Larrea tridentata indirectly interacts with co-blooming annuals with species-specific effects that map onto arthropod and plant communities

Here I show that Larrea interacts with plants, but also with other trophic levels and indirectly for pollinators. We explore the relative importance of plant-plant, plant-arthropod, and plant-pollinator interactions as the foundation plant shifts into a reproductive life stage.

Introduction

Foundational plants have positive structural influences onto their associated plant communities. In arid environments, shrubs can act as keystone facilitators, directly benefiting associated plants via multiple mechanistic pathways across all life stages (Filazzola and Lortie, 2014), such as stress amelioration, improved water and nutrient availability (Whitford et al, 1994) and seed trapping (Flores and Jurado, 2003). Most studies only consider plant-plant, and few pay attention to indirect interactions that arise from proximity or how foundation plants influence other trophic levels. Interactions for pollinators between plants forms a continuum from competition to facilitation. If they both flower, then they likely interact for pollinators. Bruno et al. (2003) predicts temporal flips in relative interactions from competition to facilitation, however few studies have documented the effect. Another major objective of this study is to test for relative changes in interactions when foundational plants shift into a reproductive life stage. Interactions have been documented to vary between years, but they can also vary within a year. A shift by such a dominant plant may alter the reproductive success of its neighbours. However, the same attributes that make a plant act as a magnet are the same that may cause it to compete. This is an indirect interaction, which is mediated by insects. Facilitation of shared pollinators can be particularly important in deserts because harsh environmental conditions can lead to large spatial variation in floral abundances and pollinator populations (Rathcke, 1983).

Mechanisms? Understanding mechanisms is important because… Facilitation pathways that don’t involve co-blooming are critically understudied. There are several pathways to facilitation: Change in behaviour (attracting more to area), change in population (concentrate pop). By sampling arthropod community, can try to see the pathways better. Community level interaction pathways very complicated. During the flowering period of the focal plant it may not be possible to separate interactions for pollinators from those that do not require co-blooming. If the shrub is providing wind-blocking or habitat, may see facilitation without flowers. On the competition side, it may not be possible to separate competition due to parasitizing pollinator visits versus interference. While one study has tested for mechanistic differences (Jacobsen), this is a test for sequential mutualism.

Harsh environments are a good place to study these interactions because plants tend to benefit more from amelioration. The question of mechanisms in this case is a insight into the potential intense environmental and interspecific pressures. Deserts are typified by harsh climate, high species specialization, short blooming periods, all of which may intensify positive and negative interactions. There have been no studies testing for interspecific facilitation in deserts, however intraspecific density has be shown to be positive (Roll et al, 1997). Interspecific studies have primarily focused on cacti systems (Fleming 2001 Sonoran). Competition for pollination is a driving factor for diverging phenologies, and the evolution of specialized mutualisms. Harsh environmental conditions strongly constrain when flowers can bloom in the desert. And they are apparently really specialized. But there isn’t really empirical evidence for the interactions between pollinators. Researchers have found that within season flowering phenologies within the desert are predictable (Jennings, 2001). Ambroisa dumosa increased seed set in annuals, however it is not possible to know if this was due to pollinator visits or a more direct sort of facilitation. Few studies looked at the indirect interactions of shrubs, indirect ones are understudied despite nurse plant literature being dominated by shrubs (Filazzola). Chung et al removed the flowers from Rosa multiflora (2014), but found no effect on bee visitation rates to co-blooming annuals. Shading by the shrub Lonicera decreased visitation and pollen deposition to annuals beneath it (McKinney, 2011). The majority of facilitation studies involving shrubs involve invasive species, however this is about foundation species. Reid found cushions facilitated.

Include a conceptual diagram of the mechanisms.

Creosote bush, Larrea tridentata (Zygophyllaceae) is a generalist shrub - the full pollinator guild contains 22 specialist pollinators and more than 80 generalists (Minkley et al, 1999). The associated guilds are highly variable over space and most shrubs only interact with 20% of the full guild, but there is a stable core guild (Cane and Minkley). L. tridentata has a large range and abundance. It has been a dominant flowering shrub for 25 000 years (Batancourt 1990), and individuals that are several thousand years old have been documented (Vasek, 1980). It both competes through allelopathy. It has been documented to act as a nurse shrub as well. It is a very reliable flowerer. Whether it competes for pollinators is not known. If they facilitate their understory, than they may be able to buffer their associates from a pollinator decline. But if they outcompete them, then their associates may be extra vulnerable. Understanding interactions for pollination at a community level is critical for understanding potential impacts of any decline. Larrea offers a lot of resources to pollinators. Second largest pollinator guild.

Here we test for the influence of larrea onto its commonly co-occurring annual Malacothrix glabrata. Larrea and Malacothrix overlap at beginning and ends of their phenology. This makes it an interesting system to test for changes in interactions for pollinators. Malacothrix was chosen as a phytometer because it commonly co-occurs with L. tridentata, and was the only annual growing sufficiently nearby in high enough abundances to use for experiments. Introduce more of the study here.

Hypothesis and Predictions

Therefore, we also look for responses in the associated arthropod communities. I predict that they will compete prior to Larrea blooming due to interference, and that Malacothrix will show increased visitation when larrea is blooming. More visits underneath than in open areas. To disentangle effects via blooms, we tested before and during full bloom. If larrea acts as a magnet, we expect to see an increase in arthropod abundance in pan traps.

Study site and weather.

The Mojave Desert is a biodiversity hotspot supporting 659 species of bees (Saul-Gershenz et al, 2012) and 1680 species of vascular plants (Rundel and Gibson, 2005). It’s desert, below precipitation etc. etc. The study site is located in Sunset Cove on the USNRS reserve Granites, elevation. The site is a gently sloping cove, shrub and cactus dominated community. The most abundant shrub is Acamptappus spheorpus, but larrea likely has the greatest biomass. Also common is ambrosia salsola, eriogrnum fascilatum, the chollas, thamnosa montana. The most common flowering annuals present during the study period were small Boraginace, crpythanta sp, Fremont phacelia, wallaces wooly daisy, gilia. Later more phacelia, Malacothrix, fremonts pincushion were common. The study took place between April 10th, 2017 and May 5th, 2017.

Methods:

Experimental design

Microsites were located in a paired fashion; one inside the dripline of the focal plant (“shrub”) and one a minimum of 1.5 m away in an open area (“open”), both on the south side of the shrub to ensure the plants were not shaded. The paired sites were used to minimize potential differences due to environmental heterogeneity. *Malacothrix glabrata*, desert dandelion, was used as a phytometer. Phytometer, definition here, are commonly employed in agricultural studies to measure pollination services.

Visitation to phytometer

The phytometers were gathered from nearby (<3 km) populations where it seasonally coexists with *L. tridentata* at the beginning of each study day. These were transplanted into 15 cm diameter black pots. Transplants with similar floral number, size and habit were paired and one pot was placed per microsite. The mean number of flowers of M. glabrata was 10.

Pollinator activity to phytometers was captured using Polaroid Cube+ HD cameras. They capture video in 1080p, and have an approximate run time of 1.5 hours.

Six shrub/open pairs were tested each day between the hours of 11:30 am and 3:30 pm to capture peak pollinator activity. Ten day of trials (60 shrub/open pairs) were prior to L. tridentata blooming. Individuals with fewer than five open blooms were considered non-blooming. The same shrubs were retested after the shrubs entered full bloom. The average number of blooms for ‘blooming’ treatment was 300.2 ± 176.72SD. The minimum tested was 102, the maximum was 1080. In two cases, a focal shrub did not bloom and was replaced by a new blooming shrub. The experiment began April 10th and the final day was May 5th resulting in 20 days of visitation footage. Five videos were omitted due to disturbance or battery failure.

The blooms of the *Malacothrix* were snipped to be equal in number between shrub and open sites, but left to vary between replicates, except for the first two days of the study.

Floral density can influence pollinator visitation (Bosch and Waser, 2001), we ensured that there were no blooming Malacothrix in the direct vicinity of the experiment. Heterospecific annual floral density and annual species richness were measured within a 0.25 m2 quadrat in each microsite. The number of heterospecific shrubs and cacti in bloom were counted within a 2 m radius of each microsite.

Video footage was reviewed in lab. Pollinator visitation, the number of flowers visited, duration of visit, and identity of visitor. A pollinator visit was defined as when an insect flew onto the flower, touching the reproductive organs. We also tracked when insects crawled onto the flower and touched other parts of the plant. Visitation rates are a commonly used proxy of pollination (cite).

Pollen deposition

To quantify how pollen deposition changes with proximity of L. tridentata, I collected stigma from M. glabrata at a nearby site (3 km) with a naturally occurring population of Malacothrix and L. tridentata. It was not possible to do this at my main study site because I could not ensure that the Malacothrix had not been pollinated prior to moving them to my site.

I collected three stigma from each of three flowers from one Malacothrix (nine stigmas per plant) growing each of under the dripline and in a nearby open area, 298 in total. Open area at least 1 m away from dripline of any larrea. Only 13 pairs were tested because a heatwave followed by a wind storm killed the Malacothrix. The distances to the three closest Malacothrix neighbours were measured and to the nearest L. tridentata. The number of Malacothrix flowers per plant were counted, and each Larrea was rated on a Likert scale (1 to 5) to quantify how in bloom it is. The x, y and z were quantified – this with the Likert scale forms a proxy for the number of flowers. The stigmas were stored individually in micro centrifuge tubes filled with denatured alcohol.

The tubes were spun down in a centrifuge at 4200 rpm for 4.5 minutes and the pellet pipetted onto the slide. This along with the stigma were mounted in fuchsin jelly (Kearns book).

At 100 x magnification, 10 longitudinal transects (18 mm by x mm) of pollen were counted per slide. Heterospecific pollen grains were imaged using a Canon 60D SLR with 60mm macro lens into microscope afocally. All stigma were also imaged.

Heterospecific pollen were identified using a reference collection created of 38 species from surrounding sites in 2017 and 2018. This reference collection was photographed using Lumenera microscope camera at 100 x and 400x and the size of grains were measured using Infinity Analyze to aid identification. The digitized reference collection was uploaded to global pollen project (DOI) and the slides are in Lortie Lab at York University.

Arthropod community sampling

We used pan traps to sample the arthropod communities associated with the microsites, as well as aid insect identification in videos. The pan traps consisted of yellow, white and blue six inch diameter plastic bowls (Solo brand). These were filled with water with a few drops of Dawn original dish detergent added. Three traps, one of each colour were placed in a triangular shape per microsite, slightly embedded in the ground to prevent blowing away. They were deployed by 10 am and picked up after 5:30 pm on sunny days to capture peak pollinator activity. Shrubs were videoed and pan trapped on different days as to not influence pollinator visitation to *Malacothrix*. There were placed under 6 shrub/open pairs per day, 9 days pre-blooming and 10 blooming. Percent vegetation cover was recorded in a 0.25 m2 quadrat. The arthropods were stored in 91% isopropyl alcohol. Mites and springtails were excluded from analyses. Bees and syrphid flies were identified to genus or species, rest to minimum of family except thrips, orthopterans and arachnids. Chalcids, and other parasitoids (<2mm) were left at family or subfamily due to their small size. RTU (recognizable taxonomic unit) is a suitable proxy for diversity analyses. A full list of RTU are used is in appendix. The groups are exclusive, for example, wasps in the genus Miscophus are in the family Crabronidae, but are only counted in the rtu Miscophus, not Crabronidae. Using RTU limits resolution, however many species have not been described and useful keys are often lacking. Nymphs were included in analyses provided they could be identified to order. The specimens are located within our collection in Lortie Lab at York University. Each pinned specimen has unique ID. Data available: Braun, 2018.

Arthropod use of Larrea

L. tridentata was observed in 15-minute time periods. Four individuals were observed per day, 10 days pre-blooming and 10 days blooming. The number of visits and identity of the visitors were recorded and visitors were collected when possible to aid identification. The part of the plant that was visited was recorded (branch, flower, understory – which includes the ground itself and plants growing under the shrub), and the general behaviour of the visitor – landing, touchdown (land then fly away), hovering/inspecting, crawling (understory only).

To measure the microclimates of the microsites, 16 HOBO pendant data loggers were used to record temperature and light availability. Eight loggers were placed under *L. tridentata* and eight in open areas for between March x and May 14th. They take readings every ½ hour. I calculated the average daily maximum and minimum temps and light availability.

Weather data

A Campbell weather station ([www.wrcc.dri.edu/ucnrs](http://www.wrcc.dri.edu/ucnrs)) in the adjacent Granite Cove provided hourly environmental data. Data logged between 10 am and 5:00 pm were used to correspond with study timings. The mean of hourly wind speed (m/s), mean temperature (ºC) and mean solar radiation were calculated for that time period daily.

Analysis

Video test

We fitted a GLMM (lme4) using a negative binomial error distribution to account for overdispersion. To maintain the count structure of the data I included the number of *M. glabrata* blooms as a predictor variable, and the length of the video as an offset. It is not uncommon to convert to visits/hour/flower, however this makes the assumption that pollinators respond linearly to the number of flowers and that the slope of the relationship does not change with any treatment. This method allows for more information to be maintained by not standardizing (cite). Due to the repeated measures study design – we included the rep id as a random effect. The rep ID is the ID of the focal plant + microsite (i.e. 270 open). I also calculated, and built models for plants visited and flowers visite. I fitted gamma GLMM for visit duration and proportion of flowers visited per visit. I calculated this at the rep level. RTU (recognizable taxonomic unit): these were honeybees, solitary bees, lepiodeptera, syrphid, bombyliid and other. These were integrated into models and using post-hoc tests I determined if there were RTU specific responses. Models were compared to null models of random intercept models using likelihood.

Insect communities

GLMM, again shrub identifier as a random effect. Species richness was modelled using a linear mixed model.

Beetles from the family Melyridae made up ~1000 of the total arthropods captured, so we ran analyses with them excluded, included and on their own because their high numbers really swamped out the responses from other insects.

Diversity indices were calculated using the r package vegan. To see if communities were different under shrubs, used rda ordination methods. Alpha diversity: Beta diversity, arthropod community turnover was also calculated for shrub open and pre-post. Hemipteran nymph were lumped together. Other nymphs were added to family as long as they could be IDed (example, coccinelidae, ladybug larvae).

Pollen

RII

*To enable contrasts between plant responses to cushions and arthropods and to assess the biological importance of statistically significant differences, the relative intensity of interactions (Rii) effect size metric was also calculated with cushion designated as the treatment and open as the control and compared via t-tests* [*[42]*](http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0037223#pone.0037223-Armas1)*. This metric is symmetric around 0, ranges from −1 to +1, and negative values denote relative competition whilst positives denote facilitation. The GLMMs were conducted using the lme4 package in R.2.10.1, and all other statistical analyses were conducted using R. 2.10.0.*

Veg

Linear models for percent cover and neg binomial GLMM annual richness and annual bloom density (glmer.nb, lme4)

**Results**

Camera test

A total of 697 flying floral visitors were recorded from 303 hours of video observation for a total of 925 flower visits. 61 of 235 observation periods had no flying visitors.

There was a negative effect of the shrub microsite (Figure 3a) and of blooming on both foraging bouts and total flowers visited, and a positive effect of the conspecific floral density. There was no effect of annual floral density. There was no influence of microsite of visit duration or the proportion of flowers visited per visit, but a positive influence of blooming on both visit duration and the proportion of flowers visited.

Hoverflies (Syrphidae: Scaeva and Toxomerus) (%) were the most frequent visitor. Others were next – beetles and individuals that were too small to adequately classify, as well as a few muscid flies. After them were solitary bees. After that flies in the Bombyliidae family (mainly Anthrancinae, Usiinae and Bombyliinae).

Rtu differences

RDA of visitor identity?

Effects on arthropod communities

Positive effect of shrub on species richness, negative effect of blooming (Table). There was a significant interaction between microsite and blooming, significantly more abundant pre-blooming, but no significant different after blooming. All arthropods together, no microsite effect and negative blooming. Melyridae alone had a significant microsite\*blooming interaction:

There is a significant correlation of insect abundance between shrub/open microsites (p = 4.41e-07, 0.4576805).

Indicator species analysis. Species accumulation curve. CA

Predatory and parasitoid wasps were more abundant in shrub microsites. Velvet ants were only associated with open sites.

No significant correlation between visitation and abundance, or diversity from pan traps.

No difference in bee abundance in pan trap with blooming or microsite. Barely caught any syrphids. There was a significant decrease in micro beeflys with flowering.

Plant-plant facilitation

Percent cover of ground vegetation was significantly greater in shrub microsites before and after blooming. Prior to blooming, no significant different in annual floral density or plant species richness. Significant decrease in richness and annual floral density with blooming.

Co-blooming foundation plants

Prior to blooming, there was no difference in the number of blooming shrubs in a radius around the shrub and open microsites. There is a significant increase in the number of surrounding shrubs with larrea blooming, and after larrea blooms there is a significant difference between shrub and open sites. Logical given the open sites were chosen to be away from shrubs.

Visitation to larrea

The number of flowers and the height of the shrub (Pearson’s, 0.3185, p = 0.03511), number of flowers and width (Pearson’s, 0.462, p = 0.001595) and width and height (Pearson’s, 0.6915, p = 2.02e-07), all tested using cor.test function in r.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Pre-blooming | Blooming | Total |
| All insect uses | 138 | 400 | 538 |
| Uses touching plant | 57 | 232 |  |
| Uses not touching plant | 81 | 168 |  |
| Understory uses | 20 | 15 |  |
| Flower uses | NA | 197 |  |
|  |  |  |  |

Visitors and insect uses of L. tridentata was significantly different after blooming.

The most frequent floral visitors to L.tridentata were bees (115): Apis mellifera (54 visits), Centris rhodapus (35), Hesperapis larrea (30), Eucera sp. (11) and other solitary bees (39) including Hoplitis and Megachile. Visitation by all visitors was positively associated with flower number, height and width. Visitation to larrea much greater. 17.13 floral visits to the plants per hour.

Climate amelioration

Data logger data analysis goes here

Relative effects

RII

**Discussion**

Larrea tridentata interacts with multiple trophic levels of its surrounding communities.

* Larrea influences both plants and insects that it associates with
* Differential effects of the different communities
* No effect on pollinator abundances – thus likely behavioural?
* Just because it concentrates insects doesn’t mean that benefits plants
* Facilitates vegetation growth but competes for pollinators
* Is there a temporal structure to the data?
* Dilution effect – not only was larrea flowering – surround shrubs and cactus were as well
* So yes, there might be a temporal effect but really that effect is of the dominant, foundational plants all flowering, and potentially life cycle shifting of certain pollinators
* What happened to the syrphids?
* Unavailability of rainfall likely prevents annuals from adjusting their phenology. The warmth and moisture required would put a hard limit on when they can flower.
* Lack of correlation between shrub abundances and visitation suggests that differences in visitation driven by something else ie foraging preferences vs concentrating local abundances. They were tested on different days however. But not far apart.
* Lack of visitation to Malacothrix not due to lack of bees – Larrea was visited. If the bees that visited were oligolectic but main visitor to Larrea was the honeybee. A generalist.

In the Mojave, shrubs (Ambrosia dumosa, windpollinated) positively influenced the seed set of annuals growing under the canopy (Holzapfel, 1999).

Table: Total arthropod abundance for each treatment

|  |  |  |  |
| --- | --- | --- | --- |
|  | Shrub | Open | Total |
| Pre-blooming | 935 | 973 | 1908 |
| Blooming | 692 | 777 | 1469 |
| Total | 1627 | 1750 | 3377 |

Table 2: Mean ± SD, arthropod abundance per shrub for each treatment, 3 pan traps. Including flower beetles.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Shrub | Open | Total |
| Pre-blooming | 17.31481 ± 7.947526 | 18.01852 ± 10.074235 | 17.66667 ± 9.037823 |
| Blooming | 11.53333 ± 6.217708 | 12.95000 ± 7.601126 | 12.24167 ± 6.951205 |
| Total | 14.27193 ± 7.630035 | 15.35088 ± 9.177678 |  |

Table 3: Total arthropod abundance without Melyrid beetles

|  |  |  |  |
| --- | --- | --- | --- |
|  | Shrub | Open | Total |
| Pre-blooming | 783 | 510 | 1293 |
| Blooming | 435 | 359 | 794 |
| Total | 1218 | 869 | 2087 |

Mean plant visits per hour. This is just the mean number of potential foraging bouts. ± the standard deviation.

|  |  |  |
| --- | --- | --- |
|  | Open | Shrub |
| Pre-blooming | 4.2955249 ± 4.621614 | 2.9976793 ± 3.134733 |
| Blooming | 1.2526164 ± 1.376179 | 0.9458532 ± 1.271302 |

Mean number of flowers visited per hour. ± standard deviation.

|  |  |  |
| --- | --- | --- |
|  | Open | Shrub |
| Pre-blooming | 5.758404 ± 7.547992 | 3.776575 ± 4.742340 |
| Blooming | 1.722185 ± 2.218946 | 1.268643 ± 2.047149 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Percent cover | | Annual Richness | | Annual bloom density | |
|  | **χ**2 value | |  | |  | |
|  |  | p – value | **χ**2 value | p – value | **χ**2 value | p – value |
| Microsite | **165.399** | **<0.0001** | 0.7071 | 0.40 | 0.6009 | 0.438 |
| Blooming | **34.180** | **<0.0001** | 2.7010 | 0.10 | **13.3646** | **0.0003** |
| Microsite \* blooming | **22.806** | **<0.0001** | NA | NA | NA | NA |

Table 2: GLMM results for pan trap abundances. Non-significant interactions were removed models.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Insect abundance (Melyridae: Dastyine excluded) | | Insect abundance (Melyridae: Dastyine included) | | Insect species richness | |
|  |  | |  | |  | |
|  | **χ**2 value | p – value | **χ**2 value | p – value | **χ**2 value | p – value |
| Microsite | 13.4645 | **0.0008** | 1.7201 | 0.1897 |  |  |
| Blooming | 11.3074 | **0.0002** | 33.6643 | **<0.0001** |  |  |
| Microsite \* Blooming | 3.8427 | **0.04996** | NA | NA |  |  |

Supplemental Data

* All camera stuff with all visitors included, along with model outputs