tr>
<td><em>Ambrosia</em> Growth (cm)</td>
<td>48</td>
<td>46.5</td>
<td>-0.012ns</td>
<td>0.052*</td>
</tr>
<tr>
<td>Final Biomass (g)</td>
<td>48</td>
<td>19</td>
<td>-0.039*</td>
<td>0.002ns</td>
</tr>
<tr>
<td><em>Larrea</em> Growth (cm)</td>
<td>48</td>
<td>15.4</td>
<td>-0.001ns</td>
<td>0.014*</td>
</tr>
</tbody>
</table>
Table 9: Two-way ANOVA Two Species Response Surface Experiment. Two-way ANOVA shows that *Ambrosia*’s biomass and growth is dependent upon total seedling density and frequency of *Ambrosia* seedlings. The frequency of *Larrea* has a significant effect on the biomass of *Larrea* seedlings.

<table>
<thead>
<tr>
<th></th>
<th><em>Ambrosia</em> Biomass</th>
<th></th>
<th><em>Ambrosia</em> Height</th>
<th></th>
<th><em>Larrea</em> Biomass</th>
<th></th>
<th><em>Larrea</em> Height</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Effect</strong></td>
<td>df</td>
<td>F</td>
<td>P</td>
<td>df</td>
<td>F</td>
<td>P</td>
<td>df</td>
</tr>
<tr>
<td>Density</td>
<td>2</td>
<td>4.834</td>
<td><strong>0.012</strong></td>
<td>2</td>
<td>4.834</td>
<td><strong>0.014</strong></td>
<td>2</td>
</tr>
<tr>
<td>Frequency</td>
<td>5</td>
<td>3.100</td>
<td><strong>0.039</strong></td>
<td>5</td>
<td>3.100</td>
<td>&lt;<strong>0.001</strong></td>
<td>5</td>
</tr>
<tr>
<td>Density*Frequency</td>
<td>10</td>
<td>0.736</td>
<td>0.324</td>
<td>10</td>
<td>0.736</td>
<td>0.324</td>
<td>10</td>
</tr>
</tbody>
</table>
Figure 12: Mean log final biomass (A) and mean log growth (B) of *Ambrosia* from the response surface experiment with three total densities and four levels of abundance of *Ambrosia* over a three month time interval. *Ambrosia* has significantly higher biomass and growth at low frequencies and densities.

**Larrea** responses

The linear regression model showed that *Larrea’s* average final biomass was decreased (p=0.002) when the competitor was *Ambrosia*. Specifically, when there were fewer *Larrea* seedlings in a pot, there was a higher frequency of *Ambrosia* seedlings, which reduced the average final biomass of *Larrea*. The density of *Larrea* did not have
any significant effect on conspecifics (Table 8). The density of Larrea significantly increased conspecific growth (P=0.008), but Ambrosia did not affect Larrea’s growth.

The two-way ANOVA showed that an increase of the frequency of Larrea from 50 to 100% had a negative effect on the average final biomass of Larrea (p=0.021, Figure 13) but there was no effect of an increase in density of seedlings. Neither density nor frequency of Larrea seedlings had a significant effect on the average height growth of Larrea. None of the interactions were significant.

Figure 13: Mean log final biomass and of Larrea from the response surface experiment with three densities four levels of abundance of Larrea over a three month time interval. Larrea has higher biomass with low densities of its competitor.

Three Species Experiment: Competition among Ambrosia, Larrea and Eriogonum

Ambrosia Responses

The linear regression model showed that Ambrosia limited the average final biomass of conspecifics more than its competitors (p=0.048, Table 10). When Ambrosia competed with conspecific seedlings for resources its average final biomass decreased (p=0.003, Table 10), indicating intraspecific competition. However, neither the densities
of Larrea or Eriogonum had a significant effect on Ambrosia’s average final biomass or growth.

The two-way ANOVA showed that the frequency of Ambrosia was a significant factor determining its average final biomass (p=0.027; interaction p=0.009). Ambrosia dumosa pots containing 75% Ambrosia seedlings had less average final biomass than pots containing 25% Ambrosia (p=0.031) regardless of the combination of the other species in the pot (Table 11; Figure 14). When high, density was the factor that decreased the growth of Ambrosia (p=0.002), while the frequency of Ambrosia was not a significant factor. There were no significant results found in response to Larrea frequency. None of the interactions were significant.
Table 10: Regression analysis for growth variables measured during the three-species response surface experiment. Variables indicate final biomass and height growth over the three month time period. Regression analysis for growth variables measured during the three-species response surface experiment. Variables indicate final biomass and height growth over the three month time period. Each variable was regressed against the density of Ambrosia, the density of Larrea and the density of Eriogonum. The $R^2$ value is listed and p-value for each factor is in parenthesis under the competition coefficient. All response variables were log-transformed to meet assumptions of normality. Asterisks represent a p-value of <0.05.

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>N</th>
<th>$R^2$</th>
<th>Density Ambrosia</th>
<th>Density Larrea</th>
<th>Density Eriogonum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Final Biomass (g)</strong></td>
<td>Ambrosia</td>
<td>69</td>
<td>20</td>
<td>-0.043*</td>
<td>-0.38 ns</td>
</tr>
<tr>
<td><strong>Growth (cm)</strong></td>
<td>Ambrosia</td>
<td>69</td>
<td>44</td>
<td>-0.032*</td>
<td>-0.031 ns</td>
</tr>
<tr>
<td><strong>Final Biomass (g)</strong></td>
<td>Larrea</td>
<td>53</td>
<td>19</td>
<td>-0.027 ns</td>
<td>-0.019 ns</td>
</tr>
<tr>
<td><strong>Growth (cm)</strong></td>
<td>Larrea</td>
<td>53</td>
<td>14.6</td>
<td>0.016 ns</td>
<td>0.019 ns</td>
</tr>
<tr>
<td><strong>Final Biomass (g)</strong></td>
<td>Eriogonum</td>
<td>60</td>
<td>14.2</td>
<td>-0.047 ns</td>
<td>-0.060 ns</td>
</tr>
<tr>
<td><strong>Growth (cm)</strong></td>
<td>Eriogonum</td>
<td>60</td>
<td>20.4</td>
<td>-0.051 ns</td>
<td>0.011 ns</td>
</tr>
</tbody>
</table>
Table 11: Two-way ANOVA Three Species Response Surface Experiment. Two-way ANOVA shows that *Ambrosia*’s biomass and growth is dependent upon total seedling density and frequency of *Ambrosia* seedlings. *Eriogonum*’s density is highly dependent upon the number of seedlings in a pot.

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>F</th>
<th>P</th>
<th>Effect</th>
<th>df</th>
<th>F</th>
<th>P</th>
<th>Effect</th>
<th>df</th>
<th>F</th>
<th>P</th>
<th>Effect</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>2</td>
<td>0.620</td>
<td>0.543</td>
<td>Ambrosia</td>
<td>2</td>
<td>7.486</td>
<td><strong>0.002</strong></td>
<td>Larrea</td>
<td>2</td>
<td>2.574</td>
<td>0.089</td>
<td>Eriogonum</td>
<td>2</td>
<td>7.487</td>
<td><strong>0.002</strong></td>
</tr>
<tr>
<td>Frequency</td>
<td>5</td>
<td>2.850</td>
<td><strong>0.027</strong></td>
<td>Height</td>
<td>5</td>
<td>0.523</td>
<td>0.757</td>
<td>Height</td>
<td>5</td>
<td>1.969</td>
<td>0.106</td>
<td>Height</td>
<td>5</td>
<td>0.988</td>
<td>0.436</td>
</tr>
<tr>
<td>Density*</td>
<td>10</td>
<td>2.947</td>
<td><strong>0.009</strong></td>
<td>Biomass</td>
<td>10</td>
<td>1.802</td>
<td>0.093</td>
<td>Biomass</td>
<td>10</td>
<td>1.321</td>
<td>0.255</td>
<td>Biomass</td>
<td>10</td>
<td>1.972</td>
<td>0.061</td>
</tr>
<tr>
<td>Frequency</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

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Figure 14: Average final biomass and average height growth of *Ambrosia* from the response surface experiment with three densities of combinations of *Ambrosia*, *Larrea*, and *Eriogonum* seedlings and four levels of abundance of *Ambrosia* over a three month time interval. Proportions that overlap represent 25a%=double *Larrea* (relative to *Ambrosia*), 25b%=double *Eriogonum* (relative to *Ambrosia*), 75a%=No *Larrea*, 75b%=No *Eriogonum*. 
Eriogonum responses

The linear regression model showed that *Eriogonum* had a significantly negative effect on the average final biomass of conspecifics (p=0.035) but its average final biomass was not significantly affected by heterospecifics. *Eriogonum* decreased conspecific average final biomass (Table 10). *Eriogonum*’s average final biomass was reduced by *Ambrosia* and *Larrea* but this interaction was not significant due to variation around the mean. There were no significant effects of average height growth found for *Eriogonum*.

The two-way ANOVA showed that density but not relative abundance, had a significant effect on the average final biomass of *Eriogonum* (p=0.005). Specifically, pots with 8 seedlings had a higher average final biomass than pots with a density of 32 (Figure 15). Ultimately, *Ambrosia* nor *Larrea* had a significant negative or positive effect on *Eriogonu*’s performance due to variation around the means.
Figure 15: Average final biomass and average height growth of Eriogonum from the response surface with three densities of combinations of Eriogonum, Larrea, and Ambrosia seedlings and four levels of abundance of Eriogonum over a three month time interval. Frequencies that overlap represent 25a%=double Larrea (relative to Eriogonum), 25b%=double Ambrosia (relative to Eriogonum), 75a%=No Larrea, 75b%=No Ambrosia.

DISCUSSION

Effect of Density and Frequency on Ambrosia and Larrea Interactions

The two species response surface experiment with Ambrosia and Larrea shows that both intraspecific and interspecific interactions operate simultaneously to influence Ambrosia’s competitive ability. Intraspecific competition was a greater factor for Ambrosia’s average final biomass but not a significant factor influencing Ambrosia’s average height growth. Because growth increased when the neighbor was Larrea, Larrea demonstrated a facilitative effect over Ambrosia’s average height growth. At the field
site in the Colorado Desert, *Ambrosia* is the numerically dominant shrub. While adults are known to facilitate their own seedlings (Miriti, 2007), seedlings can survive well in isolation of other species (McAuliffe, 1988). High intraspecific competition could be an indication that biotic stress overrides abiotic relief for *Ambrosia* under plant canopies.

The facilitative effect of *Larrea* on *Ambrosia* is surprising because *Larrea* is known to secrete allelopathic chemicals which inhibit elongation of *Ambrosia* seedlings (Mahall and Callaway, 1992). Mahall and Callaway’s experiment did not manipulate frequency, which may explain these opposing results. The negative effects of *Ambrosia* on intraspecific competition may be stronger than negative effects attributable to *Larrea*. These pairwise species interactions among seedlings may change the ontogeny of these species. For example, Hamerlynck et al. (2002) found that adult *Ambrosia* are found at greater abundances on soils where *Larrea* cannot establish, but are suppressed when the two species co-occur, which implies a change in the relative competitive abilities of these two species over time.

*Larrea* seedling performance was greatest in the presence of conspecifics. Although *Larrea* appears to facilitate *Ambrosia*’s initial establishment, *Ambrosia* reduced *Larrea*’s local dominance by reducing *Larrea*’s average final biomass. Specifically, when *Larrea* was at low densities *Ambrosia* reduced *Larrea*’s biomass. Conversely, when *Larrea*’s density was high there was less competition from *Ambrosia* seedlings, thus, biomass increased. In contrast, intraspecific competition was a greater factor for *Larrea*’s average height growth. While it is well documented that *Larrea* seedlings aggregate in natural microhabitats (Bowers et al., 2004; McAuliffe, 1988), *Larrea* are
spatially segregated as adults. This segregation could be due to biotic interactions (Fonteyn and Mahall, 1981), the capacity for competitive advantages to shift with development (Bengtsson et al., 1994), abiotic factors such as geomorphology (McAuliffe, 1994; Schenk et al., 2003) or distance to water sources (Schwinnig et al., 2011). These mechanisms are supported by the demonstration that Larrea inhibits the root growth of neighboring conspecifics (Mahall and Callaway, 1991), and limits root overlap with other species (Brisson and Reynolds, 1994). The resulting segregated spatial pattern reduces contact with competitors allowing individuals to stably coexist.

*Interactions Among Ambrosia, Larrea and Eriogonum*

Facilitated microhabitats commonly contain more than two species, which can be mutually beneficial for the collective group when nonhierarchical competition exists (Allesina and Levine, 2011). An indirect relationship was not explicitly determined among the three species used in this study, although Ambrosia did not significantly reduce Larrea’s final biomass as in the two-species experiment. While not significant, Eriogonum had a positive effect on Larrea’s height growth and average final biomass. Thus, the introduction of Eriogonum appears to have offset some of the negative impact of Ambrosia on Larrea. Indirect effects are common but may not be detected because over time direct negative effects can be ameliorated by indirect positive effects (Miller, 1994). This suggests that species identity, and not simply an interspecific identity can influence Larrea persistence.

Ambrosia’s average growth and average final biomass is adversely affected by intraspecific competition such that high densities of Ambrosia appear to promote density-
dependent mortality of conspecifics. *Ambrosia* seedlings can occur at high densities under *Ambrosia* canopies (*personal observation*); however, seedlings do not appear to persist at high densities in this microsite (*personal observation*). The current experiment suggests that high densities of *Ambrosia* limit conspecific establishment, which supports results found in the two-species experiment. Introduction of other species as seedlings at low densities may reduce the adverse intra- and interspecific impacts of *Ambrosia*. None of the results for *Larrea* were significant suggesting that a lower competitive significance of this common species.

Similarly, this experiment also suggests that intraspecific competition limits *Eriogonum*’s average final biomass more than interspecific competition. In chapter 2, *Eriogonum* demonstrated significantly greater germination than all other species independent of microhabitat; however, annual visits to the study site do not show recruitment of this species. The current experiment suggests that high densities of *Eriogonum* may reduce their seedling success.

Overall, results from the three species experiment show that density and frequency can work together or independently to affect seedling performance of *Ambrosia*. Competition between *Ambrosia* and *Larrea* is lessened by the introduction of *Eriogonum*, which does not appear to be affected by the presence of *Ambrosia* or *Larrea*. Taken together, this experiment suggests that coexistence can occur among these three species.
Implications

*Ambrosia* and *Larrea* are dominant species throughout US southwestern deserts and suffered skewed mortality due to drought. *Eriogonum* has a more limited distribution, which is exacerbated by the extensive mortality it suffered at the Colorado Desert field site. Recruitment of these species is likely to be affected by facilitator-seedling or seedling-seedling interactions. Their relative competitive abilities for nutrients and growth are important for their coexistence. Results from this study suggest that a mixed species microhabitat may deter some of the negative impacts of *Ambrosia* on the initial establishment of *Larrea*. Other studies suggest that if *Larrea* does not succumb to competitive exclusion, it may become more competitive with *Ambrosia* over time. Thus, identifying species, such as *Eriogonum* that will lessen the competitive interaction between *Ambrosia* and *Larrea* is important if restoration is necessary. Restoration may be required for community recovery after high mortality because natural regeneration has been slow.
GENERAL CONCLUSIONS

The objective of my research was to examine biotic and abiotic factors that may affect seedling regeneration. Shrub mortality has been extensive throughout US southwestern deserts, but the roles of underlying heterogeneity and plant facilitation in promoting seedling establishment have not been examined at my study site. To address this issue, I identified nitrogen availability and microhabitat variation associated with potential facilitator identity as key variables that could influence seedling establishment and early survival. First, I was interested in determining the role of facilitators in affecting soil nitrogen distributions and their capacity to provide suitable microhabitats for regeneration of woody perennials. Second, I was interested in determining the extent to which competition among seedling cohorts.

To understand plant influences on nitrogen availability, I measured heterogeneity in nitrogen distributions in locations under direct plant control and in locations independent of plant control. I focused on the microhabitats controlled by the three dominant species at the study site, Ambrosia, Larrea and Tetracoccus to serve as candidate facilitators because their distribution and abundance could provide sufficient safe sites for novel seedling recruitment. Results indicated that Ambrosia, Larrea and Tetracoccus have distinct controls over nitrogen mineralization rates that are detectable.
considering significant edaphic variation in nitrogen availability. This result suggests that loss of any of these species removes nitrogen concentrations that are unique to these species. This result, however, is restricted to the ammonium form of nitrogen and fluctuates seasonally and annually. I confirmed that nitrogen concentrations decrease with distance from shrub canopies and that nitrogen mineralization rates are higher during the winter than the summer. The winter season is when the greatest pulse of recruitment occurs, thus nitrogen availability may have a crucial role in where seedlings establish and their success as juveniles. However, there were fluctuations in nitrogen availability among winter and summer seasons that were not comparable with rainfall totals averaged among the nearby weather stations. This suggests that rainfall patterns are spatially and temporally dispersed or that available moisture does not always predict when nitrogen will become available. Through examining the open location ≥1.5 m away from shrub canopies, I was able to show that there is significant heterogeneity in nitrogen availability within the landscape to which species may also respond. This responsiveness may or may not be mediated by facilitator effects.

In order to determine species effects on the germination of woody perennials, and to isolate the effects of canopy light and NH$_4^+$: NO$_3^-$ ratios associated with the dominant species, I conducted field and greenhouse experiments. In chapter 2, seeds were sowed under the canopy, at the canopy edge and 150 cm from *Ambrosia*, *Larrea* and *Tetracoccus* in the field. These experiments showed that neither species identity or canopy microhabitats influenced seed germination. Species identity was the only factor that influenced germination rates. These results suggest that germination is not sensitive
to the range of microhabitats examined; seedlings can emerge in a broader range of microhabitats than suggested by juvenile distributions. Factors that enhance seedling growth and survival may more strongly influence juvenile distributions than germination.

In chapter 3, I examined factors that would affect the seedling stage of development in two-species and three-species combinations. Using similar light moisture and nitrogen levels found under *Ambrosia* canopies, I examined the impact of density and frequency of neighboring seedlings in a controlled environment. Using response surface experiments, I showed that *Ambrosia*’s establishment was sensitive to the frequency of conspecifics and the total density of seedlings in microhabitat. *Ambrosia*’s establishment decreased when neighboring seedlings were conspecific and also at high seedling densities. Negative frequency dependence had an adverse impact on the establishment of *Ambrosia*. When *Ambrosia* populations were mixed with less competitive species at low densities, conspecific establishment increased.

*Larrea* decreased conspecific growth through intraspecific competition and at the same time *Ambrosia* decreased *Larrea*’s biomass. *Larrea* increased in biomass when the frequency of *Ambrosia* seedlings is low. When there were three species competing for resources, *Ambrosia* and *Eriogonum* both experienced greater intraspecific competition, while *Larrea* did not respond to changes in species identity, frequency or density. The presence of *Eriogonum* appears to lessen competition for *Larrea*, whereas *Ambrosia* limited itself more than its competitor(s) when sharing a microhabitat with one or two other species. These results suggest the frequency of *Ambrosia* seedlings will greatly impact conspecific performance.
Overall this research contributes to the overall understanding of how biotic and abiotic factors that influence seedling establishment. Through these experiments I demonstrated that shrubs have distinct control over nitrogen availability under their plant canopies but their control lessens with distance and is sensitive to environmental perturbations. Nitrogen distributions are dynamic, which may explain in part why seedling recruitment is so sporadic. Shrub species examined in this study do not require facilitated establishment at the seedling stage of establishment, but where they establish may have consequences for their success as seedlings. *Ambrosia*’s competitive ability as a seedling and its capacity to establish in open microsites may explain why it is the numerically dominant shrub at the study site and why unlike many other woody seedlings, it successfully establishes in the open, in isolation of other species. Taken, together these results suggest that microhabitats for germination do not limit community recovery at this site; however, seedling response to microhabitat variation will be vital to seedling success.
REFERENCES


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