

Braun Spring Progress Report

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Drivers and consequences of ant community assembly in deserts

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Chapter	Title	Timeline
1	The relative importance of habitat filtering and biotic interactions on desert ant	Summer 2022 – Complete data analysis, write chapter and submit
2	An examination of the consequences of partitioning between ant species on cactus fitness	<p>April – May 2022: Flowering period field work: Conducted surveys, baiting experiments and set up exclusion experiment</p> <p>Aug – 2022: Collect cactus fruit and measure plant fitness</p> <p>Sept 2022 – December 2022: Extract video data and measure seeds</p>
3	Effect of <i>Bromus</i> invasion on granivorous ant community assembly	<p>Pilot experiment August 2022</p> <p>April 2023 – Measure annual community</p> <p>Summer 2023 – Sample ant community, conduct ant-seed</p>

General Introduction

Understanding the mechanisms that maintain species coexistence and determine patterns of community assembly are fundamental topics of community ecology. Ants are a model system to study assembly processes because ant communities are often strongly structured by environmental conditions (Retana and Cerdá 2000, Gibb et al. 2015a) and competition (Camarota et al. 2016), though the latter is the subject of continuing debate (Andersen 2008, Tschinkel and King 2017). Ants are one of the most dominant insect groups in terrestrial ecosystems and in deserts, many species are keystone taxa through their strong influence on most ecological processes (Whitford 2000). Ants also engage in diverse interactions with many species of plants including pollination, plant defense, seed dispersal and herbivory (Hölldobler & Wilson, 1990; Rico-Gray & Oliveira, 2007). Ant-plant interactions are often facultative and diffuse i.e. are context-dependent and comprised of multiple interactions between multiple species (Rico-Gray & Oliveira, 2007). An inevitable outcome of these diffuse interactions is that functionally similar ants are using the same plant resources. Niche theory predicts that organisms partition resource use to avoid competition (Schoener 1974, Carothers and Jaksic 1984). As in many other species, ant coexistence is mediated through niche differences and performance trade-offs that reduce the fitness differences between species (Chesson, 2000). Therefore, the outcome of resource partitioning may be important drivers of community dynamics that scale beyond the ant community.

The overarching hypothesis examined in this thesis is that the drivers of ant distributions and species coexistence i.e. spatial and resource partitioning among ant species scales to the plant community. An additional goal is to describe how interactions with the plant communities can influence ant community assembly. My first chapter **seeks to understand** how ant communities are assembled, and how environmental stress and vegetation influence the functional composition of ant communities. **Species coexistence in harsh environments is still not well understood because coexistence has focused on the impacts of lethal stress (Kim & Ohr, 2020).** Combining taxonomic with trait-based approaches can provide insight into the mechanisms driving environmental and biotic filtering, as well as the spatial scale at which these processes

operate for ant communities (Fichaux et al. 2019). The second chapter addresses how temporal, spatial and temperature-mediated partitioning of ant foraging behaviors alters nectar-mediated interactions between ants, plants, herbivores and pollinators. The goal of the third chapter is to understand how invasive plants impact ant community assembly that in turn impact annual plant seed bank composition. This work will contribute to an understanding of how ant communities are assembled in deserts and explore the consequences of interactions among ants on several aspects of plant community structure.

Chapter 1: The relative importance of habitat filtering and biotic interactions on desert ant community assembly

The extent to which community assembly mechanisms are deterministic processes i.e. niche based is a central topic in ecology. There are two major hypothesized processes that predict contrasting but non-exclusive patterns in the trait and spatial distributions of coexisting species. Environmental filtering structures communities by only allowing only organisms capable of surviving local conditions to persist within the local species pool (Vellend 2010). This mechanism can lead to a convergence in ecological attributes or traits among species in that area. In deserts, traits that allow organisms to cope with environmental stressors such as water stress and temperature extremes are common. In contrast, limiting similarity predicts that competitive exclusion results among ecologically similar species (Abrams 1983). This mechanism can lead to a divergence in traits among coexisting species. These processes work together but at differing scales, at large scales habitat filtering is expected to be stronger than biotic interactions, whereas at the fine scales that interactions actually occur at, biotic interactions prevail.

Functional traits reflect aspects of morphology, life history or physiology that enable an organism to be successful in an environment (Violle et al., 2007). Plant ecologists have relatively recently incorporated the role of facilitation by foundation plants into trait-based community assembly theory (Schöb et al. 2012). In deserts, shrubs reduce environmental stress by reducing daytime heat and retaining warmth overnight, creating locally stable micro-climates (McIntire and Fajardo 2014). Shrub facilitation often increases the cover and biomass of annual plants (Holzapfel et al. 2006, Pugnaire et al. 2011), as well as arthropod species richness and abundance

beneath the canopy (Braun et al., 2021; Braun & Lortie, 2020). Surface complexity can act as a filter on ant traits, for example longer-legged ant species are more successful on rugose surfaces than short-legged species (Kaspari and Weiser 1999). Therefore, at fine scales, foundation shrubs and ground-covering vegetation may alter taxonomic composition, trait distributions and coexistence patterns of the ant community.

There is also the capacity for the relative importance of environmental and biotic filtering mechanisms to change along environmental gradients. The stress gradient hypothesis proposes that competitive interactions decrease in importance as environmental stress increases (Bertness and Callaway 1994). There is extensive empirical support for the stress gradient hypothesis in plant communities (Lortie and Callaway 2006, He et al. 2013), however, in animal communities, tests are relatively rare. Due to the ecological importance and dominance of ants in arid ecosystems, understanding how their communities may change under increasing stress and along climatic gradients is important research to predicting the outcome of global change on these ecosystems.

Questions: How do abiotic and biotic interactions shape the taxonomic and functional composition, as well as co-occurrence, of ant communities? Does the relative importance of environmental filtering increase with stress?

Hypothesis: Desert ant communities are assembled through niche-based processes.

Predictions: 1) There will be evidence for environmental filtering on ant communities through the following responses to environmental gradient

- a) alpha and beta diversity
- b) trait convergence i.e. decreases in functional diversity and functional divergence
- c) community trait composition
- d) specific trait by environment interactions

2) The trait distributions of co-occurring ants will be more dispersed than expected (biotic filtering).

3) Effect size measure of the trait dispersion of co-occurring ants will decrease with stress (stress gradient).

Methods: Ant communities were sampled using pitfall traps at nine sites in the San Joaquin Valley, California, USA that span a distance of ~200 km from north to south. The shrub species *Ephedra californica* (Ephedraceae) and *Atriplex* sp. are the dominant perennial species at six of the sites and the remaining three sites are relatively open with few shrubs. White plastic drink cups (12.4 cm tall, 9 cm diameter) were placed with the top of the cup flush with the ground. In order to prevent vertebrate bycatch, 0.5-inch hardware cloth was placed horizontally within the trap and a piece of aluminum flashing was elevated three cm above the trap to shelter the trap. The traps were filled to a depth of three cm with 100% propylene glycol. Propylene glycol is a biodegradable, non-toxic preservative that does not evaporate and preserves DNA (Nakamura et al., 2020). At shrub sites, traps were placed at 12 pairs of shrub/open microsites and pairs were located at least 10 m apart. Shrub microsites were located beneath the canopy of a foundation shrub at the center of a 0.5 m quadrat placed just inside the dripline of the shrub. Open microsites were located randomly at least 2 m away from shrub microsites. At sites without shrubs in collections areas (i.e. within 500 -1000 m of collections), pitfalls traps were deployed every 10 m in open areas along two transects located at least 10 m apart. At each site, 24 pitfall traps were deployed continuously for 72 hours per sampling event. The traps were deployed in different locations within the study site each sampling instance to avoid repeated measures. Throughout the season, 648 traps were deployed totaling 46 656 trap-hours (24 traps per site * 3 sampling events * 72 hours). During each sampling instance, microsite-level vegetation characteristics were estimated. At each pitfall trap location we measured the percent cover of ground-covering vegetation, rocky cover, woody cover and bare ground. Vegetation height was measured at the center of the quadrat and the dominant vegetation type was recorded. At shrub microsites, we measured the longest dimension of the shrub canopy axis, its perpendicular width, and the height of the focal shrub to the tip of the highest green tissue (Lortie et al., 2018). Site-level vegetation characteristics were measured each month by placing 0.5 m quadrats every 4 m in ten 25 m transects distributed around the site and repeating the microsite level measurements. Each sampling location was georeferenced using a handheld GPS unit.

Ants were first identified to genus using Fisher and Cover (2007), and to species using AntWiki keys (www.antwiki.org). Representative individuals of each species were also barcoded and compared to BINs in BOLD systems. I measured the following morphological trait data from the ant specimens: Weber's length (Brown 1953), which is the length of the longest diagonal on the thorax and a measure of overall worker body size. Femur length relates to foraging ability in complex environment and thermoregulation (Feener Jr et al. 1988), mandible length and head size with relate to diet, scape length relates to chemosensory abilities and eye size relates to forage behaviours (Weiser and Kaspari 2006). The latter six traits were divided by Weber's body length to remove the impact of body size.

I measured each of the seven traits in up to six individuals per species, per site (265 individual ants) to capture the intraspecific variation between populations. The majority of functional ecology papers use traits measured at the species-level (de Bello et al., 2021), however traits captured at the population-level can better reflect environmental filtering (Jung et al., 2010; Violle et al., 2012) and may represent local adaptation (Kawecki & Ebert, 2004). I dissected each ant and affixed them to microscope slides using Elmer's glue. I placed each ant mount slide on top of a stage micrometer slide and took focus-bracketed photographs using a Canon 60D DSLR camera with a 60 mm macro lens and Canon EF 25 II extension tube. Helicon Focus software was used to combine the focus stacks into single images. I imported each composite image into ImageJ software, used the micrometer divisions within the image to calibrate the measurement scale within the software and measured each trait using the software.

I extracted local estimates of water stress during the sampling period from NASA's ECOsystem Spaceborne Thermal Radiometer Experiment on Space Station (EcoStress) (Meerdink et al. 2019) data for each site and study period. This 70 m resolution satellite data provides the evaporative stress indicator (ESI), a measure of plant water-stress based on temperature and evapotranspiration. I extracted climate data for each site from WorldClim, and calculated deMartonne's aridity index ($\text{aridity} = P/(T+10)$ where P = annual precipitation and T = mean annual precipitation. (Fick & Hijmans, 2017).

I converted the abundance-based data to incidence-based because ants forage socially and this leads to clustering of individuals from the same colony (Longino & Coddington, 2002). I calculated functional richness and divergence indices within the population-level trait means using X package (citation). I used GLMM to model alpha, beta and functional diversity in response to the environmental variables using site as a random effect (glmmTMB, citation).

I calculated the community weighted mean (CWM) value for each trait at each pitfall trap. CWM using incidence-based data is the mean value of the trait among the species present. To relate environmental variables and CWM, I carried out a db-RDA with the CWM trait values as the response and environmental variables as predictors using the capscale function of the vegan package in R. I conditioned the model on Site, and also conducted the permutation tests within strata of sites to account for within site correlation structures when assessing significance.

Four corner analysis is a formal test for trait by environmental interactions. It relates species traits, species abundances and environmental conditions to determine the relation of species traits to the environment (Legendre et al. 1997). I used a GLM based approach to calculating the fourth corner (Brown et al. 2014) but this method cannot incorporate intraspecific trait values. *I calculated the fourth corner correlation using code modified from Peres-Neto 2017.*

Results and Discussion

A total of 15519 individual ants from 11 species were collected and identified. All species are native to California. The most abundant species are *Solenopsis xyloni*, the native Southern fire ant, and *Pheidole hyatti*, the big-headed ant. These two species are both considered ecologically dominant in deserts. I have completed the trait measurements and I am currently working through the analyses. I used GLMM to model the drivers of ant species richness. Species richness driven by vegetation height at the site-level (more height, less richness), and aridity (higher aridity, lower richness). Loss of species with aridity.

Beta diversity turnover component driven by x and x.

Function diversity driven by x and x. Functional richness driven by x and x. The increase/decrease in functional diversity with x suggests a convergence/divergence in trait values with increasing stress. This is consistent/inconsistent with predictions that

The db-RDA on community weighted mean trait values showed that ESI significantly drives mean trait values (stat). Trait composition also varies between months (p).

Fourth corner without intraspecific trait values included showed significant trait * env interaction, ESI shows strong relationships with femur and mandibles. These traits are associated with...

These results show that water stress acts as an environmental filter on ant communities. Femur length looks like it helps – femur length is related to thermoregulation.

Next steps

The next step is to explore the potential importance of biotic interactions. I will create co-occurrence matrices for each pair of species. I will use a null model approach and compare the observed community to a random community, and calculate the standardized effect size (SES) that describes the degree of aggregation or segregation from random. If trait divergence promotes species coexistence, we should see that the variation among co-occurring species is larger than random i.e. trait overdispersion (Weiher and Keddy 1995).

In order to estimate the range of climatic conditions each ant species experiences, I will use presence data extracted from the Global Ant Biodiversity Informatics database (Guenard et al. 2017), a curated database of ant species occurrence compiled from the literature and biodiversity databases. I will use these datasets to create environmental niche models (ENM) using MaxEnt (Elith et al. 2011). Outputs from ENM models includes an importance score for each environmental variable used in the model, and a suitability estimate for a given pixel. Niche theory predicts that species abundance reflects their performance under environmental conditions, however, community assembly studies virtually never incorporate the full range of conditions a species experiences into the analyses.

I would also like to better contrast the impacts of intraspecific variation in traits to interspecific variation in traits. My goal is to finalize these analyses and write this as a paper over the summer.

Chapter 2: An examination of the consequences of resource partitioning between ant species on cactus fitness

Indirect defensive mutualisms with ants are important to many species of plants. These interactions are often mediated by extra-floral nectaries (EFN) which are nectar secreting glands in plants not directly involved in pollination. Plants provide nectar to ants, who in return defend the plant, modifying the interaction between herbivores and plants which can indirectly influence plant fitness. EFN have been reported in 3941 species of plants within 108 families and have arisen 457 times independently (Weber and Keeler 2013) indicating that this trait is adaptive in a wide range of situations. EFN-ant interactions are a model system for evolutionary study globally (Heil and McKey 2003) but not well studied in deserts (Aranda-Rickert et al. 2014). Ant species differ in their quality as defenders and in some cases confer no net benefit to the plant due to their negative influences on other mutualisms (Melati and Leal 2018). In the Sonoran desert, native fire ants *Solenopsis xyloni* are the most aggressive herbivore removers for

Ferocactus but also reduce seed set through interference with pollinator visitation (Ness 2006). This trade-off has been documented across multiple systems (LeVan et al. 2014, Ohm and Miller 2014) suggesting it is relatively common. Conversely, ant interference has been reported to improve pollination effectiveness by only reducing visitation by less effective pollinators (González et al. 2013). Therefore, there is the capacity for the interactions between ant species to influence both the outcomes of the ant-defense system mutualism and pollination mutualisms within the same system.

There are typically multiple ant species within a site that engage in defensive mutualisms with a single species of plant. In some communities, a single ant species will interact with an individual plant or even a single ant colony (Lanan and Bronstein 2013), however, in others, multiple ant species will defend the same individual plant (Miller 2007). Variation in ant attendance may be related to competition and resource partitioning among ant species. Ants partition resources through foraging at different times, at different temperatures and by foraging within specific territories. Therefore, there may be differences in plant defense that depend on the distribution and overlap of the ant species within a site. The size of the cactus may relate to the identity of the defenders if larger plants that produce more resources also attract the most effective or abundant defender. The goal of this chapter is to understand the drivers of dominance and competition between ant species, and how the outcome of this partitioning impacts cacti in terms of plant fitness, herbivory and pollination. This research will improve our understanding of how diffuse mutualisms are maintained within harsh environments.

Questions:

Are the benefits to plants driven more strongly by ant species-identity or environmental context?
How is ant activity on plants and the ground partitioned? Are plant resources or temperature a stronger driver of ant activity on plants?

Hypothesis: Ant species differ in their effectiveness as plant defenders. Therefore, resource partitioning between ant species influences plant fitness by differentially modifying plant interactions with arthropod herbivores and pollinators.

Predictions: 1) Plants with higher levels of ant activity will have higher fitness

2) Plants with ants will have fewer herbivores

3) Ant activity on plants and dominance on bait cards will depend on temperature and the time of day

4) Plants that produce more EFN resources be defended by the dominant ant

5) Plants defended by the dominant species will have higher fitness

Methods: Silver cholla (*Cylindropuntia echinocarpa*) is an EFN-bearing cactus that is widespread in California deserts (Pemberton 1988). 2020-21 was an exceptional drought year in California (Williams et al., 2022) and 2022 was the third most severe drought in the last 40 years at the preserve. This severely limited my sample size and impacted my work. I was able to locate 50 plants with buds within a 200 m by 50 m area of Granite Cove (34.780 N, -115.65 W, 1293 masl) on the property of the University of California Sweeney Granites Desert Research Station.

To test for the influence of ant exclusion on silver cholla fitness, I applied Tanglefoot resin to the base of 20 cholla individuals. Tanglefoot resin is frequently used by ecologists to exclude ants from plants because it forms a physical barrier to their movement e.g. (Del-Claro et al., 2019; O'Dowd & Catchpole, 1983).

I conducted 18 repeated surveys of the 50 focal silver cholla over three weeks, distributed evenly across three different time blocks: morning (8:00 to 10:00 AM), midday (12:00 to 2:00 PM) and evening (5:30 to 7:30 PM). Each survey, I recorded the number and identity of ants and all other arthropods on the plants. I collected representative insect specimens to identify to species in lab.

EFN are located on flowers, buds and vegetative growth. I attempted to exclude ants and other insects from EFN by filling the space between spines with cotton and bagging with a muslin bag so that I could quantify EFN sugar production, but was not successful. As proxies for the total EFN resources available from each plant, I counted the number of buds at the beginning of the experiment and at several times over the three weeks. I counted the number of open flowers each sampling day. I also counted the vegetative growth by counting the number of new segments on

each cactus individual. I also counted the number of shrubs, cacti, silver cholla and shrubs/cacti with green growth within a 1.5 m radius of each focal plant. I measured the longest dimension of the cacti canopy axis, its perpendicular width, and the height.

EFN-bearing cacti may employ strategies to minimize EFN nectar production. I had planned a set of inducibility experiments which involved measuring EFN flow in response to mechanical damage and *Chelinidea vittiger* herbivory. Plants typically already had herbivores on them, and my attempts to keep ants away from the EFN to measure nectar were unsuccessful. I was not able to try again due to the drought and ongoing vertebrate damage to many silver cholla in the area.

To determine the influence of ant interference to pollinator visitation, I recorded pollinator visitation to flowers using Polaroid Cube+ video cameras at 8 plants (half exclusion treatments) per day for four days (total of 32 1.5 hour videos). I recorded the number of ants present when I placed the cameras and the number of open flowers.

In order to understand how sugar-feeding ants are distributed and to measure ant species richness independently of those found on cactus, I conducted a baiting experiment at two study sites: Granite Cove and Sunset Cove. At Granite Cove, the area included the silver cholla study area. Sunset Cove is located on the other side of a rocky mountain ridge from Granite. At Granite Cove I completed 18 baiting trials and at Sunset Cove I completed 17 baiting trials. Each trial, I placed 20 baits cards approximately 15 m apart in one or two transects located haphazardly using different starting locations with the site each baiting trial. I placed the baits in open areas as well as under the canopy of perennial plants in order to sample a range of microhabitats. Baits consisted of nickel-sized amount of honey placed onto the centre of paper index cards. The use of honey baits to attract sugar seeking ants is widespread in ant community ecology e.g. (Dáttilo & MacGregor-Fors, 2021; Feener Jr. & Schupp, 1998). Baits were distributed during three different time blocks: morning, midday and evening (7:45 AM to 10:00 AM, 11:45 to 2:00 PM and 5:00 PM to 7:30 PM). I checked the baits 15 minutes after placement, and half hourly after that for 4-6 checks per instance. I counted any ants at the baits, recorded their identity and any interspecific interactions. I collected specimens of the ants to verify their identity. All bait

locations were georeferenced using a handheld GPS. I also recorded the microsite (open, beneath large perennial), and the percent cover of organic material, dried rooted plant material, perennial plants, large rock and bare ground in a 0.5 by 0.5 m area around the bait card. I monitored temperature continuously across the site using 16 HOBO onset data loggers measuring temperature every half hour.



I attempted a nectar addition experiment to understand how ant activity on cactus is mediated by sugar resource availability. At Sunset Cove, I chose 80 silver cholla; half had no signs of new vegetative or reproductive growth (and thus no EFN resources) and half had vegetative and/or reproductive growth. I added two 5 ml tubes of 20 Brix honey water to half of the cholla, thus the gradient was 20 + nectar/growth, 20 + nectar/0 growth, 20 growth/0 nectar, 20 0 growth/0 nectar. The honey water tubes were attractive to ants and I observed 5 species feeding. Unfortunately, the tubes were destroyed twice by rodents and all of plants with growth suffered from intense vertebrate herbivory and those without new growth began to turn brown and dry out as the site continued to dry. There were not enough healthy plants at the site to use different ones and so I was unable to carry out this experiment. In the future I will protect the nectar in rodent proof containers, and use arboreal pitfall traps to measure the ant community response to increased resource availability.

Results and discussion

The ant exclusion treatments were not completely effective as ants were still on the plants.

The most abundant herbivores on silver herbivores were *Chellidea vittager* and *Narnia femorata* both large hemipterans that specialize on cactus.

Ant attendance at the honey baits was sparse. Of the ~750 individual baits placed, only 51 attracted ants, and ants were present during 135 of the 3140 checks. 11 morphospecies were detected using baits and the majority were *Forelius* sp. Species turnover at baits occurred seven times and only one interspecific interaction was recorded. The results of the baiting experiment suggest that ant populations are strongly depressed by the two years of drought.

Forelius sp. was the ant species most frequently observed on cactus and was generally observed midday. *Forelius*, also known as the high noon ant, likes disturbed areas and has a high heat tolerance (citations).

Next steps: I plan to return in August to count and collect fruit as an estimate of cactus fitness. I will count the number of seeds per fruit. I will create spatial maps of the baiting data and overlay the cactus. I will analyse this year's data to understand patterns of plant defense, however, I will need a field season with some rainfall so that I can use a larger study site and so that ant abundance will be higher.

Chapter 3: Effect of *Bromus* invasion on granivorous ant community assembly

Seeds, like nectar, are a high-quality source of energy in desert ecosystems. Invasive grasses including red brome (*Bromus rubens*) and cheatgrass (*B. tectorum*) are a major threat to biodiversity in Western USA and reduce the abundance and diversity of native plants (citations).

Bromus invasion also reduces ant diversity (Ostoja et al., 2009). The replacement of native plants with exotic plants alters the availability of native seeds to granivores, however, the impacts of this replacement on granivorous ant community assembly are not yet known. Granivorous ants in arid environments collect large numbers of seeds and can impact the composition of seed banks (cite). Size-matching between seeds and harvester ant body size is frequently reported (Kaspari, 1996; Pfeiffer et al., 2006; Retana et al., 1994). Therefore the composition of the seed bank can depend on the body size and dietary preferences of granivores present at the site (Pirk et al., 2009). Increases in *Bromus* cover may alter both the abundance and the size distribution of seeds available to ants leading to the loss of ant species or changes to foraging habits. These effects may be more pronounced in more stressful environments where the background levels of productivity are already lower. For example, *Messor* ant colonies are more selective with the seeds they forage in higher resource, less arid sites (Segev et al. 2020). *Bromus* could also indirectly impact ant communities through altering competitive interactions between granivores. *Bromus*'s invasion in the Great Basin Desert is attributed to escape from granivorous generalist rodents (Lucero et al. 2019). A classic example of species coexistence through resource partitioning is among granivorous rodents and ants in deserts (Brown and Davidson 1977, Davidson 1977b, a). Excluding rodents dramatically changes the plant community, leading to a strong increase in large-seeded plants (Brown and Heske 1990), and excluding either group as a whole increases the population of the other (Brown and Davidson 1977). Feeding trials have shown that generalist rodents prefer native seeds to *Bromus* (Lucero 2018) and this consumer pressure may be an additional pathway of change to the native communities that impacts granivorous ants. Therefore, multiple direct and indirect consequences of *Bromus* invasion are likely to impact native granivores. This could be an example of how invasive species alter biodiversity by interrupting coexistence mechanisms.

Questions: Does invasion by *Bromus* alter ant-seed interactions?

Hypothesis: Brome invasion and environmental stress jointly reduce seed availability which modifies resource use among granivorous ants.

Predictions: 1) Seed size – worker body-size matching will increase with decreasing *Bromus* cover because workers can be more choosy with higher resource availability

2) Seed removal rates will increase with *Bromus* invasion

3) There will be evidence for environmental filtering from *Bromus* invasion on ant body sizes

Experimental Approach:

I will use six sites that form a gradient of *Bromus* invasion and aridity. To characterize large-scale vegetation patterns at the site, I will calculate the normalized difference vegetation index (NDVI) from high resolution National Agriculture Imagery Program (NAIP) imagery. Shrubs strongly facilitate *Bromus* (Lucero et al., 2021), therefore I will also determine shrub cover at the study sites using supervised classification on the NAIP imagery. In the spring I will measure the abundance and richness of annual plants present at the sites. I will determine their seed sizes using plant trait databases such as TRY and Kew (Royal Botanic Gardens Kew, 2022; TRY, 2022). I will use pitfall traps deployed beneath shrubs and in opens areas to determine patterns in the abundance and diversity of ant species at the sites. I will measure Weber's body length on a subset of individual ants of each species at each site.

I will measure granivore seed size preferences and seed removal rates by offering seed baits in Petri dishes protected from vertebrates using cages. The seed baits will be one of three sizes of native seed or *Bromus*. I will place these both under shrubs and in open areas at least 5 m apart along transects (4 seed size treatments * 2 microsites * 15 replicates). These seed trays will be paired with unprotected trays to measure rodent removal rates and will be monitored by camera traps. I will estimate removal rates by weighing the remaining seeds after 48 hours. I will also conduct observations of the ant seed trays to determine which ant species are directly interacting with the seeds. I will measure the seed bank to determine the range of sizes of seeds that have escaped granivore predation. I will measure small-scale brome cover using quadrats along transects, at pitfall trapping sites and at seed tray locations.

Structural equation modelling is a multivariate technique that allows researchers to parse direct and indirect causal effects (Fan et al., 2016). I will use SEM to understand the direct and indirect

responses of seed size, ant size and removal rates to abiotic factors (aridity and ESI/water stress) and biotic factors including *Bromus* invasion and microsite.

Timeline: I plan to at least pilot this experiment in August 2022. I will visit the sites in spring of 2023 to measure the annual communities, and return in July 2023 to conduct the seed size experiments.

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