NURSING BACK TO HEALTH: SHRUBS FACILITATE THE RESTORATION OF NATIVE FORBS WITH REDUCTIONS IN NON-NATIVE COMPETITION IN AN INVASED ARID SHRUBLAND

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Abstract

Restoring native species to invaded arid ecosystems is challenging as non-native species often limit native species establishment, which limits success. Nurse plant facilitation may be utilized to improve native species establishment through reductions in abiotic and biotic stresses but this has not yet been tested for native forb restoration in invaded arid ecosystems. Five native forb species were seeded in shrub and open microsites, with and without exclosures. Non-native removals were done to determine if shrubs could facilitate native forbs establishment in an invaded arid shrubland. Shrubs facilitated native species, and interestingly, most natives co-existed with non-natives. However, non-native removals had a large positive effect for two less competitive native species. Exclosures overall were not effective at increasing native density. Seeding natives with shrubs and non-native removals are recommended as effective strategies for increasing native species establishment in invaded arid systems.
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**General Introduction**

Introductions of non-native species are a major contributing factor to the current biodiversity crisis (Sala et al., 2000; Dirzo & Raven, 2003; Barnosky et al., 2011). The negative effects of non-native species on ecosystems are well documented, and include reductions in native biodiversity, economic costs, loss of traditional landscapes, species extinctions, and alteration of ecosystem functions (Manchester & Bullock, 2000; Henderson et al., 2006; Flory & Clay, 2010; Vila et al., 2011). These negative effects on natives can occur through a number of mechanisms including competitive interactions, predation or herbivory, habitat alteration, spread of disease, and hybridization (Manchester & Bullock, 2000; Gioria & Osborne, 2014). Non-native species gain dominance in introduced ecosystems through a number of mechanisms. For a non-native species to become established in an ecosystem in the first place, it must pass through different barriers limiting successful establishment such as geographical (humans introduce the species outside of its native range), survival barrier (depends on the environmental conditions at the site), and dispersal barriers (limiting extension into surrounding areas) to name a few examples (Blackburn et al., 2011). If introduced species have a fitness advantage or are able to exploit an unfilled niche they may be able to become established and dominant (MacDougall et al., 2009). Early arrival at a site or even earlier emergence within the season by non-native species also gives a competitive advantage over native species and contributes to their dominance (Cleland et al., 2015; Stuble & Souza, 2016). Some non-native species have shown rapid evolution within their introduced range by developing mechanisms of herbivory defence such as the novel weapons hypothesis (Callaway & Ridenour, 2004). This can occur when herbivores within the introduced range do not consume the non-native species and thus these non-natives are ‘free’ to allocate resources to allelopathic chemicals that directly interfere with
native plant species that have not been exposed to these ‘weapons’ previously (Callaway & Ridenour, 2004). These complex and interacting mechanisms of non-native species introductions must be addressed when managing for conservation of native species.

Restoration ecologists and land managers commonly focus on reducing the negative effects of non-native species introductions (Genovesi, 2005; Suding, 2011; Catford, 2016). Ecological restoration is defined as “the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed” (p. 3 Society for Ecological Restoration, 2004). With increased degradation, through non-native species introductions, for example, from anthropogenic causes the need for restoration ecology has been increasingly recognized (Hobbs & Norton, 1996; Hobbs & Harris, 2001; Jorgensen, 2013). During restoration efforts, manipulations are applied with the goals of transitioning a degraded ecosystem into a recovered one. The goals for a restoration project are guided by reference systems that are non-degraded analogues or historical conditions, if the data are available (White & Walker, 1997; Swetnam et al., 1999; Asbjornsen et al., 2005). However, with climate change and further introductions of non-native species it may be impractical to use historical or reference systems for restoration goals (Hughes et al., 2005). Anthropogenic disturbances, particularly non-native species introductions, have shifted some ecosystems so far from their historical trajectories that they may now be considered “novel ecosystems” and it may be impossible to return these ecosystems to their historical conditions (Hobbs et al., 2006; Hobbs, 2007; Seastedt et al., 2008; Hobbs et al., 2009). Instead, alternate goals have been proposed including considering restoring for resilience and resistance (Funk et al., 2008; Hobbs et al., 2009), hybrid ecosystems, which allow the persistence of some non-natives as well as native species (Hobbs et al., 2014), and restoring for structure and function of
ecosystems (Mitsch, 2012; Higgs et al., 2014; Standish et al., 2014; Awasthi et al., 2016).

However, a continuing major challenge for land managers is the negative effects exerted by the non-native species limiting restoration of native targets (Flory and Clay, 2010; Ketenring & Adams, 2011; Jauni & Ramula, 2015). Thus, determining methods to increase native biodiversity and restore ecosystem function in invaded ecosystems is an important challenge for advancing restoration ecology in these systems.

To achieve successful restoration outcomes, ecological theories are often used to inform and guide managers on what manipulations to apply. Community ecology theory has been readily applied to restoration projects including succession, alternative stable states, filters, life history, recruitment limitations, and connectivity, to name just a few (Suding et al., 2004; Young et al., 2005; Lake et al., 2007). Competitive interactions have previously dominated community ecology theory with very little attention given to positive or facilitative interactions (Bertness & Callaway, 1994; Callaway, 1995; Bruno et al., 2003). Similarly, competitive interactions are commonly the focus for restoration projects, particularly in invaded ecosystems, because manipulations attempt to remove competitive non-natives or identify competitive native species that are able to persist within these landscapes (Young et al., 2005; Brown et al., 2008).

However, within the last two decades, research has shown that positive interactions can have an important role in structuring communities especially in high stress environments such as alpine, salt marsh, or desert ecosystems (Bertness & Callaway, 1994; Bertness & Hacker, 1994; Callaway, 1995; Bruno et al., 2003). These positive interactions could therefore play an important role in restoration, especially in stressful environments (Padilla & Pugnaire, 2006; Brooker et al., 2008; Ren et al., 2008). Facilitation occurs between two or more species when
one species benefits, but neither is harmed (Bertness & Callaway, 1994; Stachowicz, 2001; Bruno et al., 2003). Facilitation is commonly observed with “nurse plants” which reduce harsh conditions within their canopy allowing other plant species to establish (Franco & Nobel, 1989; Holmgren et al., 1997; Flores & Jurado, 2003). Nurse plants reduce stress within their canopy through a number of mechanisms including reducing temperature extremes and herbivory, and increasing soil moisture and nutrients (Filazzola & Lortie, 2014). Thus, in high stress ecosystems, the positive effects of nurse plants may be a necessary consideration for the successful establishment of native species in restoration projects.

Positive interactions have been applied previously to some restoration projects. For example, soil mutualisms between native species and mycorrhizal fungi have been used to improve the establishment of native species (Zhang et al., 2011). Nucleation - planting species in clusters or islands - has also had success in reforestation projects by mimicking natural processes of succession (Carriere et al., 2002; Reis et al., 2010; Holl et al., 2011). Nurse plants have also been used as tools for restoration, but currently have only focused on reforestation (Gomez-Aparicio et al., 2004; Gomez-Aparicio, 2009; Zwiener et al., 2014) and have not been applied to other disturbances or ecosystems. However, it is likely that nurse plants would be able to increase the establishment of other functional group targets in restoration projects and in other ecosystems. These positive effects of nurse plants may help overcome many of the negative effects of non-native species on native plant species and thus increase target establishment within invaded ecosystems.
The purpose of this thesis was to apply a first test of the ability for shrubs to act as nurse plants and facilitate the establishment of native forbs in an invaded arid shrubland. Facilitation by shrubs helps to structure communities in desert ecosystems, and thus restoration projects must account for their effects. Globally, arid ecosystems are becoming increasingly susceptible to species invasion (Smith et al., 2000; Brooks et al., 2004). Therefore, research is needed that examines restoration of native forb species into invaded arid ecosystems. Here, we conducted the first test by seeding and measuring the response of five native forb species in two microsites (shrub and open) with and without a reduction in consumer pressure (through the use of exclosures) and with and without competition from non-natives (removing or leaving non-native neighbours intact). These effects were tested within a single year, and therefore, could not detect the possibility for year effects influencing the outcome of these interactions. Nonetheless this was (to our knowledge) the first study to test the use of shrubs for facilitating the establishment of native forbs into an invaded ecosystem and will serve as a test of the capacity of this seeding method to be used in future restoration projects.
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Summary

Restoring invaded ecosystems by increasing native establishment is a common focus for land managers, however, establishing native species in these ecosystems has had limited success. Previous work on reforestation has successfully used nurse plants to increase restoration success, but this method has not been applied to forb restoration in invaded ecosystems. It is hypothesized that shrubs will facilitate native forbs through reductions in consumer pressure, abiotic stress, and competition from non-native species in an invaded arid shrubland. The following predictions were tested: i) shrubs will facilitate native forbs, ii) native forbs will increase with non-native removals, and iii) reductions in consumer pressure through exclosures will be most effective in open microsites. To test this, five native forb species were seeded in shrub and open microsites, with and without exclosures and with and without non-native removals. Shrubs had either a positive or neutral effect on the biomass of all species, but a negative effect on abundances. Interestingly, most natives co-existed with non-natives species, however, two native species were found to have a large positive effect of non-native removals. Exclosures provided positive effects for both biomass and abundance of a few species. Seeding native forbs within shrub canopies is a successful strategy for improving the establishment of native species. Removing non-natives may also be required to improve the success of less competitive native species. Thus, seeding with shrubs and with non-native removals is recommended as the most effective strategy for increasing the establishment of native forbs. Future studies should test if these positive effects persist over time and at larger spatial scales.
**Introduction**

Invasion by non-native species has been described as one of the main contributing factors to the current biodiversity crisis (Sala et al., 2000; Dirzo & Raven, 2003; Barnosky et al., 2011). The effects of non-native species on introduced communities have been well documented and include reducing biodiversity, biomass, and ecosystem function (Flory & Clay, 2010; Kettenring & Adams, 2011; Vila et al., 2011). Therefore, restoring invaded ecosystems has been a focus for land managers (Genovesi, 2005; Suding, 2011; Catford, 2016), but controlling non-natives has been challenging and resulted in limited success. This mixed success has been attributed to studies that have limited applicability to land management by only examining small plots, short time frames, or too few species (Kettenring & Adams, 2011; Belnap et al., 2012). Restoration efforts can also result in secondary invasions either by only removing target invaders, but leaving other invaders, or by restoration efforts on degraded lands, which may have the unintended consequence of promoting invasion (Allen et al., 2005; DeMeester & Richter, 2010; Pearson et al., 2016). A major limitation to a number of restoration projects is that non-native species removals are not followed up with planting natives which may allow for re-invasion or limited success (Kettenring & Adams, 2011). Re-establishing native species within heavily invaded sites may be difficult as non-native species often exert negative effects (Flory & Clay, 2010; Kettenring & Adams, 2011; Jauni & Ramula, 2015). These can include soil legacies (Grman & Suding, 2010) and niche pre-emption (Dickson et al., 2012), which allows non-native species to gain dominance. Therefore, identifying methods of increasing the establishment and persistence of native species within invaded landscapes is important for the success of these restoration projects.
Positive interactions among plants can increase establishment in high stress environments. This occurs when a benefactor species or “nurse plant” alters environmental conditions within its canopy which favours establishment by other plants. In arid environments, shrubs commonly act as nurse plants through a number of abiotic mechanisms including reducing temperature extremes, increasing soil moisture and improving soil conditions such as nutrients and microbial biomass and activity (Hortal et al., 2013; Rodriguez-Echeverria et al., 2013; Filazzola & Lortie, 2014; Rodriguez-Echeverria et al., 2016). Shrubs may also indirectly facilitate species under their canopy by providing protection from consumers, especially if the shrub possesses traits such as dense branching or physical/chemical defenses to deter consumers (Barbosa et al., 2009; Perea & Gil, 2014). Shrub facilitation, therefore, could be utilized to improve restoration outcomes as positive interactions can increase the establishment of native species (Padilla & Pugnaire, 2006). Nurse plants, mainly shrubs, have successfully been applied to reforestation projects due to their ability to limit abiotic stress and protect from consumer pressure (Gomez-Aparicio et al., 2008; Rey et al., 2009; Bueno & Llambi, 2015). However, this method has not yet been applied to restoration efforts looking to increase native forb species in invaded ecosystems.

The use of shrub facilitation to restore natives in an invaded ecosystem may be challenged by interactions from non-natives and consumers. Previous work has shown that nurse plants can facilitate invasive species (Cavieres et al., 2008; Rodriguez-Buritica & Miriti, 2009) and this may reduce the likelihood of natives being able to establish. Additionally, consumer pressure can reduce the overall success of restoration efforts if they preferentially feed on natives (Vavra et al., 2007; Orrock et al., 2008). Conversely, in some systems if consumer pressure is eliminated it
can lead to competitive dominance of a few highly competitive invasive species (Osem et al., 2007; Heard & Sax, 2013; Gross et al., 2015). Restoration efforts looking to apply facilitation as a mechanism to support native reintroductions need to consider the potential co-occurring interactions with competitors and consumers. In California, many ecosystems have become heavily invaded with Mediterranean annual grasses that outcompete native species (Seabloom et al., 2003; Cox & Allen, 2008; HilleRisLambers et al., 2010) and these problematic invaders are expected to increase in dominance (McKinney & Cleland, 2014). There has been limited success in attempts to restore native species to invaded arid ecosystems and planting natives with shrubs may be an effective strategy to improve restoration outcomes.

Shrubs can facilitate the establishment of native forbs in an invaded arid shrubland, but their ability to do so has never been tested. Here, we conducted the first test by seeding and measuring the response five native forb species in two microsites (shrub and open) with and without a reduction in consumer pressure (through the use of exclosures) and with and without competition from non-natives (removing or leaving non-native neighbours intact). Although our current design captures a single growing season and, therefore, cannot detect potential year effects, this is (to our knowledge) the first study using shrub facilitation to restore seeded native forbs in an invaded ecosystem. Thus, we aim to test the capacity for this seeding method to be used for future restoration projects in arid systems. We hypothesized that shrubs facilitate native forb establishment through a reduction in consumer pressure and abiotic stress, when competition from non-native species is removed in an invaded arid shrubland. The following predictions were tested in the field: i) shrubs will facilitate native plant species establishment, ii) native species establishment will increase in both microsites with the removal of non-native plant
species and, iii) reductions in consumer pressure through exclosures is more important in the open where there is not a shrub-refuge effect. Collectively, this examines both the direct effects of shrubs as restoration agents and the indirect effects of non-native plant species and consumers on the potential contributions of shrubs to native forbs.
Methods

Study Site

The experiment was conducted at Panoche Hills Recreation Area in California, USA (36°41.776’N, 120°47.886’W, 650 m.a.s.l.). This site has sandy loam soils, and it is an arid shrubland with a Mediterranean climate located within the San Joaquin Desert and is heavily invaded with non-native grasses including *Bromus madritensis* ssp. Rubens, *Bromus diandrus*, *Bromus hordeaceus*, and *Schismus barbatus*. The most dominant native shrub within the landscape and the focal shrub in this study is *Ephedra californica*. Other less abundance shrubs species include *Juniperus sp.*, *Ericameria sp.*, and the non-native shrub *Marrubium vulgare*. The overall landscape consists of steep hills and canyons, but the study site was conducted on a plateau. Native forb species comprise a very small proportion of the total plant biomass (1%) and are generally restricted to south facing slopes where Mojave Desert species may be found including *Chaenactis fremontii*, *Caulanthus inflatus*, *Malacothrix glabrata*, and *Salvia sp.* Other native forb species include *Amsinckia tessellata*, *Calachortus clavatus*, *Castilleja brevistyla*, *Cryptantha sp.*, *Erinogonum sp.* *Lasthenia sp.*, *Lepidium Nitidum*, *Monolopia lanceolata*, and *Phacelia tanacetifolia*. The growing season is determined by seasonal rains that usually begin in October and last until March. The site on average receives 18.5 cm of rain annually and has a mean July maximum temperature of 38.2°C (Panoche Road California weather station (CA2265A6), Western Regional Climate Center (WRCC 2016), 36.72° N, 120.75° W, 619 m.a.s.l.) The 2015-2016 growing season began in October 2015 and ended in April 2016 (end of flowering and beginning of senescence). This was an El Niño year that results in above average rainfall for the study site and in California in general, with the study site receiving 20.1 cm of rain for the whole growing season. The site was grazed with 600 sheep beginning in April and
continued into May 2016. Other herbivores at this site include the black-tailed jackrabbit (*Lepus californicus*), desert cottontail (*Sylvilagus audubonii*), Heermann’s kangaroo rat (*Dipodomys heermanni*), and the San Joaquin antelope squirrel (*Ammospermophilus nelson*).

**Study Species**

The following five native forb species were selected for seeding into this site: (*Amsinckia tessellata* (A. Gray), *Caulanthus lasiophyllus* (Hook & Arn) Payson, *Lepidium nitidum* (Torrey & A. Gray), *Monolopia lanceolata* Nutt., and *Phacelia tanacetifolia* Benth.). *Caulanthus lasiophyllus* and *Lepidium nitidum* were collected at a nearby location to Panoche Hills in May 2015 (36.53° N, 120.69° W, 509 m. a.s.l.). The other three species were purchased from S&S Seeds Inc. located in Carpinteria, California in 2015 (approximately 400 km away from the study site). These species needed to be purchased due to low seed availability at the site and surrounding area for these species. All five species can found at Panoche Hills in very low densities or are thought to have occurred here previously (R. O’Dell, personal communication, May 2014). *Amsinckia tessellata* and *Phacelia tanacetifolia* are both within the Boraginaceae family. *Amsinckia tessellata* is a common weedy species that produces yellow flowers that coil back, and produces bristly hairs over the stem and flowers from March until June (Kelly and Ganders 2016). *Phacelia tanacetifolia* can range in height from 15 – 100 cm and is erect with few branches and is covered in short hairs. It has bell-shaped purple flowers that are dense and branched and flowers from March to May (Walden et al. 2016). *Caulanthus lasiophyllus* and *Lepidium nitidum* are in the Brassicaceae family. *Caulanthus lasiophyllus* is a common desert species that produces small white terminal flowers that are clustered and flowers from March until June (Al-Shehbaz 2016). *Lepidium nitidum* produces white flowers and is often found
alkaline soils and flowers from February until May (Al-Shehbaz 2016). Monolopia lanceolata is in the Asteraceae family and is an erect plant that may be simple or branched with a yellow ray or disk flower and is often found in grassland habitats, flowering between February and June (Johnson 2016). Ephedra californica is a shrub species native to California and found in the Ephedraceae family. It has yellow-green needle like leaves with dense branching, produces cones, and is scattered throughout arid shrublands globally (Ickert-Bond 2016).

Baseline estimates of germination, germination rate, and viability were conducted on all five species (Appendix A). Germination percentage and germination rate were tested in growth chambers programmed to simulate the shrub and open conditions associated with this specific study site (temperature and relative humidity). These estimates were derived from HOBO ProV2 loggers deployed in the field in the preceding season, 2014 (Appendix A). Germination was not significantly different between growth chamber simulated microsites (Fig. A1). For two species (Phacelia tanacetifolia and Monolopia lanceolata), days to 50% germination occurred significantly earlier when seeds were germinated in open simulated conditions ($\chi^2 = 15.95$, df= 5, p<0.007) (Fig. A1). Percent viability was approximately 100% for three of the five species (Fig. A2).

**Study Design**

Sixty shrub-open paired sites were used in this study (N = 120). Shrub-open paired sites were randomly selected from a representative live shrub (at least 50% live canopy) size class for the plateau. Half of the paired shrub-open sites (thirty) had animal exclosures erected in January 2014. Exclosures were circular in shape and constructed with a 70 cm diameter using 21 ga
galvanized poultry netting buried 10 cm below ground and 1.2 meters above the surface. Exclosures were built on the north side of shrubs as the north side of the shrub has been shown to have a greater facilitative effect (Castro et al., 2002; Schafer et al., 2012). The paired open exclosures were located 2 m north of the shrub. Half of all pairs were randomly selected to be used for the non-native removal treatment. On October 24, 2015, seed from the five forb species were added to each treatment (microsite, exclosure, removals) in a fully orthogonal design with 10 replicates (N = 80). Twenty paired sites were randomly selected to be controls (no seed added) that were fully orthogonal for each treatment (n = 5). One gram of seed from each of the five native species (mean number of seeds in 1 g: *Amsinckia tessellata* = 235.2 ± 7.612 SE; *Caulanthus lasiophyllus* = 3019 ± 8.916 SE; *Lepidium nitidum* = 752.4 ± 4.261 SE; *Monolopia lanceolata* = 737.8 ± 7.832 SE; *Phacelia tanacetifolia* = 772 ± 5.301 SE) was evenly added to hand-dug furrows within the center of each plot (N = 80) to avoid potential edge effects. On January 15, 2016 all non-native emerging vegetation was hand-pulled from removal treatment plots. Additional removals of emerging non-native vegetation were performed throughout the season as needed. Shrub size (area and volume) and canopy cover were measured and included as covariates.

Micro-environmental conditions including air temperature, light, and relative humidity were measured in shrub and open sites using HOBO pro-v2 loggers (six loggers per microsite). The sensor for each logger was placed approximately 5 cm above the ground. Microsites were randomly selected for placement of the loggers. Loggers within the shrub were placed on the north side. Photosynthetically active radiation (PAR) was also recorded using a Licor LI-250A light meter quantum sensor on February 4, 2015 (a clear, sunny day) between 10 am and 12 pm.
Soil moisture levels (top 10 cm of soil) were measured using a Delta SM150 probe on January 16, 2016 and again on March 23.

**Data Collection**

Plant surveys of abundance, biomass (above and below-ground), and average number of flowers for each species were completed for all plots on March 12-13, 2016. Biomass was collected by harvesting a randomly selected individual plant from each species within a plot. Both above-ground biomass and below-ground biomass were harvested for analyses. The biomass samples were then dried in Yamato DNK900 drying ovens at 85°C for 7 days before weighing using a Mettler Toledo XS204 Excellence XS analytical balance. Flowers or fruits were counted from three random individuals of each species from each plot. These counts were then averaged for each species within each plot.

**Statistical Analyses**

To test for evidence of shrubs altering microclimatic conditions within their canopy, environmental variables including soil moisture, temperature, and relative humidity were compared among microsites. Soil moisture data (converted from a percentage to a proportion) from January and March were compared in separate models between the three factors (microsite, exclosures, removals) using Generalized Linear Models (GLMs) fit to a binomial distribution and the logit link function. Data for temperature (°C) and relative humidity (percent converted to a proportion) were subset to include only the growing season from these loggers (November – April) and two hours mid-day (12:00 – 14:00) because this time frame is the most variable time period. Total, early (November-January), and late (February-April) growing season measures
were contrasted to within-season variability. T-tests (t.test function) were performed to examine the differences between shrub and open microsites for temperature and variance throughout the growing season. Differences between shrub and open microsites in relative humidity and variance throughout the growing season were compared using GLMs fit to a binomial distribution with the logit link function. A GLM was required for these comparisons as the data does not assume a normal distribution required for t-tests.

Each response variable (abundance, above-ground biomass, below-ground biomass, and mean number of flowers) was compared for the three factors (microsite, exclosure, removals) and their interaction terms. Variable selection for covariates was performed using a correlation matrix corrplot function (corrplot package) to remove collinear variables, and best subsets regression using the regsubsets function (leaps package) to remove variables with low $R^2$ values. The covariates shrub volume and shrub area were found to be highly collinear as well as shrub canopy density and PAR so only shrub area and PAR were included in the remaining models (Appendix B). Variable selection through best subsets regression showed that all of the covariates (shrub area, PAR, or soil moisture) explained a low proportion of the variation for all responses and so they were not included in the final models (Appendix C). Initially, statistical models were performed with species, microsite, exclosure and removals as fixed factors (Appendix D). However, the significant interactions with species as a factor were noticeably due to species-specific differences. Additionally, due to low sample sizes for one species in particular (Monolopia lanceolata), multiple comparisons would not be possible in the subsequent post hoc analysis for this model. Therefore, statistical models were performed separately for each species, rather than including species as a factor, to take into consideration any species-specific differences. Each species can be considered independent, and whether or not corrections for
multiple comparisons is required to reduce Type I error can be debated (Cabin and Mitchell 2000; Quinn and Keough 2002). Due to the fact that we are not interested in differences between species per se, we are considering the influence of species identity to be an independent factor, and in the interest of saving statistical power, it was decided that separate models for each species would be performed as has been previously conducted in other studies (Maestre et al., 2001; Maestre et al., 2003; Poyry et al., 2005; Bischoff et al., 2006; Tomiolo et al., 2015; Zhang et al., 2015). To test for differences in abundance among the three factors (microsite, exclosure, and removal), GLMs fit to a negative binomial distribution (to account for over-dispersion) using the glm.nb function (MASS package) were performed using the logit link function (MASS package). Above and below ground biomass, and mean number of flowers analyses were performed using an ANOVA using function aov. Normality and heterogeneity of variances were determined using Shapiro-Wilks (shapiro.test function) and Levene’s test of homogeneity of variances (leveneTest function) respectively. To achieve normality and/or homogeneity in variances the following transformations were performed: Caulanthus lasiophyllus, Monolopia lanceolata, and Phacelia tanacetifolia above and below-ground biomass were log transformed, Lepidium nitidum above-ground biomass was log transformed, Amsinkica tessellata below-ground biomass was square-root transformed, Monolopia lanceolata mean number of flowers was log transformed, and Phacelia tanacetifolia mean number of flowers was square-root transformed. Interactions between factors could not be calculated for Lepidium nitidum mean number of flowers due to a low number of flowering individuals. Diversity estimates including species richness and Simpson’s diversity index were calculated to test for differences among treatments. Richness was tested using a GLM fit to a Poisson distribution with the log link function. Simpson’s diversity estimates were analyzed using a GLM fit to a binomial distribution
and the logit link function.

To compare the ecological effect of the three factors (microsite, exclosure, removals) on the response of native forbs, an effect size estimate RII (the Relative Interaction Index; Armas et al. 2004) was calculated using the following equation: \[ RII = \frac{(t-c)}{(t+c)} \] where \( t \) is the plant response in the “treatment” (i.e. shrub, exclosure, and non-native removal) and \( c \) is the plant response in the “control” (i.e. open, no-exclosure, and non-natives present). RII values range from +1 to -1 where positive values indicate a treatment effect and negative values indicate a control effect whereas values that are not significantly different from zero indicate a neutral effect. Bootstrapped RII values (999 iterations) were calculated for each factor and response variable among all species (Filazzola, A., 2016 https://dx.doi.org/10.5281/zenodo.60810). All statistical analyses were performed in R (R development core team 2015) and the script can be found in Appendix E.
Results

Micro-environmental contrasts between shrub and open microsites

Shrub and open microsites differed in the mean and variance of the micro-environmental variables measured (temperature, and relative humidity). These differences occurred across the entire growing season and by early and late growing season contrasts. Temperature was significantly different between the shrub and open microsites for the entire growing season ($T_{2373} = 2.48$, $p = 0.013$) with shrubs being significantly warmer in the early season ($T_{1594.3} = -3.88$, $p = <0.001$, $\bar{x}_{\text{shrub}} = 5.23^\circ C \pm 0.06$, $\bar{x}_{\text{open}} = 4.32^\circ C \pm 0.08$) and cooler in the late season ($T_{1091.4} = 3.10$, $p = 0.002$, $\bar{x}_{\text{shrub}} = 11.7^\circ C \pm 0.07$, $\bar{x}_{\text{open}} = 12.7^\circ C \pm 0.13$) compared to the open microsite (Fig. F1). Temperature variability was also significantly different between shrub and open microsites with the shrub being consistently less variable (entire growing season: $T_{447.48} = 6.81$, $p = <0.001$, $\bar{x}_{\text{shrub}} = 29.8 \pm 2.85$, $\bar{x}_{\text{open}} = 51.7 \pm 1.48$; early growing season: $T_{341.78} = 2.01$, $p = 0.045$, $\bar{x}_{\text{shrub}} = 28.7 \pm 1.87$, $\bar{x}_{\text{open}} = 33.8 \pm 1.67$; late growing season: $T_{172.18} = 7.23$, $p = <0.001$, $\bar{x}_{\text{shrub}} = 31.4 \pm 1.87$, $\bar{x}_{\text{open}} = 75.6 \pm 5.62$). Relative humidity was consistently lower within the shrub compared to the open (entire growing season: $\chi^2_{2691} = 19.5$, $p = <0.001$, $\bar{x}_{\text{shrub}} = 81.6\% \pm 0.15$, $\bar{x}_{\text{open}} = 89.4\% \pm 0.14$; early growing season: $\chi^2_{1619} = 9.28$, $p = 0.002$, $\bar{x}_{\text{shrub}} = 83.7\% \pm 0.16$, $\bar{x}_{\text{open}} = 91.7 \pm 0.19$; late growing season: $\chi^2_{1066} = 21.1$, $p = <0.001$, $\bar{x}_{\text{shrub}} = 80.0\% \pm 0.27$, $\bar{x}_{\text{open}} = 87.0\% \pm 0.25$) (Figure F1). In contrast, the variation in humidity was consistently higher in the shrub when compared to the open (entire growing season: $T_{252.75} = -4.28$, $p = <0.001$, $\bar{x}_{\text{shrub}} = 184 \pm 3.20$, $\bar{x}_{\text{open}} = 85.8 \pm 2.80$; early growing season: $T_{146.19} = -2.18$, $p = 0.031$, $\bar{x}_{\text{shrub}} = 91.9 \pm 1.99$, $\bar{x}_{\text{open}} = 47.8 \pm 2.10$; late growing season: $T_{72.85} = -9.21$, $p = <0.001$, $\bar{x}_{\text{shrub}} = 457 \pm 5.89$, $\bar{x}_{\text{open}} = 52.4 \pm 2.26$). Soil moisture was recorded early in the growing season (January) and at the end of
the growing season (March) in both shrub and open conditions and at neither time was a significant difference between microsites detected.

**Shrub facilitation**

Shrubs had an overall positive or neutral effect for all species and most response variables. Above-ground biomass increased for three of the five species examined (*Lepidium nitidum, Monolopia lanceolata*, and *Phacelia tanacetifolia*), and was not different between shrub and open microsites for the other two species (Table 1, Fig. 1). Conversely, shrubs only facilitated the below ground biomass of *Lepidium nitidum*, while all the other species were neutral (Table 2, Fig. 1). Shrubs decreased the abundance of two species (*Lepidium nitidum, Monolopia lanceolata*) (Table 3, Fig. 2) despite increasing their above-ground biomass, and in the case of *Lepidium nitidum*, below-ground biomass as well. The average number of flowers per individual increased in shrub microsites in comparison to open microsites for a single species (*Phacelia tanacetifolia*) (Table 4, Fig. G1). The shrub understory increased species richness ($\chi^2 = 4.08$, df = 1, $p = 0.043$, $\bar{x}_{\text{shrub}} = 2.86 \pm 0.19$, $\bar{x}_{\text{open}} = 3.60 \pm 0.24$), but not Simpson’s diversity.

**Consumer pressure reduction**

Consumer pressure was reduced through the use of exclosures at shrub and open microsites and this treatment had a positive or neutral effect on all species and all response variables measured. Above-ground biomass was greater with exclosures for two species (*Caulanthus lasiophyllus, Phacelia tanacetifolia*; Table 1, Fig. 1). Similarly, the below-ground biomass of *Caulanthus lasiophyllus* was greater in exclosures than without (Table 2, Fig. 1). Abundance was greater in the exclosures for most species (Table 3, Fig. 2). Exclosures had higher *Caulanthus lasiophyllus* abundance although above- and below-ground biomass remained neutral. The mean number of
flowers per individual was greater for two species (*Caulanthus lasiophyllus* and *Phacelia tanacetifolia*) when in exclosures compared to outside of exclosures (Table 4, Fig. G1).

**Removing non-native competition**

The effect of removing non-native competitors on the five native forb species was also positive or neutral for all species and response variables measured. Above-ground biomass increased for two species (*Amsinckia tessellata*, *Monolopia lanceolata*) when non-natives were removed Table 1, Fig. 1). Removing non-natives did not increase the below-ground biomass for any species (Table 2, Fig. 1). Non-native removes resulted in an increased in the abundance of a single species (*Caulanthus lasiophyllus*) (Table 3, Fig. 2). Non-native removals did not result in an increase in the mean number of flowers per individual of any species (Table 4, Fig. G1).

**Relative effect of each treatment**

Comparisons between the three treatments were made using the Relative Interaction Index (RII) to determine the treatments that were most effective for establishing native forbs. Treatments were bootstrapped and significance assessed when 95% confidence intervals were greater or less than zero. Shrub facilitation positively affected above- and below-ground biomass, but not abundance for native forbs (Fig 2). Exclosures were not related to biomass, but increased native forb abundance (Fig 2). Removal of non-natives increased above-ground biomass and plant abundance, but not below-ground biomass (Fig 2). None of the treatments had an effect on the mean number of flowers per individual (Figure F2).
Discussion

Restoration of invaded arid ecosystems is of increasing concern globally and novel techniques that are easily implemented are required to enhance the probability of success. These results demonstrate that shrubs can be used to facilitate the establishment of native forbs and thus may be an effective restoration strategy for invaded arid landscapes. However, removals may be required for some less competitive native target species, and shrubs were not effective at reducing consumer pressure. Thus, our hypothesis that shrubs facilitate native forb establishment through a reduction in abiotic stress was supported, but not through reductions in competition or consumer pressure. Contrary to our prediction, removals of non-natives were species specific and benefited only some of the target native species. Positive effects of exclosures were also species specific and there was no significant interaction with microsite suggesting shrubs are not deterring herbivory. Seeding native forbs and removing non-native competition is recommended as an effective strategy to increase native forbs at least at this site. However, we could not test for inter-annual consistency of these trends because we did not test over multiple years. Both facilitative and competitive interactions are necessary considerations in restoration projects for native forbs. Future studies should examine if the shrub effect continues to support restoration goals over time.

*Facilitation varies with response variable and species identity*

The mechanism of facilitation by shrubs on native biomass was likely due to favourable changes in abiotic conditions under the shrub canopy and not protection from consumer pressure. Micro-environmental amelioration, rather than biotic mechanisms, has been identified as the main mechanism of facilitative shrub interactions in other studies (Pugnaire et al., 2004; Gomez-
Aparicio et al., 2005; Gomez-Aparicio et al., 2008; Howard et al., 2012). In our study, changes in temperature and potential soil conditions likely resulted in the positive effect of shrubs on native forb biomass. Shrubs provided a warmer microclimate in the early season that can protect from frost and provided protection from hot temperatures later in the season (Gomez-Aparicio et al., 2008; Valles et al., 2011; Tian & Wang, 2015), however, shrubs did not increase relative humidity or soil moisture. The lack of a positive effect of *Ephedra californica* on soil moisture and humidity may be due to the canopy structure of this species of shrub’s, where its needle-like leaves likely permitted high air and moisture exchanges that reduced the soil moisture and humidity levels (Kropfl et al., 2002; Gomez-Aparicio et al., 2005; Keyes & Maguire, 2008). Additionally, our soil moisture readings were recorded within the top 10 cm of soil, but previous reports of increased soil moisture with facilitation occurred deeper within the soil (Li et al., 2010; Prieto et al., 2011). Although we did not measure soil nutrients or soil microbe levels in this study, previous research has shown that shrubs can alter each of these and have a strong influence on shrub associated plants (Rodriguez-Echeverria et al., 2013; Rodriguez-Echeverria et al., 2016). Thus, this is another possible mechanism to explain the increased biomass observed in this study. The abiotic mechanisms of shrub facilitation on the native forbs were more important than protection from consumers for restoration in an invaded ecosystem.

Shrubs facilitate productivity, but not fitness of the native forbs within their canopy. Other studies have shown that shrubs increase biomass of plants and this can be attributed to higher resources within the shrub allowing increased growth (Padilla & Pugnaire, 2006; Valles et al., 2011; Rathore et al., 2015). Increased biomass is important as it indicates greater competitive ability, survival, and fitness (Howard & Goldberg, 2001; Keddy et al., 2002). Biomass may be a
more important metric than abundance for restoration purposes, because increasing the competitive ability and growth of native species are desirable outcomes when attempting to restore invaded landscapes and ensure persistence of the target species (Miller, 1987; Donohue et al., 2010). Increasing the abundance of natives is a common goal in restoration projects (Ruiz-Jaen & Aide, 2005; Thorpe & Stanley, 2011; Wortley et al., 2013), however, if there are many individuals with low biomass, the native species may not be able to persist long term and the restoration project may not succeed (Miller, 1987; Donohue et al., 2010). Thus, our result of a positive effect of shrubs on native forb biomass, but not abundance, is viewed as a desirable outcome for restoration and not unexpected. This is because higher growth within the shrub likely resulted in increased intraspecific competition and fewer, but larger individuals (Ungar, 1992; Mangla et al., 2011; Saha et al., 2014). Above-ground, but not below-ground biomass, was different between microsites suggesting that competition occurs above-ground and that resource partitioning exists among the shrub and understorey species (Ba et al., 2006; Armas et al., 2009). There was no evidence of shrubs increasing the reproductive output of the native forb species in this study although this has been reported in a previous study (Shumway, 2000). Future studies should consider treatments that increase the reproductive output of native species because it likely increases the success of the restoration project and ensure that natives persist in the landscape over the long-term.

A consistent pattern observed in this study is that facilitative outcomes are species specific. Species-specific traits for both nurse species and the beneficiary species can impact the outcome of plant-plant interactions (He et al., 2012; Noumi et al., 2015). Traits for nurse plants that should be considered prior to planting include allelopathy and canopy structure (Tian & Wang,
For instance, the shrub *Larrea tridentata* can facilitate native annuals by providing shade and reducing heat stress, but can inhibit root elongation of perennial plants (Mahall & Callaway, 1992). Traits of target species that should be considered include shade tolerance, competitive ability and stress tolerance (Tian & Wang, 2015). Additionally, some species have been found to prefer found either the shrub or open microsite and thus will have a negative effect when placed in the non-preferred microsite (Liancourt et al., 2005; Alday et al., 2014). Therefore, species selection for restoration projects of both the nurse plant and the target species is important to ensure the success of seeding species with nurse plants.

*Shrubs did not reduce consumer pressure*

Protection from herbivores by nurse plants is a common form of facilitation for other plant species. However, this study found that shrubs did not deter consumer pressure at this site. Previous studies have found reducing consumer pressure to be the most important facilitation mechanism in dryland savannas (Louthan et al., 2014; Torres & Renison, 2015). Although there is no evidence in this study that shrubs could reduce consumer pressure, an alternative explanation could be that the grazing intensity by sheep at the site may have been too high and there was a collapse of facilitation (Smit et al., 2007; Abdallah & Chaieb, 2012; Soliveres et al., 2012). Sheep also prefer to eat a diverse diet and may have targeted the native forbs regardless of shrub cover because the study site was largely dominated by annual grasses (Parsons et al., 1994). Grazing or herbivory treatments have been suggested as a restoration strategy to reduce non-native biomass (La Pierre et al., 2010; Heard & Sax, 2013; Gross et al., 2015). However, this could introduce unintended negative effects by changing the dominance of the functional group of the invader (Kimball & Schiffman, 2003; Stahlheber & D’Antonio, 2013). Additionally, it can have negative effects if the invaders, but not the native species, are resilient
to consumer pressure (Kimball & Schiffman, 2003; Reisner et al., 2013). Although there has been some success using herbivores and grazing for restoration (Hayes & Holl, 2003; La Pierre et al., 2010), we did not find a positive effect of consumer pressure and found that all species benefit from being within the exclosure for at least one response variable. This suggests that grazing and/or herbivory may not be effective at increasing native forbs at invaded arid ecosystems (Gomez-Aparicio et al., 2008).

Native forbs can coexist with non-natives

Most species were able to coexist with non-natives and did not display a negative effect when non-natives were present. However, a few species Amsinkcia tesselata and Monolopia lanceolata experienced lower above-ground biomass with non-natives, and Caulanthus lasiophyllus was more abundance with non-native removals. The difference in above-ground biomass would suggest that the competition with the two species was for above-ground resources. Although only a few species benefit from the removals, we found a large positive effect of non-native removals for all response variables except for below-ground biomass, which had a neutral effect. Thus, it is recommended that non-native removals be performed in restoring native forbs to invaded sites in the future, as they may prevent some species from becoming established (Flory & Clay, 2010; Dickson et al., 2012; Bennett et al., 2014). It has been suggested that natives and non-natives do not differ in competitive ability, but generally natives are able to outperform non-natives in low resource conditions (Daehler, 2003). Given that the native species in this study were able to coexist with the non-natives under relatively high resource conditions (i.e. El Niño wet year), it may be possible that these species may actually outcompete and not just coexist with non-natives in lower resource years and this should be investigated in future studies. Furthermore, other studies have shown that native species tend to be seed limited which may suggest that current
levels of dominance by non-natives may not simply be the result of direct competition (Seabloom et al., 2003). Therefore, adding natives should be a focus for restoration on top of removing non-native species. Additionally, when removals are attempted, all non-native species should be removed and not just “problematic” invaders as selectively removing a dominant invader can result in secondary invasions or another invasive species becoming dominant (D’Antonio & Meyerson, 2002; Allen et al., 2005; Cox & Allen, 2011). Restoration of native forbs is dependent on removing all non-natives despite potential co-existence, particularly given that arid ecosystems have such a high variability in climate.

**Future directions**

Our study provides evidence that shrubs can be an effective tool for establishing native forbs to an invaded arid shrubland through abiotic stress amelioration, but not by reductions in consumer pressure. However, as this study was conducted in one year and in experimental sized plots, future studies should replicate over multiple years and at larger plot sizes than those used in this study. Brief temporal and spatial scales are a limitation of this and many other restoration projects that needs to be addressed in future research (Kettenring & Adams, 2011). Inter-annual effects are critical considerations for restoration projects in general (Young et al., 2005) because variation among years can influence treatment effects and study outcomes (Brooks, 2000; Bakker et al., 2003; Vaughn & Young, 2010; Wilson, 2015). Although we found a trend with shrub facilitation and removals benefiting some species, these trends may change in future seasons. This experiment is nonetheless the first to test the capacity for shrubs to be used in restoration planning to improve restoration outcomes for planting native forbs in an invaded arid shrubland. Further investigation into the possibility of using this as a planting strategy and initial results appear promising at this time.
General Conclusions

Shrub facilitation can increase the establishment of native forb species in an invaded arid shrubland suggesting nurse plants can be an effective tool for restoration. The positive effect of shrubs on the native forbs was attributed to reductions in abiotic stress rather than protection from herbivory. Importantly, biomass of the native forbs increased with shrub facilitation which is likely to increase the success of restoration over the long term because biomass has been correlated with competitive ability and persistence with non-natives (Miller, 1987; Howard & Goldberg, 2001; Keddy et al., 2002; Donohue et al., 2010). Consumers did not have an effect on the facilitative outcome of shrubs on native forbs and this may be because: consumers do not play an important role in structuring this community, the study shrub species does not inhibit herbivory, or the intensity of grazing was too high resulting in a collapse in this interaction (Smit et al., 2007; Abdallah & Chaieb, 2012; Soliveres et al., 2012). Surprisingly, removing non-natives did not have a universal positive effect for all forb species in this study. Only two species experienced a positive effect of removing non-natives, although this effect was large and may be necessary when attempting to establish less competitive species. Planting native forb species with nurse plants and non-native species removals was an effective strategy for establishing native species during the year of the study and is recommended to be used as a seeding strategy in future restoration projects. However, the potential influence of year effects and spatial scale of the plots should be examined further.

Year effects, or inter-annual variations in the success of plant establishment, are known to influence restoration success (Brooks, 2000; Bakker et al., 2003; Young et al., 2005; Vaughn & Young, 2010; Wilson, 2015). Such contingencies in management are best addressed by timing planting during favourable conditions, but it is often not practical for land managers who may
not have the resources to capitalize on these opportunities (Young et al., 2005). Nonetheless, it is important to understand the influence of inter-annual variation in order to better predict restoration outcomes. Contingencies have been reported in previous studies of nurse plant facilitation where depending on the level of stress, facilitative outcomes may not be observed, or if there is too much stress the nurse plant may not facilitate at all (Maestre et al., 2009; le Roux & McGeoch, 2010; Dangles et al., 2013; Koyama & Tsuyuzaki, 2013). This has important implications for applying nurse plant facilitation as a restoration strategy for seeding native species as the expected positive effects on establishment may not occur depending on annual climatic variations (i.e. drought years). It is important that future studies examining the use of nurse plants as a seeding strategy investigate these trends over multiple years to determine how inter-annual climatic variation can influence the facilitative effects. Additionally, future studies should investigate if the role of spatial scale influences the positive effect of shrubs by seeding across larger areas. It is expected that shrubs will facilitate at all spatial scales and that they may even promote nucleation or increase the resilience/resistance to invasion within their canopy. A previous study showed that planting native forbs within patches in an invaded grassland was a successful strategy for increasing resistance to invasion and allowed dispersal into the surrounding invaded matrix (Grygiel et al., 2014). This could be used as a planting strategy to accelerate restoration efforts and increase success. By planting forbs in concentrated patches within the shrub canopy, there is the possibility of increasing restoration success not only by facilitating the establishment of native species through facilitative mechanisms of nurse plants, but also by mimicking the natural processes of succession through nucleation, and help resist invasion within these patches. This method of planting within patches has been shown to be both
ecologically and cost effective for reforestation efforts (Holl et al., 2011), and it could be a method of reducing the cost of restoration of invaded arid landscapes.

Increasing native biodiversity is an important challenge for land managers of invaded ecosystems, but is essential for improving the functioning of the ecosystem (Hobbs et al., 2009; Higgs et al., 2014). Despite the limitations in study design, these results have described a novel method for successfully increasing the establishment of native forbs within an invaded arid landscape. Although it is impractical to eliminate all non-natives from this heavily invaded site we have demonstrated a way to increase the native biodiversity within this site through the use of nurse plants. With climate change predicting the negative impacts of non-native species to increase (Smith et al., 2000), it is necessary to develop novel techniques to mitigate these effects on biodiversity and ecosystem function. Increasing native forb establishment within this landscape dominated by invasive Mediterranean grasses has effectively increased the ecosystem function and biodiversity within the area. This has implications towards restoring plant-pollinator interactions which are of importance in the agriculturally significant San Joaquin region (Forup et al., 2008). Given the global loss of diversity that has been attributed to invasions by non-native species, identifying methods of preserving and increasing natives within these landscapes is essential. Facilitation by nurse plants could be an example of an effective method for re-introducing native biodiversity to these ecosystems.
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Tables
Table 1: ANOVA results for above-ground biomass for five native forb species between microsites, exclosures, and non-native removal treatments. Bold values indicate significance at the α<0.05 level.

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<tr>
<th>Model</th>
<th>Amsinckia tessellata</th>
<th>Caulanthus lasiophyllus</th>
<th>Lepidium nitidum</th>
<th>Monolopia lanceolata</th>
<th>Phacelia tanacetifolia</th>
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<td>Df</td>
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Table 2: ANOVA results for below-ground biomass for five native forb species between microsites, exclosures, and non-native removal treatments Bold values indicate significance at the $\alpha<0.05$ level.

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</table>
Table 3: Results of Generalized Linear Models (GLMs) comparing the abundance of five native forb species between microsites, exclosures, and non-native removals. Bold values indicate significance at the α<0.05 level.

<table>
<thead>
<tr>
<th>Model</th>
<th>Amsinckia tessellata</th>
<th>Caulanthus lasiophyllus</th>
<th>Lepidium nitidum</th>
<th>Monolopia lanceolata</th>
<th>Phacelia tanacetifolia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Df</td>
<td>$\chi^2$</td>
<td>p</td>
<td>Df</td>
<td>$\chi^2$</td>
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Table 4: ANOVA results for the mean number of flowers for five native forb species between microsites, exclosures, and non-native removal treatments. Bold values indicate significance at the $\alpha<0.05$ level.

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<tr>
<th>Model</th>
<th>Amsinckia tessellata</th>
<th>Caulanthus lasiophyllus</th>
<th>Lepidium nitidum</th>
<th>Monolopia lanceolata</th>
<th>Phacelia tanacetifolia</th>
</tr>
</thead>
<tbody>
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<td>p</td>
<td>Df</td>
<td>F</td>
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<td>0.286</td>
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</tr>
</tbody>
</table>
Figures

Figure 1 Mean above and below-ground biomass for five native forbs seeded within shrub and open microsites, with and without exclosures and non-native species. Error bars presented are standard error. Significance at $\alpha<0.05$ denoted by $*** = \leq 0.001$, $** = \leq 0.01$, $* = \leq 0.05$. 
Figure 2: Mean abundance (±SE) for five native forb species within shrub and open microsites, with and without exclosures and non-native species. Significance at α<0.05 denoted by *** = ≤ 0.001, ** = ≤0.01, * = ≤ 0.05.
Figure 3: Relative Interaction Index (RII) values for above and below-ground biomass and abundance among the three treatments: microsite, exclosures and non-native removals. Values shown are means ± 95% bootstrapped confidence intervals. Values that are significantly greater than zero indicate positive effects, while values that are significantly lower than zero indicate negative effects. Any value that is not significantly different from zero is a neutral effect.
Appendices

Appendix A: germination and viability

To estimate baseline germination for each species, seeds were germinated in Sanyo MLR-351-H growth chambers that simulated the shrub and open environmental conditions using data from HOBO ProV2 loggers in the field for the 2015 growing season (January – May 2015) at Panoche Hills. The growth chambers were programmed using environmental data from January 23 to February 23, 2015 specifically as this is the critical period for seed germination. Five replicates of 20 seeds per species per microclimate were placed in Petri-dishes lined with moistened filter paper. Seeds were watered every 2-3 days to ensure they were kept moist and germination was recorded every 2-3 days for 14 days. A seed was considered germinated when the radicle or coleoptiles were visible. Once germinated, seeds were removed from the Petri dish to reduce the likelihood of protracted interference effects. Total germination (the number of seeds germinated) and days to 50% germination (the number of days needed to reach 50% of the total germination) were recorded as response variables. Days to 50% germination can be used as a proxy measure for germination rate. Generalized Linear Models (GLMs) fit to a normal distribution with the identity link function in order to test for a difference between species, microsite (shrub and open) and the species by microsite interaction. Statistical analyses were performed using JMP 10 (SAS Institute Inc. 2012).

The viability of seeds from each species was also determined using tetrazolium chloride (tz) viability analyses. 100 seeds were randomly selected for viability testing (25 seeds per replicate). Seeds are first soaked in deionized water for 24 hours, then dissected to expose the embryo, and placed in a 1% solution of tetrazolium (2, 3, 5-triphenyl-2H-tetrazolium chloride) at 25°C for 24
hours. Seeds were determined to be viable if the embryos stained pink or red (Baskin and Baskin 2001).

**Figure A1:** The number of germinated seed (top panel) and the number of days to 50% germination (bottom panel) for five native forb species in growth chambers simulating shrub and open conditions. Species codes are as follows: PHAC = *Phacelia tanacetifolia*, MONO = *Monolopia lanceolata*, CAUL = *Caulanthus lasiophyllus*, AMSK = *Amsinckia tessellata*, LIPI = *Lepidium nitidum*. Significance is denoted with different letters.
Figure A2: The percent viability for five native forb species. Species codes are as follows: PHAC = *Phacelia tanacetifolia*, MONO = *Monolopia lanceolata*, CAUL = *Caulanthus lasiophyllus*, AMSK = *Amsinckia tessellata*, LIPI = *Lepidium nitidum*. Significance is denoted with different letters.
Appendix B: Correlation matrix between covariates

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<th>5</th>
<th>6</th>
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**Figure B1:** Correlation matrix between covariates. The covariates are encoded by number: 1 = shrub area, 2 = shrub volume, 3 = shrub canopy density, 4 = PAR (photosynthetic active radiation), 5 = soil moisture in January 2016, 6 = soil moisture in March 2016. Values shown are R values.
Appendix C: Table of best subsets results

Table C1: Best subsets results for each covariate for above-ground biomass. Moist1 indicates soil moisture recorded in January 2016 and Moist2 is soil moisture recorded in March 2016. Asterisks denote when a covariate was included in the model.

<table>
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<tr>
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<th>Intercept</th>
<th>Shrub area</th>
<th>PAR</th>
<th>Moist1</th>
<th>Moist2</th>
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Table C2: Best subsets results for each covariate for below-ground biomass. Moist1 indicates soil moisture recorded in January 2016 and Moist2 is soil moisture recorded in March 2016. Asterisks denote when a covariate was included in the model.

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<th>Intercept</th>
<th>Shrub area</th>
<th>PAR</th>
<th>Moist1</th>
<th>Moist2</th>
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Table C3: Best subsets results for each covariate for abundance. Moist1 indicates soil moisture recorded in January 2016 and Moist2 is soil moisture recorded in March 2016. Asterisks denote when a covariate was included in the model.

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<th>Intercept</th>
<th>Shrub area</th>
<th>PAR</th>
<th>Moist1</th>
<th>Moist2</th>
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Table C4: Best subsets results for each covariate for average number of flowers. Moist1 indicates soil moisture recorded in January 2016 and Moist2 is soil moisture recorded in March 2016. Asterisks denote when a covariate was included in the model.

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<tr>
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Appendix D: Tables for ANOVA and GLM (Generalized Linear Model) full models

**Table 1:** ANOVA results of a full model including species as a fixed effect for above-ground biomass compared among treatments (microsite, exclosure, removal. Bold values indicate significance at $\alpha < 0.05$.

<table>
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<th>F value</th>
<th>p-value</th>
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<td>43.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Microsite</td>
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<td>35.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Exclosure</td>
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<td>15.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Removal</td>
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<td>16.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species * Microsite</td>
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</tr>
<tr>
<td>Species * Exclosure</td>
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<td>0.259</td>
</tr>
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</tr>
<tr>
<td>Species * Removal</td>
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</tr>
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<td>0.477</td>
</tr>
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<td>0.303</td>
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</table>
Table 2: ANOVA results of a full model including species as a fixed effect for below-ground biomass compared among treatments (microsite, exclosure, removal). Bold values indicate significance at $\alpha < 0.05$.

<table>
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<th>p-value</th>
</tr>
</thead>
<tbody>
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<td>0.356</td>
</tr>
<tr>
<td>Removal</td>
<td>1</td>
<td>2.86</td>
<td>0.093</td>
</tr>
<tr>
<td>Species * Microsite</td>
<td>4</td>
<td>0.977</td>
<td>0.421</td>
</tr>
<tr>
<td>Species * Exclosure</td>
<td>4</td>
<td>1.16</td>
<td>0.329</td>
</tr>
<tr>
<td>Microsite * Exclosure</td>
<td>1</td>
<td>1.00</td>
<td>0.318</td>
</tr>
<tr>
<td>Species * Removal</td>
<td>4</td>
<td>0.590</td>
<td>0.617</td>
</tr>
<tr>
<td>Microsite * Removal</td>
<td>1</td>
<td>0.022</td>
<td>0.882</td>
</tr>
<tr>
<td>Exclosure * Removal</td>
<td>1</td>
<td>0.411</td>
<td>0.522</td>
</tr>
<tr>
<td>Species * Microsite * Exclosure</td>
<td>4</td>
<td>0.488</td>
<td>0.744</td>
</tr>
<tr>
<td>Species * Microsite * Removal</td>
<td>4</td>
<td>0.003</td>
<td>1.00</td>
</tr>
<tr>
<td>Species * Exclosure * Removal</td>
<td>3</td>
<td>0.306</td>
<td>0.821</td>
</tr>
<tr>
<td>Microsite * Exclosure * Removal</td>
<td>1</td>
<td>0.291</td>
<td>0.590</td>
</tr>
<tr>
<td>Species * Microsite * Exclosure * Removal</td>
<td>3</td>
<td>0.596</td>
<td>0.618</td>
</tr>
</tbody>
</table>
Table 3: GLM (Generalized Linear Model) results of the full model (including species) comparing abundance by the three treatments (microsite, exclosure, removal). Bold values indicate significance at the $\alpha < 0.05$ level.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Df</th>
<th>$\chi^2$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>4</td>
<td>114</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Microsite</td>
<td>1</td>
<td>4.67</td>
<td>0.031</td>
</tr>
<tr>
<td>Exclosure</td>
<td>1</td>
<td>79.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Removal</td>
<td>1</td>
<td>5.24</td>
<td>0.022</td>
</tr>
<tr>
<td>Species * Microsite</td>
<td>4</td>
<td>12.5</td>
<td>0.014</td>
</tr>
<tr>
<td>Species * Exclosure</td>
<td>4</td>
<td>29.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Microsite * Exclosure</td>
<td>1</td>
<td>4.34</td>
<td>0.037</td>
</tr>
<tr>
<td>Species * Removal</td>
<td>4</td>
<td>31.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Microsite * Removal</td>
<td>1</td>
<td>2.05</td>
<td>0.152</td>
</tr>
<tr>
<td>Exclosure * Removal</td>
<td>1</td>
<td>1.75</td>
<td>0.185</td>
</tr>
<tr>
<td>Species * Microsite * Exclosure</td>
<td>4</td>
<td>1.25</td>
<td>0.870</td>
</tr>
<tr>
<td>Species * Microsite * Removal</td>
<td>4</td>
<td>5.35</td>
<td>0.253</td>
</tr>
<tr>
<td><strong>Species * Exclosure * Removal</strong></td>
<td><strong>3</strong></td>
<td><strong>20.4</strong></td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>Microsite * Exclosure * Removal</td>
<td>1</td>
<td>0.453</td>
<td>0.501</td>
</tr>
<tr>
<td>Species * Microsite * Exclosure * Removal</td>
<td>3</td>
<td>0.257</td>
<td>0.992</td>
</tr>
</tbody>
</table>
Table 4: ANOVA results of a full model including species as a fixed effect for mean number of flowers per individual compared among treatments (microsite, exclosure, removal). Bold values indicate significance at $\alpha < 0.05$.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Df</th>
<th>F value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>4</td>
<td>43.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Microsite</td>
<td>1</td>
<td>5.96</td>
<td>0.012</td>
</tr>
<tr>
<td>Exclosure</td>
<td>1</td>
<td>37.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Removal</td>
<td>1</td>
<td>0.490</td>
<td>0.485</td>
</tr>
<tr>
<td>Species * Microsite</td>
<td>4</td>
<td>3.20</td>
<td>0.015</td>
</tr>
<tr>
<td>Species * Exclosure</td>
<td>4</td>
<td>12.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Microsite * Exclosure</td>
<td>1</td>
<td>1.04</td>
<td>0.310</td>
</tr>
<tr>
<td>Species * Removal</td>
<td>4</td>
<td>0.636</td>
<td>0.640</td>
</tr>
<tr>
<td>Microsite * Removal</td>
<td>1</td>
<td>0.651</td>
<td>0.421</td>
</tr>
<tr>
<td>Exclosure * Removal</td>
<td>1</td>
<td>2.95</td>
<td>0.090</td>
</tr>
<tr>
<td>Species * Microsite * Exclosure</td>
<td>4</td>
<td>1.11</td>
<td>0.354</td>
</tr>
<tr>
<td>Species * Microsite * Removal</td>
<td>4</td>
<td>0.261</td>
<td>0.903</td>
</tr>
<tr>
<td>Species * Exclosure * Removal</td>
<td>3</td>
<td>1.13</td>
<td>0.338</td>
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<tr>
<td>Microsite * Exclosure * Removal</td>
<td>1</td>
<td>0.002</td>
<td>0.969</td>
</tr>
<tr>
<td>Species * Microsite * Exclosure * Removal</td>
<td>3</td>
<td>0.095</td>
<td>0.963</td>
</tr>
</tbody>
</table>
Appendix E: R code script for statistical analyses and plots

## statistical analyses included in Chapter 2 seed addition manuscript

library(corrplot)
library(leaps)
library(car)
library(MASS)
library(lsmeans)
library(metafor)
library(dplyr)

data <- read.table("C:\Users\Amanda\Documents\PhD\Chapter 2 seed addition\Ch 2 seed addition final survey 2016.csv", header=T, sep=",") ## plant data
str(data)
data2 <- read.table("C:\Users\Amanda\Documents\PhD\Chapter 2 seed addition\HOBOdata\HOBO data compiled 2016.csv", header=T, sep=",") ## HOBO data
str(data2)
attach(data2)

## variable selection

## correlation matrix used to determine if covariates are collinear
comp <- cbind(data$area, data$vol, data$canopy, data$PAR, data$moist1, data$moist2)
comp2 <- cor(comp)
library(corrplot)
corrplot(comp2, method="number")
## volume and area are correlated, remove volume
## canopy, area and volume are correlated, remove canopy
## PAR correlations likely due to differences in microsite so will retain

## best subsets to determine if covariates explain a significant amount of variation
r1 <- regsubsets(abundance~area+PAR+moist1+moist2, data=data, nbest=10)
summary(r1) ## shows variables in each model
plot(r1, scale="r2") ## produces plot
## together all covariates have an r2 value of 0.0019, very low so will remove from future models

r2 <- regsubsets(above~area+PAR+moist1+moist2, data=data, nbest=10)
summary(r2)
plot(r2, scale="r2")
## together all covariates have an r2 value of 0.017, very low and will remove from future models

r3 <- regsubsets(below~area+PAR+moist1+moist2, data=data, nbest=10)
summary(r3)
plot(r3, scale="r2")
## together all covariates have an r2 value of 0.011, very low and will remove from future models

r4 <- regsubsets(flowersavg~area+PAR+moist1+moist2, data=data, nbest=10)
summary(r4)
plot(r4, scale="r2")
## together all covariates have an r2 value of 0.041, very low and will remove from future models
data2 <- subset(data, status == "native")
air <- subset(data2, above > 0)
ground <- subset(data2, below > 0)
m1 <- aov(log(above) ~ species * micro * exclosure * removal, data = air) # log transform for normality and homogeneity of variances
shapiro.test(m1$residuals) ## normal
leveneTest(log(above) ~ species * micro * exclosure * removal, data = air) ## variances are homogenous
summary(m1) ## all interactions with species are significant. Will re-run separate models for each species
## cannot conduct pairwise comparisons with Monolopia due to low sample size.

m2 <- aov(below ~ species * micro * exclosure * removal, data = ground)
shapiro.test(m2$residuals)
leveneTest(below ~ species * micro * exclosure * removal, data = ground) # normal and has heterogeneity of variances
summary(m2) # only effect was for species and microsite

m3 <- glm.nb(abundance ~ species * micro * exclosure * removal, data = data2)
summary(m3)
anova(m3) # species is main significant factor

flowers <- subset(data2, flowersavg > 0)
m4 <- aov(sqrt(flowersavg) ~ species * micro * exclosure * removal, data = flowers)
shapiro.test(m4$residuals)
leveneTest(sqrt(flowersavg) ~ species * micro * exclosure * removal, data = flowers) # could not normalize, homogeneity of variances
summary(m4)

# subsetting the data by species to run models separately by species
amsk <- subset(data, species == "amsk") # subset for Amsinckia tessellata
caul <- subset(data, species == "caul") # subset for Caulanthus lasiophyllus
lepi <- subset(data, species == "lepi") # subset for Lipidium nitidum
mono <- subset(data, species == "mono") # subset for Monolopia lanceolata
phac <- subset(data, species == "phac") # subset for Phacelia tanacetifolia

# convert percent cover data into proportion data for stats
percents <- (data$percent.cover / 100)

## testing each response variable and each species separately

## biomass

##amsinckia biomass
amsk1 <- subset(amsk, both > 0) # total biomass subsetting for values greater than zero (i.e. an individual was found in the plot)
mass <- aov(both ~ micro * exclosure * removal, data = amsk1) # removed covariates as they do not significantly influence the model or have high explanatory power
summary(mass)
leveneTest(both ~ micro * exclosure * removal, data = amsk1) # tests for homogeneity of variances - non. sig = homogeneous, in package car
shapiro.test(mass$residuals) # data is normal

amsk2 <- subset(amsk, above > 0) # above ground biomass
amass <- aov(above ~ micro * exclosure * removal, data = amsk2)
summary(amass)
leveneTest(above ~ micro * exclosure * removal, data = amsk2)
shapiro.test(amass$residuals) # normal
amsk3 <- subset(amsk, below > 0) ## below ground biomass
bmass <- aov(sqrt(below) ~ micro * exclosure * removal, data = amsk3) ## sqrt transformation to meet the assumption of normality and homogeneity of variances
summary(bmass)
leveneTest(below ~ micro * exclosure * removal, data = amsk3) ## sqrt transformation achieved homogenity of variances
shapiro.test(bmass$residuals) # normality was acheived with sqrt transformation

## shrub covariates stats including area, moist1, moist2, PAR, and canopy were not included as they were not significant for any species or response

## Caulanthus biomass
caul1 <- subset(caul, both > 0) ## total biomass
grr <- log10(caul1$both) ## log transform to meet the assumptions of normality
mass <- aov(grr ~ micro * exclosure * removal, data = caul1)
leveneTest(grr ~ micro * exclosure * removal, data = caul1) # homogeneous
summary(mass)
shapiro.test(mass$residuals) # normal after transformations
caul2 <- subset(caul, above > 0) # above ground biomass
frr <- log10(caul2$above) # log transform to normalize
mass <- aov(frr ~ micro * exclosure * removal, data = caul2)
summary(mass)
shapiro.test(mass$residuals)
leveneTest(frr ~ micro * exclosure * removal, data = caul2) # homogenous
caul3 <- subset(caul, below > 0) # below ground biomass
urr <- log10(caul3$below) # log transform to normalize
mass <- aov(urr ~ micro * exclosure * removal, data = caul3)
summary(mass)
shapiro.test(mass$residuals)
leveneTest(urr ~ micro * exclosure * removal, data = caul3) # homogenous

## shrub only covariates were not significant and were not included

## Lipidium
lepi1 <- subset(lepi, both > 0) # total biomass
hh <- sqrt(lepi1$both) # square root transform to normalize
mass <- aov(hh ~ micro * exclosure * removal, data = lepi1)
summary(mass)
shapiro.test(mass$residuals)
leveneTest(hh ~ micro * exclosure * removal, data = lepi1) # homogenous
lepi2 <- subset(lepi, above > 0) # above ground biomass
hh <- log10(lepi1$above) # log transform for normality
mass <- aov(hh ~ micro * exclosure * removal, data = lepi2)
summary(mass)
shapiro.test(mass$residuals)
leveneTest(hh ~ micro * exclosure * removal, data = lepi2) # homogenous
lepi3 <- subset(lepi, below > 0) # below ground biomass
mass <- aov(below ~ micro * exclosure * removal, data = lepi3)
summary(mass)
shapiro.test(mass$residuals) ## normal
leveneTest(below ~ micro * exclosure * removal, data = lepi3) # homogenous
# no shrub covariates were significant and were omit

```R
# Monolopia
mono1 <- subset(mono, both >0) # total biomass
mm <- log10(mono1$both) # log tranform to normalize
mass <- aov(mm ~ micro*exclosure*removal, data=mono1)
summary(mass)
shapiro.test(mass$residuals)
leveneTest(mm ~ micro*exclosure*removal, data=mono1) ## homogenous

mono2 <- subset(mono, above >0) # above ground biomass
nn <- log10(mono2$above) #log transform to normalize
mass <- aov(nn ~ micro*exclosure*removal, data=mono2)
summary(mass)
shapiro.test(mass$residuals)
leveneTest(nn ~ micro*exclosure*removal, data=mono2) # homogenous

mono3 <- subset(mono, below >0) # below ground biomass
mass <- aov(log(below) ~ micro*exclosure*removal, data=mono3) # log transform to normalize and achieve homogeneity of variances
summary(mass)
shapiro.test(mass$residuals)
leveneTest(below ~ micro*exclosure*removal, data=mono3)
```

```R
# Phacelia
phac1 <- subset(phac, both>0) # total biomass
mass <- aov(log(both) ~ micro*exclosure*removal, data=phac1) # log transform to normalize
summary(mass)
shapiro.test(mass$residuals) ## normal
leveneTest(log(both) ~ micro*exclosure*removal, data=phac1) # homogenous

phac2 <- subset(phac, above>0) # above ground biomass
mass <- aov(log(above) ~ micro*exclosure*removal, data=phac2) # log transform to normalize
summary(mass)
shapiro.test(mass$residuals)
leveneTest(log(above) ~ micro*exclosure*removal, data=phac2) # homogenous

phac3 <- subset(phac, below>0) # below ground biomass
mass <- aov(log(below) ~ micro*exclosure*removal, data=phac3) # log transform
summary(mass)
shapiro.test(mass$residuals)
leveneTest(log(below) ~ micro*exclosure*removal, data=phac3) # homogenous
```

```R
## abundance

## Amsinckia
numbers <- glm.nb(abundance ~ micro*exclosure*removal, data=amsk) ## over dispersed with poisson so used negative binomial distribution(residual deviance > x2 DF)
summary(numbers)
anova(numbers, test="Chisq")
```

```R
## Caulanthus
numbers <- glm.nb(abundance ~ micro*exclosure*removal, data=caul)
summary(numbers)
anova(numbers, test="Chisq")
```
## Lipidium

```r
numbers <- glm.nb(abundance ~ micro*exclosure*removal, data=lepi)
summary(numbers)
anova(numbers, test="Chisq")
```

## Monolopia

```r
numbers <- glm.nb(abundance ~ micro*exclosure*removal, data=mono)
summary(numbers)
anova(numbers)
```

## Phacelia

```r
numbers <- glm.nb(abundance ~ micro*exclosure*removal, data=phac)
summary(numbers)
anova(numbers, test="Chisq")
```

## brome

### average number of flowers per individuals

#### Amsinckia

```r
flour <- subset(amsk, flowersavg>0) #only including plots with flowers
petal <- aov(flowersavg ~ micro*exclosure*removal, data=flour)
summary(petal)
shapiro.test(petal$residuals) # normal
leveneTest(flowersavg ~ micro*exclosure*removal, data=flour) # homogenous
```

#### Caulanthus

```r
flour <- subset(caul, flowersavg>0)
petal <- aov(flowersavg ~ micro*exclosure*removal, data=flour)
summary(petal)
shapiro.test(petal$residuals) # normal
leveneTest(flowersavg ~ micro*exclosure*removal, data=flour) # homogenous
```

#### Lipidium

```r
flour <- subset(lepi, flowersavg>0)
petal <- aov(flowersavg ~ micro*exclosure*removal, data=flour)
summary(petal)
shapiro.test(petal$residuals) # normal
leveneTest(flowersavg ~ micro*exclosure*removal, data=flour) # homogenous
```

### due to unbalanced design from low numbers of individuals flowering, interactions could not be calculated

#### Monolopia

```r
flour <- subset(mono, flowersavg>0)
petal <- aov(log(flowersavg) ~ micro*exclosure*removal, data=flour)
summary(petal)
shapiro.test(petal$residuals) # normal
leveneTest(log(flowersavg) ~ micro*exclosure*removal, data=flour) # log transform to meet assumption of homogeneity of variances
```

#### Phacelia

```r
flour <- subset(phac, flowersavg>0)
petal <- aov(sqrt(flowersavg) ~ micro*exclosure*removal, data=flour) # sqrt transform to normalize
summary(petal)
leveneTest(sqrt(flowersavg) ~ micro*exclosure*removal, data=flour) # homogenous
shapiro.test(petal$residuals)
# normal after transformation
## diversity estimates

```r
## richness
test <- subset(data, abundance > 0) # remove species that have 0 values

richness <- aggregate(test$abundance, by=list(test$number, test$exclosure, test$micro, test$removal), length) # sum the number of species per plot
colnames(richness) <- c("number", "exclosure", "micro", "removal", "richness") # rename columns

rich <- glm(richness ~ micro*exclosure*removal, family=poisson, data=richness)
summary(rich)
anova(rich, test="Chisq")

## Simpson's diversity
total.abd <- aggregate(data$abundance, by=list(data$number), sum) # sum total species in each plot
t1 <- rbind(total.abd, total.abd, total.abd, total.abd, total.abd, total.abd, total.abd, total.abd, total.abd, total.abd, total.abd, total.abd, total.abd, total.abd) # duplicate to match original dataset
t2 <- t1[order(t1[,1]),] # order the sequence as plots

data2 <- data # rename data
data2["simp"] <- (data2$abundance/t2[,2])^2 # divide each species abundance by total plot abundance

simpson <- aggregate(data2$simp, by=list(data2$number, data2$exclosure, data2$micro, data2$removal), sum) # sum each species value

colnames(simpson) <- c("number", "exclosure", "micro", "removal", "Simpson index", "diversity") # rename columns

div <- glm(diversity ~ micro*exclosure*removal, family=binomial, data=simpson)
summary(div)
anova(div, test="Chisq")

## environmental variables

## soil moisture

cold <- (data$moist1/100) ## converting % soil moisture to proportions
warm <- (data$moist2/100)

jan <- glm(cold ~ micro*exclosure+removal, family=binomial, data=data) ## first soil moisture reading (moist1) took place in January
summary(jan)
anova(jan, test="Chisq")

march <- glm(warm ~ micro*exclosure+removal, family=binomial, data=data) ## second soil moisture reading (moist2) took place in march
summary(march)
anova(march, test="Chisq")

march2 <- glm(warm ~ micro*exclosure+removal+moist1, family=binomial, data=data) ## second soil moisture reading with moist1 as a covariate
summary(march2)
anova(march2, test="Chisq")

##HOBO data
```
growing <- subset(data2, month %in% c("November", "December", "January", "February", "March", "April"))  ##
subsetting data by time periods
early <- subset(growing, month %in% c("November", "December", "January"))  ## early growing season
late <- subset(growing, month %in% c("February", "March", "April"))  ## late growing season

fhour <- factor(hour)
is.factor(fhour)  ## treat hour as a factor not a number

time1 <- subset(early, fhour %in% c("12", "13", "14"))  ## subsetting data by mid-day where there is the greatest
variation in temp/RH
time2 <- subset(late, fhour %in% c("12", "13", "14"))
timeall <- subset(growing, fhour %in% c("12", "13", "14"))

#temperature
#entire growing season
growth <- t.test(temp ~ micro, data = timeall)
growth
#early season
bird <- t.test(temp ~ micro, data = time1)
bird

#late season
hot <- t.test(temp ~ micro, data = time2)
## variation in temperature
#entire growing season
growing2 <- na.omit(growing)
variation <- aggregate(growing[, "temp"],
by = list(micro = growing[,"micro"], removal = growing[, "removal"], julian.date = growing[, "julian.date"]), var)
m1 <- t.test(x ~ micro, data = variation)
m1
#early variation
variation <- aggregate(early[, "temp"],
by = list(micro = early[,"micro"], removal = early[, "removal"], julian.date = early[, "julian.date"]), var)
m2 <- t.test(x ~ micro, data = variation)
m2

#late variation
variation <- aggregate(late[, "temp"],
by = list(micro = late[,"micro"], removal = late[, "removal"], julian.date = late[, "julian.date"]), var)
m3 <- t.test(x ~ micro, data = variation)
m3

#humidity
data2[,"humidity"] <- (data2$RH/100)
#entire growing season
m1 <- glm(humidity ~ micro, family = binomial, data = timeall)
anova(m1, test = "Chisq")

#early season
bird <- glm(humidity ~ micro, family = binomial, data = time1)
anova(bird, test = "Chisq")
#late season
hot <- glm(humidity ~ micro, family = binomial, data = time2)
anova(hot, test = "Chisq")
##variation

###entire growing season

growing2 <- na.omit(timeall)
variation <- aggregate(growing2[, "RH"], by=list(micro=growing2[, "micro"], julian.date=growing2[, "julian.date"]), var)
growth <- t.test(x~micro, data=variation)
growth

###early variation

early2 <- na.omit(time1)
variation <- aggregate(early2[, "RH"], by=list(micro=early2[, "micro"], julian.date=early2[, "julian.date"]), var)
growth <- t.test(x~micro, data=variation)
growth

###late variation

late2 <- na.omit(time2)
variation <- aggregate(late2[, "RH"], by=list(micro=late2[, "micro"], julian.date=late2[, "julian.date"]), var)
growth <- t.test(x~micro, data=variation)
growth

### RII calculations

### function

###permutation RII

perm.rii <- function(x, treatment, control.var, treat.var, variable, perm){
s1 <- subset(x, x[, treatment] == treat.var, select=variable) ## subset the treatment group
o1 <- subset(x, x[, treatment] == control.var, select=variable) ## subset the control group
min.samp <- min(length(s1[,1]),length(o1[,1])) ## minimum number of samples
rii.avg.total <- c() ## set up blank mean vector
rii.se.total <- c() ## set up blank mean vector
for (i in 1:perm){ ##loop the sampling of treatment and control groups and calculate RII
  set.seed(i) ## control randomization to return same values
  treat.samp <- sample(s1[,variable],min.samp)
  control.samp <- sample(o1[,variable],min.samp)
  return1 <- (treat.samp - control.samp) / (treat.samp+control.samp)
  rii.avg <- mean(return1)
  rii.se <- se(return1)
  rii.avg.total <- c(rii.avg.total, rii.avg) ## bind all the means together
  rii.se.total <- c(rii.se.total, rii.se) ## bind all the confidence intervals together
}
rii.avg <- mean(rii.avg.total)
rii.se <- mean(rii.se.total)
treat <- c(treatment)
rii.results <- data.frame(factor=treat, average=rii.avg, error=rii.se)
return(rii.results)
}

perm.rii.abd <- function(x, treatment, control.var, treat.var, variable, perm){
s1 <- subset(x, x[, treatment] == treat.var, select=variable) ## subset the treatment group
o1 <- subset(x, x[, treatment] == control.var, select=variable) ## subset the control group
min.samp <- min(length(s1[,1]),length(o1[,1])) ## minimum number of samples
rii.avg.total <- c() ## set up blank mean vector
rii.se.total <- c() ## set up blank mean vector
for (i in 1:perm){ ##loop the sampling of treatment and control groups and calculate RII
  set.seed(i) ## control randomization to return same values
  treat.samp <- sample(s1[,variable],min.samp)
  control.samp <- sample(o1[,variable],min.samp)
return1 <- (treat.samp - control.samp) / (treat.samp + control.samp)
return1[is.na(return1)] <- 0
rii.avg <- mean(return1)
rii.se <- se(return1)
rii.avg.total <- c(rii.avg, total, rii.avg) ## bind all the means together
rii.se.total <- c(rii.se, total, rii.se) ## bind all the confidence intervals together
rii.avg <- mean(rii.avg.total)
rii.se <- mean(rii.se.total)
treat <- c(treatment)
rii.results <- data.frame(factor=treat, average=rii.avg, error=rii.se)
return(rii.results)
}

## function for standard error
se <- function(x) sqrt(var(x)/length(x))
mean <- function(x) sum(x)/length(x)

## biomass

## above ground biomass
aboverii <- subset(data, above>0 & status=="native" & seed=="seed") ## subsetting the data to above ground biomass
overall.ex <- perm.rii(aboverii, "exclosure", "no exclosure", "exclosure", "above", 999) ## bootstrapped RII by exclosure
overall.micro <- perm.rii(aboverii, "micro", "open", "shrub", "above", 999)
overall.removal <- perm.rii(aboverii, "removal", "present", "removal", "above", 999)
overall.results <- rbinding(overall.micro, overall.ex, overall.removal) # combining into one data frame
overall.results[,"error"] <- overall.results[,"error"]*1.96 ## calculating 95% confidence intervals
rma.uni(overall.results[,"average"], sei=overall.results[,"error"]) ## heterogeniety tests

## not significantly different

## figure for above ground biomass RII c
error.bar <- function(x, y, upper, lower=upper, length=0.05,...){ ## calculating error bars
  if(length(x) != length(y) | length(y) != length(lower) | length(lower) != length(upper))
    stop("vectors must be same length")
arrows(x+y+upper, x-y-lower, angle=90, code=3, length=length, ...)
}
par(mar=c(4.5,4.5,1,0)) ## change margins
par(mfrow=c(1, 3)) ## makes graphs into panel style with one row and 3 columns
plot(seq(1,3,1), overall.results[,"average"], pch=19, xlab=c(0.8,3.2), ylab="RII", xaxt="n", lty=2, lwd=2) # adding error bars
error.bar(seq(1,3,1), overall.results[,"average"],overall.results[,"error"], lwd=2) # adding error bars
axis1(1, seq(1,3,1), c("microsite","exclosure","removal"), cex.axis=2) # axis labels
abline(h=0, lty=2, lwd=2) # line at zero for RII
text(2.4, 0.6, "above-ground biomass", cex=2)

## below ground biomass
belowrii <- subset(data, below>0 & status=="native" & seed=="seed") ## subsetting the data to below ground biomass
overall.ex <- perm.rii(belowrii, "exclosure", "no exclosure", "exclosure", "below", 999) ## bootstrapped RII by exclosure
overall.micro <- perm.rii(belowrii, "micro", "open", "shrub", "below", 999)
overall.removal <- perm.rii(belowrii, "removal", "present", "removal", "below", 999)
overall.results <- rbind(overall.micro, overall.ex, overall.removal) # combining into one data frame
overall.results[, "error"] <- overall.results[, "error"] * 1.96 ## calculating 95% confidence intervals

rma.uni(overall.results[, "average"], sei=overall.results[, "error"]) ## heterogeneity tests
## not significantly different

## figure for below ground biomass RII
error.bar <- function(x, y, upper, lower=upper, length=0.05,...){ ## calculating error bars
if(length(x) != length(y) | length(y) != length(lower) | length(lower) != length(upper))
  stop("vectors must be same length")
arrows(x,y+upper, x, y-lower, angle=90, code=3, length=length, ...)
}
par(mar=c(4.5,2.5,1,1)) ## change margins
x<-c(-0.2, 0, 0.2, 0.4, 0.6)
plot(seq(1,3,1), overall.results[, "average"], pch=19, xlim=c(0.8,3.2), ylim=c(-0.2,0.6), ylab="", xaxt="n", yaxt="n", lab="", cex=2, cex.axis=1.8, cex.lab=2) ## making plot
axis(2, at=x, labels=FALSE)
error.bar(seq(1,3,1), overall.results[, "average"], overall.results[, "error"], lwd=2) # adding error bars
axis(1, seq(1,3,1), c("microsite","exclosure","removal"), cex.axis=2) # axis labels
abline(h=0, lty=2, lwd=2) # line at zero for RII
text(2.4, 0.6, "below-ground biomass", cex=2)

## abundance
abundancerii<-subset(data, status=="native" & seed=="seed")
overall.ex <- perm.rii.abd(abundancerii, "exclosure", "no exclosure","exclosure", "abundance", 999) ## bootstrapped RII by exclosure
overall.micro <- perm.rii.abd(abundancerii, "micro", "open","shrub", "abundance", 999)
overall.removal <- perm.rii.abd(abundancerii, "removal", "present","removal", "abundance", 999)

overall.results <- rbind(overall.micro, overall.ex, overall.removal) # combining into one data frame
overall.results[, "error"] <- overall.results[, "error"] * 1.96 ## calculating 95% confidence intervals

rma.uni(overall.results[, "average"], sei=overall.results[, "error"]) ## heterogeneity tests
## not significantly different

## figure for abundance RII
error.bar <- function(x, y, upper, lower=upper, length=0.05,...){ ## calculating error bars
if(length(x) != length(y) | length(y) != length(lower) | length(lower) != length(upper))
  stop("vectors must be same length")
arrows(x,y+upper, x, y-lower, angle=90, code=3, length=length, ...)
}
par(mar=c(4.5,1.5,1,3)) ## change margins
x<-c(-0.2, 0, 0.2, 0.4, 0.6)
plot(seq(1,3,1), overall.results[, "average"], pch=19, xlim=c(0.8,3.2), ylim=c(-0.2,0.6), ylab="", xaxt="n", yaxt="n", lab="", cex=2, cex.axis=1.8, cex.lab=2)
axis(2, at=x, labels=FALSE)
error.bar(seq(1,3,1), overall.results[, "average"], overall.results[, "error"], lwd=2) # adding error bars
axis(1, seq(1,3,1), c("microsite","exclosure","removal"), cex.axis=2) # axis labels
abline(h=0, lty=2, lwd=2) # line at zero for RII
text(2.8, 0.6, "abundance", cex=2)

## avg number of flower
flowerrii<-subset(data, flowersavg>0 & status=="native" & seed=="seed")
overall.ex <- perm.rii(flowerrii, "exclosure", "no exclosure","exclosure", "flowersavg", 999) ## bootstrapped RII by exclosure
overall.micro <- perm.rii(flowerrii, "micro", "open","shrub", "flowersavg", 999)
overall.removal <- perm.rii(flowerrii, "removal", "present","removal", "flowersavg", 999)

overall.results <- rbind(overall.micro, overall.ex, overall.removal) # combining into one data frame
overall.results[,"error"] <-overall.results[,"error"]*1.96 # calculating 95% confidence intervals

rma.uni(overall.results[,"average"], sei=overall.results[,"error"]) ## heterogeniety tests
## not significantly different

## figure for flowersavg RII
error.bar <- function(x, y, upper, lower=upper, length=0.05,...){ # calculating error bars
  if(length(x) != length(y) | length(y) !=length(lower) | length(lower) != length(upper))
  stop("vectors must be same length")
  arrows(x,y+upper, x, y-lower, angle=90, code=3, length=length, ...)
}

par(mar=c(4.5,4.5,1,1)) ## change margins
plot(seq(1,3,1), overall.results[,"average"], pch=19, xlim=c(0.8,3.2), ylim=c(0.2,0.6), ylab="RII mean number of flowers", xaxt="n", xlab="", cex=2, cex.axis=1.8, cex.lab=2) # making plot
error.bar(seq(1,3,1), overall.results[,"average"],overall.results[,"error"], lwd=2) # adding error bars
axis(1, seq(1,3,1), c("microsite","exclosure","removal"), cex.axis=2) # axis labels
abline(h=0, lty=2, lwd=2) # line at zero for RII

## figures for raw data for above-ground biomass, below ground biomass, abundance, and average number of flowers and environmental data
## function for standard error
se <- function(x) sqrt(var(x)/length(x))
mean <- function(x) sum(x)/length(x)

## error bar function
error.bar <- function(x, y, upper, lower=upper, length=0.05,...){
  if(length(x) != length(y) | length(y) !=length(lower) | length(lower) != length(upper))
  stop("vectors must be same length")
  arrows(x,y+upper, x, y-lower, angle=90, code=3, length=length, ...)
}

pdf("biomass plots.pdf", width=16, height=10, useDingbats=F) # barplot for above ground biomass by microsite
nozeroz<-subset(data, above>0 & status=="native")
species.avg <- aggregate(nozeroz$above, by=list(nozeroz$species,nozeroz$micro), mean)
species.se <- aggregate(nozeroz$above, by=list(nozeroz$species,nozeroz$micro), se)
species.avg[,"se"] <- species.se[,3]
dat2 <- data.frame(shrub=species.avg[,6:10,3],open=species.avg[,1:5,3])
(dat2 <- as.matrix(t(dat2)))
dat3 <- data.frame(shrub=species.avg[,6:10,"se"],open=species.avg[,1:5,"se"])
(dat3 <- as.matrix(t(dat3)))

## make italic function
make.italic <- function(x) as.expression(lapply(x, function(y) bquote(italic(.(y)))))

par(mfrow=c(2, 3))
par(mar=c(1.5,4.5,2,0)) # changes margins
plot1 <- barplot(dat2, beside=TRUE, # puts shrub and open beside each other
  xlab="Microsite", ylab="Above Ground Biomass")

make.italic("Shrub")
xlab="", ylab="mean above-ground biomass (g)", col=c("Grey45","White"), axis.lty=1, ylim=c(0,1.82), cex.axis=1.4, cex.lab=1.8)
error.bar.plot1, dat2,dat3, lwd=2)
box()

#axis(1, plot1[1,]+0.5, make.italic(c("Amsinckia","Caulanthus","Lepidium","Monolopia","Phacelia")), cex.axis=1.5, padj=c(0.6,0.6,0.8,0.8,0.7))
#axis(1, plot1[1,]+0.5, make.italic(c("tessellata","lasiophyllum","nitidum","lanceolata","tanacetifolia")), cex.axis=1.5, padj=(2.3,2.0,2.3,2.2,2.2)-0.2)
legend(1.18, c("shrub","open"), pch=22, pt.bg=c("Grey45","White"), cex=1.7, bty="n")
text(8,0.15, "*", cex=1.7) #lipi sig

text(11,0.35, "*", cex=1.7) #mono sig

text(14,1.76, "****", cex=1.7) #phac sig

## barplot for above ground biomass by exclosure

nozeroz<-subset(data, above>0 & status=="native")
species.avg <- aggregate(nozeroz$above, by=list(nozeroz$species, nozeroz$exclosure), mean)
species.se <- aggregate(nozeroz$above, by=list(nozeroz$species, nozeroz$exclosure), se)
species.avg[,"se"] <- species.se[,3]

dat2 <- data.frame(exclosure=species.avg[1:5,3],no.exclosure=species.avg[6:10,3])
dat2 <- as.matrix(t(dat2))

dat3 <- data.frame(exclosure=species.avg[1:5,"se"],no.exclosure=species.avg[6:10,"se"])
dat3 <- as.matrix(t(dat3))

##make italic function

make.italic <- function(x) as.expression(lapply(x, function(y) bquote(italic(.y)))))

par(mar=c(1.5,1,2,0)) #changes margins
plot1 <- barplot(dat2, beside=TRUE, #puts shrub and open beside each other
xlab="", ylab="", col=c("Grey45","White"), axis.lty=1, ylim=c(0,1.82), yaxt='n', cex.axis=1.4, cex.lab=1.8)
error.bar.plot1, dat2,dat3, lwd=2)

x<-c(0, 0.5, 1.0, 1.5)
box()

axis(2, at=x, labels=FALSE)

#axis(1, plot1[1,]+0.5, make.italic(c("Amsinckia","Caulanthus","Lepidium","Monolopia","Phacelia")), cex.axis=1.5, padj=c(0.6,0.6,0.8,0.8,0.7))
#axis(1, plot1[1,]+0.5, make.italic(c("tessellata","lasiophyllum","nitidum","lanceolata","tanacetifolia")), cex.axis=1.5, padj=(2.3,2.0,2.3,2.2,2.2)-0.2)
legend(1.18, c("exclosure","no exclosure"), pch=22, pt.bg=c("Grey45","White"), cex=1.7, bty="n")
text(5,0.65, "*", cex=2) #caul sig
text(14, 1.4, "**", cex=2) #phac sig

## barplot for above ground biomass by removal

nozeroz<-subset(data, above>0 & status=="native")
species.avg <- aggregate(nozeroz$above, by=list(nozeroz$species, nozeroz$removal), mean)
species.se <- aggregate(nozeroz$above, by=list(nozeroz$species, nozeroz$removal), se)
species.avg[,"se"] <- species.se[,3]

dat2 <- data.frame(removal=species.avg[6:10,3],present=species.avg[1:5,3])
dat2 <- as.matrix(t(dat2))

dat3 <- data.frame(removal=species.avg[6:10,"se"],present=species.avg[1:5,"se"])
dat3 <- as.matrix(t(dat3))
## make italic function

```r
make.italic <- function(x) as.expression(lapply(x, function(y) bquote(italic(.(y)))))
```

```r
par(mar=c(1.5,1.2,1,1)) #changes margins
```

```r
plot1 <- barplot(dat2, beside=TRUE, #puts shrub and open beside each other
    xlab="", ylab="", col=c("Grey45","White"), axis.lty=1, ylim=c(0,1.82), yaxt='n', cex.axis=1.4, cex.lab=1.8)
error.bar(plot1, dat2,dat3, lwd=2)
box()
```

```r
x<-c(0, 0.5, 1.0, 1.5)
axis(2, at=x, labels=FALSE)
#axis(1, plot1[1,]+0.5, make.italic(c("Amsinckia","Caulanthus","Lepidium","Monolopia","Phacelia")),
#cex.axis=1.5, padj=c(0.6,0.6,0.8,0.8,0.7))
#axis(1, plot1[1,]+0.5, make.italic(c("tessellata","lasiophyllus","nitidum","lanceolata","tanacetifolia")),
cex.axis=1.5, padj=(c(2.3,2.0,2.3,2.2,2.2)-0.2)
legend(1,1.8, c("removal","present"), pch=22, pt.bg=c("Grey45","White"), cex=1.7, bty="n")
text(2, 0.6,"***", cex=2) #lipi sig
```

```r
text(11, 0.33, "****", cex=2) #mono sig
```

## below ground biomass

```r
## by microsite
nozeroz<-subset(data, below>0 & status=="native")
species.avg <- aggregate(nozeroz$below, by=list(nozeroz$species,nozeroz$micro), mean)
species.se <- aggregate(nozeroz$below, by=list(nozeroz$species,nozeroz$micro), se)
```

```r
species.avg[,"se"] <- species.se[,3]
dat2 <- data.frame(shrub=species.avg[6:10,3],open=species.avg[1:5,3])
dat2 <- as.matrix(t(dat2))
dat3 <- data.frame(shrub=species.avg[6:10,"se"],open=species.avg[1:5,"se"])
dat3 <- as.matrix(t(dat3))
```

## make italic function

```r
make.italic <- function(x) as.expression(lapply(x, function(y) bquote(italic(.(y)))))
```

```r
par(mar=c(5,4.5,1,0)) #changes margins
```

```r
plot1 <- barplot(dat2, beside=TRUE, #puts shrub and open beside each other
    xlab="", ylab="mean below-ground biomass (g)", col=c("Grey45","White"), axis.lty=1, ylim=c(0,0.14),
cex.axis=1.4, cex.lab=1.8)
error.bar(plot1, dat2,dat3, lwd=2)
box()
```

```r
axis(1, plot1[1,]+0.5, make.italic(c("Amsinckia","Caulanthus","Lepidium","Monolopia","Phacelia")),
cex.axis=1.5, padj=c(0.6,0.6,0.8,0.8,0.7))
axis(1, plot1[1,]+0.5, make.italic(c("tessellata","lasiophyllus","nitidum","lanceolata","tanacetifolia")),
cex.axis=1.5, padj=(c(2.3,2.0,2.3,2.2,2.2)-0.2)
legend(1,0.14, c("shrub","open"), pch=22, pt.bg=c("Grey45","White"), cex=1.7, bty="n")
text(8,0.02, "***", cex=2) #lipi sig
```

## barplot for below ground biomass by exclosure

```r
nozeroz<-subset(data, below>0 & status=="native")
species.avg <- aggregate(nozeroz$below, by=list(nozeroz$species,nozeroz$exclosure), mean)
species.se <- aggregate(nozeroz$below, by=list(nozeroz$species,nozeroz$exclosure), se)
```
species.avg[,"se"] <- species.se[,3]

dat2 <- data.frame(exclosure=species.avg[1:5,3],no.exclosure=species.avg[6:10,3])
dat2 <- as.matrix(t(dat2))
dat3 <- data.frame(exclosure=species.avg[1:5,"se"],no.exclosure=species.avg[6:10,"se"])
dat3 <- as.matrix(t(dat3))

## make italic function
make.italic <- function(x) as.expression(lapply(x, function(y) bquote(italic(.(y)))))

par(mar=c(5,1,1,0)) # changes margins
plot1 <- barplot(dat2, beside=TRUE, # puts shrub and open beside each other
     xlab="", ylab="", col=c("Grey45","White"), axis.lty=1, ylim=c(0,0.14), yaxt="n", cex.axis=1.4, cex.lab=1.8)
error.bar(plot1, dat2,dat3, lwd=2)
box()

x<-c(2,0.02,0.04,0.06,0.08,0.1,0.12,0.14)
axis(2, at=x, labels=FALSE)
axis(1, plot1[1,]+0.5, make.italic(c("Amsinckia","Caulanthus","Lepidium","Monolopia","Phacelia")), cex.axis=1.5, padj=c(0,0.6,0.8,0.8,0.7))
axis(1, plot1[1,]+0.5, make.italic(c("tessellata","lasiophyllus","nitidum","lanceolata","tanacetifolia")), cex.axis=1.5, padj=c(2.3,2.0,2.3,2.2,2.2)-0.2)
legend(1,0.14, c("exclusion","no exclosure"), pch=22, pt.bg=c("Grey45","White"), cex=1.7, bty="n")
text(5,0.1, "+", cex=2) # caul sig

## barplot for below ground biomass by removal
nozeroz<-subset(data, below>0 & status=="native")
species.avg <- aggregate(nozeroz$below, by=list(nozeroz$species,nozeroz$removal), mean)
species.se <- aggregate(nozeroz$below, by=list(nozeroz$species,nozeroz$removal), se)
species.avg[,"se"] <- species.se[,3]

dat2 <- data.frame(removal=species.avg[6:10,3],present=species.avg[1:5,3])
dat2 <- as.matrix(t(dat2))
dat3 <- data.frame(removal=species.avg[6:10,"se"],present=species.avg[1:5,"se"])
dat3 <- as.matrix(t(dat3))

## make italic function
make.italic <- function(x) as.expression(lapply(x, function(y) bquote(italic(.(y)))))

par(mar=c(5,1,1,5)) # changes margins
plot1 <- barplot(dat2, beside=TRUE, # puts shrub and open beside each other
     xlab="", ylab="", col=c("Grey45","White"), axis.lty=1, ylim=c(0,0.14), yaxt="n", cex.axis=1.4, cex.lab=1.8)
error.bar(plot1, dat2,dat3, lwd=2)
box()

x<-c(2,0.02,0.04,0.06,0.08,0.1,0.12,0.14)
axis(2, at=x, labels=FALSE)
axis(1, plot1[1,]+0.5, make.italic(c("Amsinckia","Caulanthus","Lepidium","Monolopia","Phacelia")), cex.axis=1.5, padj=c(0,0.6,0.8,0.8,0.7))
axis(1, plot1[1,]+0.5, make.italic(c("tessellata","lasiophyllus","nitidum","lanceolata","tanacetifolia")), cex.axis=1.5, padj=c(2.3,2.0,2.3,2.2,2.2)-0.2)
legend(1,0.14, c("removal","present"), pch=22, pt.bg=c("Grey45","White"), cex=1.7, bty="n")

dev.off()
nozeroz <- subset(data, status == "native")

species.avg <- aggregate(nozeroz$abundance, by=list(nozeroz$species, nozeroz$micro), mean)

species.se <- aggregate(nozeroz$abundance, by=list(nozeroz$species, nozeroz$micro), se)

species.avg[, "se"] <- species.se[, 3]

dat2 <- data.frame(shrub=species.avg[6:10,3], open=species.avg[1:5,3])
dat2 <- as.matrix(t(dat2))

dat3 <- data.frame(shrub=species.avg[6:10,"se"], open=species.avg[1:5,"se"])
dat3 <- as.matrix(t(dat3))

## make italic function
make.italic <- function(x) as.expression(lapply(x, function(y) bquote(italic(y))))

par(mfrow=c(1, 3))
par(mar=c(5,4,5,1,0)) # changes margins
plot1 <- barplot(dat2, beside=TRUE, # puts shrub and open beside each other
    xlab="", ylab="mean abundance", col=c("Grey45", "White"), axis.lty=1, ylim=c(0,30), cex.axis=1.4, cex.lab=1.8)
error.bar(plot1, dat2, dat3, lwd=2)
box()
axis(1, plot1[1,]+0.5, make.italic(c("Amsinckia","Caulanthus","Lepidium","Monolopia","Phacelia")), cex.axis=1.5, padj=c(0,6,0.6,0.8,0.8,0.7))
axis(1, plot1[1,]+0.5, make.italic(c("tessellata","lasiophyllus","nitidum","lanceolata","tanacetifolia")), cex.axis=1.5, padj=c(2.3,2.0,2.3,2.2,2.2)-0.2)
legend(1.30, c("shrub","open"), pch=22, pt.bg=c("Grey45","White"), cex=1.7, bty="n")
text(8,11, "***", cex=2) # lipi sig

## barplot for abundance by exclosure
nozeroz <- subset(data, status == "native")

species.avg <- aggregate(nozeroz$abundance, by=list(nozeroz$species, nozeroz$exclosure), mean)

species.se <- aggregate(nozeroz$abundance, by=list(nozeroz$species, nozeroz$exclosure), se)

species.avg[, "se"] <- species.se[, 3]

dat2 <- data.frame(exclosure=species.avg[1:5,3], no.exclosure=species.avg[6:10,3])
dat2 <- as.matrix(t(dat2))

dat3 <- data.frame(exclosure=species.avg[1:5,"se"], no.exclosure=species.avg[6:10,"se"])
dat3 <- as.matrix(t(dat3))

## make italic function
make.italic <- function(x) as.expression(lapply(x, function(y) bquote(italic(y))))

par(mfrow=c(1, 3))
par(mar=c(5,2,1,2)) # changes margins
plot1 <- barplot(dat2, beside=TRUE, # puts shrub and open beside each other
    xlab="", ylab="", col=c("Grey45","White"), axis.lty=1, ylim=c(0,30), yaxt='n', cex.axis=1.4, cex.lab=1.8)
error.bar(plot1, dat2, dat3, lwd=2)
box()
x<-c(0.5,10,15,20,25,30)
axis(2, at=x, labels=FALSE)
axis(1, plot1[1,]+0.5, make.italic(c("Amsinckia","Caulanthus","Lepidium","Monolopia","Phacelia")), cex.axis=1.5, padj=c(0,6,0.6,0.8,0.8,0.7))
axis(1, plot1[1,]+0.5, make.italic(c("tessellata","lasiophyllus","nitidum","lanceolata","tanacetifolia")), cex.axis=1.5, padj=c(2.3,2.0,2.3,2.2,2.2)-0.2)
legend(1,30, c("exclosure","no exclosure"), pch=22, pt.bg=c("Grey45","White"), cex=1.7, bty="n")
text(2.3, "*", cex=2) # amsk sig
text(8.15, "****", cex=2) # lipi sig
text(11.45, "****", cex=2) # mono sig
text(14.29, "****", cex=2) # phac sig

## barplot for abundance by removal
nozeroz<-subset(data, status=="native")
species.avg <- aggregate(nozeroz$abundance, by=list(nozeroz$species,nozeroz$removal), mean)
species.se <- aggregate(nozeroz$abundance, by=list(nozeroz$species,nozeroz$removal), se)

species.avg[,"se"] <- species.se[3]
dat2 <- data.frame(removal=species.avg[6:10,3],present=species.avg[1:5,3])
dat2 <- as.matrix(t(dat2))
dat3 <- data.frame(removal=species.avg[6:10,"se"],present=species.avg[1:5,"se"])
dat3 <- as.matrix(t(dat3))

## make italic function
make.italic <- function(x) as.expression(lapply(x, function(y) bquote(italic(.(y)))))

par(mar=c(5,0,1,4)) # changes margins
plot1 <- barplot(dat2, beside=TRUE, # puts shrub and open beside each other
    xlab="", ylab="", col=c("Grey45","White"), axis.lty=1, ylim=c(0,30), yaxt="n", cex.axis=1.4, cex.lab=1.8)
error.bar(plot1, dat2,dat3, lwd=2)
box()
x<-c(0,5,10,15,20,25,30)
axis(2, at=x, labels=FALSE)
axis(1, plot1[1,]+0.5, make.italic(c("Amsinckia","Caulanthus","Lepidium","Monolopia","Phacelia")), cex.axis=1.5, padj=c(0.6,0.6,0.8,0.8,0.7))
axis(1, plot1[1,]+0.5, make.italic(c("tessellata","lasiophyllus","nitidum","lanceolata","tanacetifolia")), cex.axis=1.5, padj=c(2.3,2.0,2.3,2.2,2.2)-0.2)
legend(1,30, c("removal","present"), pch=22, pt.bg=c("Grey45","White"), cex=1.7, bty="n")
text(5, 8,"****", cex=2) # caul sig

#abundance by seed number
#AMSK = 235 seeds per 1 gram
#CAUL = 2019 seeds per 1 gram
#LEPI = 752 seeds per 1 gram
#MONO = 738 seeds per 1 gram
#PHAC = 772 seeds per 1 gram

data2[grep("amsk",as.character(data2$species)), "seednumber"]<-235
data2[grep("caul",as.character(data2$species)), "seednumber"]<-2019
data2[grep("lepi",as.character(data2$species)), "seednumber"]<-752
data2[grep("mono",as.character(data2$species)), "seednumber"]<-738
data2[grep("phac",as.character(data2$species)), "seednumber"]<-772

data2[,"abunseed"] <- data2$abundance/data2$seednumber

## by microsite
nozeroz<-subset(data2, status=="native")
species.avg <- aggregate(nozeroz$abundance, by=list(nozeroz$species,nozeroz$micro), mean)
species.se <- aggregate(nozeroz$abundance, by=list(nozeroz$species,nozeroz$micro), se)
species.avg[,"se"] <- species.se[,3]

dat2 <- data.frame(shrub=species.avg[6:10,3],open=species.avg[1:5,3])
dat2 <- as.matrix(t(dat2))

dat3 <- data.frame(shrub=species.avg[6:10,"se"],open=species.avg[1:5,"se"])
dat3 <- as.matrix(t(dat3))

## make italic function
make.italic <- function(x) as.expression(lapply(x, function(y) bquote(italic(.(y)))))

par(mfrow=c(1, 3))
par(mar=c(5,4.5,1,0)) # changes margins
plot1 <- barplot(dat2, beside=TRUE, # puts shrub and open beside each other
     xlab="", ylab="mean abundance", col=c("Grey45","White"), axis.lty=1, ylim=c(0,0.04), cex.axis=1.4, cex.lab=1.8)
error.bar(plot1, dat2,dat3, lwd=2)
box()
axis(1, plot1[1,]+0.5, make.italic(c("Amsinckia","Caulanthus","Lepidium","Monolopia","Phacelia")), cex.axis=1.5, padj=c(0.6,0.6,0.8,0.8,0.7))
axis(1, plot1[1,]+0.5, make.italic(c("tessellata","lasiophyllus","nitidum","lanceolata","tanacetifolia")), cex.axis=1.5, padj=c(2.3,2.0,2.3,2.2,2.2)-0.2)
legend(1,0.04, c("shrub","open"), pch=22, pt.bg=c("Grey45","White"), cex=1.7, bty="n")
text(8,11, "*", cex=2) # lipi sig
text(11,3, "**", cex=2) # mono sig

## barplot for abunseed by exclosure
nozeroz<-subset(data2, status=="native")
species.avg <- aggregate(nozeroz$abunseed, by=list(nozeroz$species,nozeroz$exclosure), mean)
species.se <- aggregate(nozeroz$abunseed, by=list(nozeroz$species,nozeroz$exclosure), se)

species.avg[,"se"] <- species.se[,3]

dat2 <- data.frame(exclosure=species.avg[1:5,3],no.exclosure=species.avg[6:10,3])
dat2 <- as.matrix(t(dat2))

dat3 <- data.frame(exclosure=species.avg[1:5,"se"],no.exclosure=species.avg[6:10,"se"])
dat3 <- as.matrix(t(dat3))

## make italic function
make.italic <- function(x) as.expression(lapply(x, function(y) bquote(italic(.(y)))))

par(mfrow=c(5,2,1,2)) # changes margins
plot1 <- barplot(dat2, beside=TRUE, # puts shrub and open beside each other
     xlab="", ylab="", col=c("Grey45","White"), axis.lty=1, yaxt='n', cex.axis=1.4, cex.lab=1.8)
error.bar(plot1, dat2,dat3, lwd=2)
box()
x<-c(0,5,10,15,20,25,30)
axis(2, at=x, labels=FALSE)
axis(1, plot1[1,]+0.5, make.italic(c("Amsinckia","Caulanthus","Lepidium","Monolopia","Phacelia")), cex.axis=1.5, padj=c(0.6,0.6,0.8,0.8,0.7))
axis(1, plot1[1,]+0.5, make.italic(c("tessellata","lasiophyllus","nitidum","lanceolata","tanacetifolia")), cex.axis=1.5, padj=c(2.3,2.0,2.3,2.2,2.2)-0.2)
legend(1,0.04, c("exclosure","no exclosure"), pch=22, pt.bg=c("Grey45","White"), cex=1.7, bty="n")
text(2,3, "*", cex=2) # amsk sig
text(8,15, "***", cex=2) # lipi sig
text(11, 4.5, "***", cex=2) # mono sig
## barplot for abunseed by removal

```r
nozeroz <- subset(data2, status=="native")
species.avg <- aggregate(nozeroz$abunseed, by=list(nozeroz$species, nozeroz$removal), mean)
species.se <- aggregate(nozeroz$abunseed, by=list(nozeroz$species, nozeroz$removal), se)

species.avg[,"se"] <- species.se[,3]
dat2 <- data.frame(removal=species.avg[6:10,3],present=species.avg[1:5,3])
dat2 <- as.matrix(t(dat2))
dat3 <- data.frame(removal=species.avg[6:10,"se"],present=species.avg[1:5,"se"])
dat3 <- as.matrix(t(dat3))

## make italic function
make.italic <- function(x) as.expression(lapply(x, function(y) bquote(italic(.(y)))))
```

```r
par(mar=c(5,0,1,4)) #changes margins
plot1 <- barplot(dat2, beside=TRUE, #puts shrub and open beside each other
     xlab="", ylab="", col=c("Grey45","White"), axis.lty=1, ylim=c(0,0.04), yaxt='n', cex.axis=1.4, cex.lab=1.8)
error.bar(plot1, dat2,dat3, lwd=2)
box()
x<-c(0,5,10,15,20,25,30)
axis(2, at=x, labels=FALSE)
axis(1, plot1[1,,]+0.5, make.italic(c("Amsinckia","Caulanthus","Lepidium","Monolopia","Phacelia")), cex.axis=1.5, padj=c(0.6,0.6,0.8,0.8,0.7))
axis(1, plot1[1,,]+0.5, make.italic(c("tessellata","lasiophyllus","nitidum","lanceolata","tanacetifolia")), cex.axis=1.5, padj=c(2.3,2.0,2.3,2.2,2.2)-0.2)
legend(1,0.04, c("removal","present"), pch=22, pt.bg=c("Grey45","White"), cex=1.7, bty="n")
text(5, 8,"***", cex=2) # phac sig
```

## average number of flowers

```r
## by microsite
nozeroz <- subset(data, flowersavg>0 & status=="native")
species.avg <- aggregate(nozeroz$flowersavg, by=list(nozeroz$species, nozeroz$micro), mean)
species.se <- aggregate(nozeroz$flowersavg, by=list(nozeroz$species, nozeroz$micro), se)

species.avg[,"se"] <- species.se[,3]
dat2 <- data.frame(shrub=species.avg[6:10,3],open=species.avg[1:5,3])
dat2 <- as.matrix(t(dat2))
dat3 <- data.frame(shrub=species.avg[6:10,"se"],open=species.avg[1:5,"se"])
dat3 <- as.matrix(t(dat3))

## make italic function
make.italic <- function(x) as.expression(lapply(x, function(y) bquote(italic(.(y)))))
par(mfrow=c(1,3))
par(mar=c(5,4.5,1,1)) #changes margins
plot1 <- barplot(dat2, beside=TRUE, #puts shrub and open beside each other
     xlab="mean number of flowers", ylab="mean number of flowers", col=c("Grey45","White"), axis.lty=1, ylim=c(0,40), cex.axis=1.4, cex.lab=1.8)
error.bar(plot1, dat2,dat3, lwd=2)
box()
```

```r
text(5, 8,"***", cex=2) # phac sig
```
## barplot for flowers by exclosure

nozeroz <- subset(data, flowersavg > 0 & status == "native")
species.avg <- aggregate(nozeroz$flowersavg, by=list(nozeroz$species, nozeroz$exclosure), mean)
species.se <- aggregate(nozeroz$flowersavg, by=list(nozeroz$species, nozeroz$exclosure), se)

species.avg[, "se"] <- species.se[, 3]
dat2 <- data.frame(exclosure=species.avg[1:5, 3], no.exclosure=species.avg[6:10, 3])
dat2 <- as.matrix(t(dat2))
dat3 <- data.frame(exclosure=species.avg[1:5, "se"], no.exclosure=species.avg[6:10, "se"])
dat3 <- as.matrix(t(dat3))

## make italic function

make.italic <- function(x) as.expression(lapply(x, function(y) bquote(italic(.)(y))))

par(mar=c(5,0,1,1)) # changes margins

plot1 <- barplot(dat2, beside=TRUE, # puts shrub and open beside each other
                 xlab="", ylab="", col=c("Grey45", "White"), axis.lty=1, ylim=c(0,40), yaxt='n', cex.axis=1.4, cex.lab=1.8)

error.bar(plot1, dat2, dat3, lwd=2)

box()

x <- c(0,10,20,30,40)
axis(1, at=x, labels=FALSE)
axis(1, plot1[1,]+0.5, make.italic(c("Amsinckia","Caulanthus","Lepidium","Monolopia","Phacelia")), cex.axis=1.5, padj=c(0.6,0.6,0.8,0.8,0.7))
axis(1, plot1[1,]+0.5, make.italic(c("tessellata","lasiophyllus","nitidum","lanceolata","tanacetifolia")), cex.axis=1.5, padj=c(2.3,2.0,2.2,2.2,2.2)-0.2)

legend(1.4, c("shrub","open"), pch=22, pt.bg=c("Grey45","White"), cex=1.7, bty="n")

text(14,39, 

## barplot for flowers by removal

nozeroz <- subset(data, flowersavg > 0 & status == "native")
species.avg <- aggregate(nozeroz$flowersavg, by=list(nozeroz$species, nozeroz$removal), mean)
species.se <- aggregate(nozeroz$flowersavg, by=list(nozeroz$species, nozeroz$removal), se)

species.avg[, "se"] <- species.se[, 3]
dat2 <- data.frame(removal=species.avg[6:10, 3], present=species.avg[1:5, 3])
dat2 <- as.matrix(t(dat2))
dat3 <- data.frame(removal=species.avg[6:10, "se"], present=species.avg[1:5, "se"])
dat3 <- as.matrix(t(dat3))

## make italic function

make.italic <- function(x) as.expression(lapply(x, function(y) bquote(italic(.)(y))))

par(mar=c(5,0,1,1)) # changes margins

plot1 <- barplot(dat2, beside=TRUE, # puts shrub and open beside each other
                 xlab="", ylab="", col=c("Grey45", "White"), axis.lty=1, ylim=c(0,40), yaxt='n', cex.axis=1.4, cex.lab=1.8)

error.bar(plot1, dat2, dat3, lwd=2)

box()

x <- c(0,10,20,30,40)
axis(1, at=x, labels=FALSE)
axis(1, plot1[1,]+0.5, make.italic(c("Amsinckia","Caulanthus","Lepidium","Monolopia","Phacelia")), cex.axis=1.5, padj=c(0.6,0.6,0.8,0.8,0.7))
axis(1, plot1[1,]+0.5, make.italic(c("tessellata","lasiophyllus","nitidum","lanceolata","tanacetifolia")), cex.axis=1.5, padj=c(2.3,2.0,2.2,2.2,2.2)-0.2)

legend(1.4, c("exclosure","no exclusion"), pch=22, pt.bg=c("Grey45","White"), cex=1.7, bty="n")

text(5,10, "*", cex=2) # caul sig

text(14,37, "***", cex=2) # phac sig

## make italic function

make.italic <- function(x) as.expression(lapply(x, function(y) bquote(italic(.)(y))))

par(mar=c(5,0,1,5)) # changes margins

plot1 <- barplot(dat2, beside=TRUE, # puts shrub and open beside each other
xlab="", ylab="", col=c("Grey45","White"), axis.lty=1, ylim=c(0,40), yaxt='n', cex.axis=1.4, cex.lab=1.8)
error.bar(plot1, dat2,dat3, lwd=2)
box()
x<-c(0,10,20,30,40)
axis(2, at=x, labels=FALSE)
axis(1, plot1[1,]+0.5, make.italic(c("Amsinckia","Caulanthus","Lepidium","Monolopia","Phacelia")), cex.axis=1.5, padj=c(0,0.6,0.6,0.8,0.8,0.7))
axis(1, plot1[1,]+0.5, make.italic(c("tessellata","lasiophyllus","nitidum","lanceolata","tanacetifolia")), cex.axis=1.5, padj=c(2.3,2.0,2.3,2.2,2.2)-0.2)
legend(1,40, c("removal","present"), pch=22, pt.bg=c("Grey45","White"), cex=1.7, bty="n")

## temperature and humidity

```r
# temperature and humidity
data2<-read.table("C:\\Users\\Amanda\\Documents\\PhD\\Chapter 2 seed addition\\HOBOdata\\HOBO data complied 2016.csv", header=T, sep=",")
str(data2)
library(dplyr)
month <- data2 %>% group_by(micro, month) %>% summarize(temp=mean(temp), RH=mean(RH))
month.se <- aggregate(data2, by=list(data2$micro, data2$month),se)
month <- data.frame(month)
new.order <- c(7,3,5,4,6,1,7,3,5,4,6,2,1)
month[,"neworder"] <- new.order
month <- month[order(month$micro, month$neworder),]
rownames(month) <- factor(seq(1,14,1))

month <- timeall %>% group_by(micro, month) %>% summarize(temp=mean(temp), RH=mean(RH))
month.se <- aggregate(timeall, by=list(timeall$micro, timeall$month),se)
month <- data.frame(month)
new.order <- c(6, 2, 4, 3, 5, 1, 6, 2, 4, 3, 5, 1, 13)
month[,"neworder"] <- new.order
month <- month[order(month$micro, month$neworder),]
rownames(month) <- factor(seq(1,13,1))

## error bar function
error.bar <- function(x, y, upper, lower=upper, length=0.0,...)

month<-month[1:12,]
par(mar=c(4.5,4.5,1,5))
plot(seq(1,6,1)+0.1,matrix(month$micro=="shrub","temp"), pch=19, col="grey30", ylim=c(0,30), xlim=c(0,8.6,2), cex=2, cex.lab=2, cex.axis=1.9, xaxt="n", xlab="", ylab="temperature (°C)"
points(seq(1,6,1)-0.1,matrix(month$micro=="open","temp"), pch=21, col="grey70", type="l", lwd=3) ## puts a line for open
points(seq(1,6,1)+0.1,matrix(month$micro=="shrub","temp"), pch=21, col="grey30", type="l", lwd=3) ## puts a line for shrub
error.bar(seq(1,6,1)-0.1,matrix(month$micro=="open","temp"),month.se[month$micro=="open","temp"])## error bar for open
error.bar(seq(1,6,1)+0.1,matrix(month$micro=="shrub","temp"),month.se[month$micro=="shrub","temp"])## error bar for shrub
points(seq(1,6,1)-0.1,matrix(month$micro=="open","temp"), pch=19, col="grey70", cex=2) ## plots white points on top so they done have strike through
points(seq(1,6,1)+0.1,matrix(month$micro=="shrub","temp"), pch=19, col="grey30", cex=2)
axis(1, seq(1,6,1), c("Nov","Dec","Jan","Feb","Mar","Apr"), cex.axis=1.9) ##add month axis
legend(0.8, 30, c("shrub","open"), pch=19, col=c("grey30","grey70"), cex=1.5, bty="n")
```
## humidity

```
nanatime <- na.omit(timeall)
month <- nanatime %>% group_by(micro, month) %>% summarize(RH=mean(RH))
month.se <- aggregate(nanatime, by=list(nanatime$micro, nanatime$month), se)
month <- data.frame(month)
new.order <- c(6, 2, 4, 3, 5, 1, 6, 2, 4, 3, 5, 1)
month[, "neworder"] <- new.order
month <- month[order(month$micro, month$neworder),]
rownames(month) <- factor(seq(1, 12, 1))

par(mar=c(4.5, 4.5, 1, .5))
plot(seq(1, 6, 1)+0.1, month[month$micro == "shrub","RH"], pch=19, col="grey30", ylim=c(70, 100), cex=2, cex.lab=2, cex.axis=1.9, xaxt="n", xlab="", ylab="relative humidity(%)")
points(seq(1, 6, 1)-0.1, month[month$micro == "open","RH"], pch=21, col="grey70", type="l", lwd=3) # puts a line for open
points(seq(1, 6, 1)+0.1, month[month$micro == "shrub","RH"], pch=21, col="grey30", type="l", lwd=3) # puts a line for shrub
error.bar(seq(1, 6, 1)-0.1, month[month$micro == "open","RH"], month.se[month$micro == "open","RH"]) # error bar for open
error.bar(seq(1, 6, 1)-0.1, month[month$micro == "shrub","RH"], month.se[month$micro == "shrub","RH"]) # error bar for shrub
points(seq(1, 6, 1)-0.1, month[month$micro == "open","RH"], pch=19, col="grey70", cex=2) # plots white points on top so they don't have a strike through
points(seq(1, 6, 1)+0.1, month[month$micro == "shrub","RH"], pch=19, col="grey30", cex=2)
axis(1, seq(1, 6, 1), c("Nov", "Dec", "Jan", "Feb", "Mar", "Apr"), cex.axis=1.9) # add month axis
legend(5.5, 30, c("shrub", "open"), pch=19, col=c("green", "darkslateblue"), cex=1.5, bty="n")
```
Appendix F: Temperature and relative humidity throughout the growing season

Figure F1: Change in temperature (top) and relative humidity (bottom) throughout the 2016 growing season (November – April) between shrub and open microsites. Temperature and Humidity were recorded with HOBO ProV2 loggers located in shrub and open microsites.
Appendix G: Figure of mean number of flowers between microsites, exclosure and non-native removal treatments for five annual species

Figure G1: The average number of flowers per individual for five native forb species seeded in two microsites (shrub and open), with and without exclosures and with and without non-native species removals. Results shown are mean ± SE. Significance at α<0.05 denoted by *** = ≤ 0.001, ** = ≤ 0.01, * = ≤ 0.05.
Appendix G2: Relative Interaction Index (RII) values for the mean number of flowers per individuals among the three treatments: microsite, exclosures and non-native removals. Values shown are means ± 95% bootstrapped confidence intervals. Values that are significantly greater than zero indicate positive effects, while values that are significantly lower than zero indicate negative effects. Any value that is not significantly different from zero is a neutral effect.