Ecology of *Eriogonum tiehmii*

A report on arthropod diversity, abundance, and the importance of pollination for seed set; plant-soil relationships; greenhouse propagation and a seedling transplant experiment; and wild population demography

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Abstract

Eriogonum tiehmii Reveal is a rare soil specialist endemic to Nevada. It inhabits outcrops of clay-rich soil developed from a variety of interbedded sedimentary rocks. Increasing threats from novel disturbances including land use and climate change have generated interest in better understanding *E. tiehmii*'s role within its ecological community, its pollination and habitat requirements, and whether the species could be a candidate for active management approaches such as ex-situ propagation and translocation or reintroduction.

Methods: We used a combination of pitfall traps, flower observation, and pollinator exclusion to assess the abundance and diversity of arthropod communities in *E. tiehmii* habitat, the most common visitors to *E. tiehmii* flowers, and the importance of pollination for seed set. We collected and analyzed soil samples from 21 occupied and unoccupied sites to assess the physical and chemical composition of *E. tiehmii* habitat soils and conducted a greenhouse soil preference experiment to test how seeds and seedlings respond to soil variation. We also tested the viability of greenhouse propagation and seedling transplants in three unoccupied locations within the broader range of *E. tiehmii*, using methods that were promising for *E. tiehmii*'s best-studied relative, *Eriogonum crosbyae*. Lastly, we also re-located monitoring transects in *E. tiehmii* habitat established by EM Strategies in spring 2019 and monitored tagged plants in extant populations for survival, size, and reproductive output, and recorded total numbers of individuals present in designated count transects.

Results: We found that the arthropod community within and around *E. tiehmii* sites is abundant and diverse; that each of the four sites sampled contained numerous unique species, and that there was high turnover in arthropod community composition over time. *E. tiehmii* sub-population 6 had the greatest biomass of arthropods collected out of any site on a single sampling date. Diversity was highest at non- *E. tiehmii* sites in May, but declined between May to June, while diversity at *E. tiehmii* sites increased (sub-population 6) or remained relatively stable (sub-population 1) between monitoring dates. The total number of pollinator visits observed was higher at *E. tiehmii* sub-population 1 than at *E. tiehmii* sub-population 6, and open-pollination significantly increased seed production, with beetles, wasps, and flies the most likely important pollinators.

Soil chemical and physical properties differed between occupied and unoccupied sites. Occupied sites were, on average, lower in sulfur, zinc, potassium, and magnesium and, on average higher in boron, pH, and silt, among other differences, though there was high variation and some overlap in these characteristics among occupied and unoccupied sites. The soil preference experiment revealed that, on average, seedlings grown in soils from occupied sites had higher total biomass and higher root allocation than seedlings grown in soils from unoccupied sites. There was a significant positive association between emergence and survival in occupied soils, but not in unoccupied soils. Seedlings responded to different components of soil variation at different life stages. While some unoccupied soils were favorable at some life history stages, none of the unoccupied soils we tested were well-suited to growth across all life stages.

We found that it is possible to propagate *E. tiehmii* seedlings in the greenhouse, and that growing them in field soils from occupied habitat promoted high root allocation that was likely beneficial for transplant survival. Early transplant survival was promising, and comparable to that observed in our experiments with *E. crosbyae*; however, a major herbivory event in July reduced seedling survivorship to near zero in all sites. Despite this, there were some early differences between sites, with the highest transplant success in a sparsely vegetated, moderately-sloped, north-facing site with relatively higher-clay soil.

Finally, looking at dynamics in extant populations by June of 2020, we observed relatively larger changes in *E. tiehmii* plant abundance, both positive and negative, in sub-populations 2, 4, and 6B, and relatively smaller

changes in sub-populations 1 and 6A. There were relatively greater proportions of larger plants in sub-populations 1 and 6A, and greater proportions of smaller plants in sub-populations 2 and 3, and the number of inflorescences increased with plant size. A major herbivory event in September of 2020 had large impacts on extant populations, and transects will need to be measured in 2021 to fully understand the effects of this disturbance.

Conclusions: *E. tiehmii* substantially contributes to and benefits from the high abundance and diversity of arthropods and pollinators found in our sampling areas. Seedlings demonstrated sensitivity to individual soil properties and growth trends that suggest a "specialist" model of soil specialization rather than a "refuge" model, indicating that they are not simply highly stress-tolerant, but that they are specifically adapted to their preferred soil types. This was borne out by the transplant experiment, where seedlings planted into a site whose properties most closely approximated their natural habitat had the highest early survival, though an unexpected herbivory event reduced survival to near zero in the field. Our work identified a set of soil conditions that are most favorable for the growth of *E. tiehmii*. While some unoccupied sites we tested were favorable for some life history stages, we did not identify unoccupied sites that could support both establishment and growth of *E. tiehmii* seedlings. Future work could determine whether other unoccupied sites can be found with conditions that can meet *E. tiehmii's* growth requirements at all life history stages, and considering the effects of biotic interactions such as plant competition, pollination, and herbivores, will be important next steps for determining the suitability of potential habitat for this soil specialist. Finally, continued demographic monitoring would be needed to acquire the size-specific growth and survival data to create structured population models.

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Introduction

Eriogonum tiehmii Reveal is a perennial herb endemic to Esmerelda County, Nevada, USA. Its range is limited to 21 acres in the Silver Peak Range where it, like many other capitate, mat-forming species of *Eriogonum*, displays a high degree of soil specialization, exclusively occupying outcrops of clay-rich soils developed over a variety of interbedded sedimentary rocks (Robinson et al., 1976; Morefield, 1995). *E. tiehmii* is listed as a Critically Imperiled (S1-Rank) species by the Nevada Division of Natural Heritage, is classified as "sensitive" by the Bureau of Land Management, and is being considered for listing under the Endangered Species Act by the US Fish and Wildlife Service, after a 90-day review found potential listing to be warranted on July 22, 2020. The primary anthropogenic threats to the species are mining and OHV activity.

E. tiehmii occurs between 1820-1890m elevation, in areas of relatively lower vegetative height and cover surrounded by saltbrush communities, and is comprised of one population divided into 8 sub-populations (Program, 2001). Surveys from 2019 estimated a total population of approximately 44,000 plants (https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.151598/Eriogonum_tiehmii). Average monthly temperatures in *E. tiehmii* habitat (30- year normals 1981-2010, PRISM Climate Group, https://prism.oregonstate.edu/) are between -0.4 to 20.7°C, with an average of 21.5 cm of precipitation per year, which falls mostly as rain and snow in the winter and spring, with occasional summer thunderstorms. Plants flower from May to June (Tiehm, 1994).

First collected in 1983 by Arnold (Jerry) Tiehm, *E. tiehmii* was formally described and named by Dr. James Reveal in 1985 (Reveal, 1985). Little is known about its biology, ecology and habitat preferences beyond surveys of associated plant species, general site characteristics, and inferences based on its taxonomic group and extremely limited range (Tiehm, 1994). The most similar species that has previously been studied in depth is *Eriogonum crosbyae* Reveal, another *Eriogonum* subgenus *Eucycla* section *Capitata* species. *E. crosbyae* is also a soil specialist, though much more widespread, occurring primarily on soils developed over hydrothermally altered rock outcrops in northwestern Nevada, southeastern Oregon, and western Idaho. Research on *E. crosbyae's* plant-soil interactions, propagation, and potential for successful seedling transplant created a foundation of methods that may be useful in the study of these processes in *E. tiehmii* (McClinton, 2019), and that body of work provides a basis of comparison for results between these two species. Average temperatures in *E. crosbyae* habitat are similar to those of *E. tiehmii,* and fall between 1.2- 16.3°C, with an average of 25.4 cm of precipitation per year (PRISM Climate Group, https://prism.oregonstate.edu/). This precipitation also typically falls as rain and snow in the winter and spring, and during summer thunderstorms, and *E. crosbyae* plants flower from May to July.

The purpose of this work is to better understand the biology, ecology, demography, and habitat preferences of *E. tiehmii*, which will aid managers and stakeholders in making decisions about the management of this rare plant. We investigated four specific areas where additional information would be helpful for decision makers: 1) identifying arthropod communities in *E. tiehmii* habiats and understanding the importance of pollination for seed set, 2) testing greenhouse propagation and seedling transplant methods in the field, 3) describing plant/soil relationships, and 4) studying wild plant demography, including survival since 2019 in monitoring transects, abundance, plant size, and reproductive output in *E. tiehmii* sub-populations.

Activity 1: Arthropod and pollinator diversity, abundance, and the importance of pollination for seed set

1.1 Introduction

Eriogonum is the second-most speciose genus in Nevada (Holmgren et al., 1966; Cronquist et al., 1977; Herbaria, 2020). While modes of reproduction in *Eriogonum* vary between primarily self-pollinated and primarily outcrossing species, many species in this genus are known to support extensive pollinator communities (James et al., 2014). Some, such as *E. crosbyae*, form associations with pollinators that are faithful to that single species where it occurs, despite the presence of other flowering plants in the vicinity (Kaye, 1990), while other *Eriogonum* species are pollinated by generalist species. Pollination is a crucial ecological interaction, as effective pollinators may play a role in increasing the seed production of rare species, and *E. tiehmii* may support a community of pollinators and other insects in this unique ecosystem.

We and others have observed that *E. tiehmii* receives numerous insect visitors to open flowers in May and June. However, the composition of the arthropod community, importance of pollination for seed set, and degree of similarity between arthropod communities within and outside of *E. tiehmii* habitat has not previously been quantified or described. We specifically asked: 1) how many species, and how many individual arthropods, are observed in *E. tiehmii* habitat and visiting *E. tiehmii* flowers? 2) are the same arthropods abundant in *E. tiehmii* and non-*E. tiehmii* habitat? and 3) what effect does pollination have on seed production? For questions 1 and 2, we also asked how arthropod abundance and composition changed over the course of the flowering season, sampling these communities in May and June.

1.2 Methods

We used two methods to quantify arthropod diversity and abundance in *E. tiehmii* (ERTI) (ERTI 1, ERTI 6A) and adjacent non-*E. tiehmii* (NT) sites (NT.1: 37.81685, -117.85571; NT.6A: 37.80353, -117.86016). See Appendix 1 for a map of all sampling sites. First, we sampled insect and arachnid abundance (for simplicity, referred to as collectively as arthropods) in these two habitat types using pitfall traps, which sample flying arthropods as well as ground dwelling ones. Secondly, we used timed flower observations to quantify visitors to flowers in both habitat types (hereafter, arthropods observed visiting flowers are referred to as pollinators). We selected non-*E. tiehmii* sites by locating populations of predominantly yellow-colored flowers that could be found within 60-100m of *E. tiehmii* plants. Forb and shrub species present in these non-*E. tiehmii* areas included *Stanleya pinnata, Mentzelia albicaulis, Eriogonum ovalifolium, Chaenactis douglasii* and *Krascheninnikovia lanata*.

Arthropod diversity and abundance

We sampled arthropod diversity and abundance at two *E. tiehmii* sub-populations and two adjacent non- *E. tiehmii* sites at the beginning (May 25-26, 2020) and peak (June 8-9, 2020) of the flowering season using pitfall traps (Southwood and Henderson, 2009; McCravy, 2018). Ten 12.7 cm diameter x 5.08 cm height plastic bowls were painted yellow to approximate the color of *E. tiehmii* flowers and placed at each site. Bowls were filled halfway with a dilute mixture of water and odorless eco-friendly dish soap (approximately 350 mL water and 15mL soap), and buried with 1" of the rim exposed to prevent them from being tipped over. Bowls were placed in similar densities and formations in both *E. tiehmii* and non- *E. tiehmii* sites. In *E. tiehmii* sites, bowls were randomly located along previously- established 100-meter transects, with 2-3 bowls per transect. In non- *E. tiehmii* sites, bowls were placed in zig-zag patterns across two approximately 4,000m^2 areas adjacent to *E. tiehmii* sub-populations, with bowls at least 10m apart.

Bowls were left in place for 24 hours, and arthropod specimens were then collected and placed in vials filled with a 70% alcohol and water solution. Individuals were separated into morphospecies (unique taxa) and each morphospecies was identified to order, family, and when possible, genus and species.

Arthropod diversity and abundance was tabulated and analyzed using R (R Core Team, 2020). We used principle coordinates analysis to visualize how community composition varied between sites and over time, using the vegdist() function in the vegan package (Makowski, 2019) to calculate a dissimilarity matrix using Bray distances, and the pco() function in the labdsv package (Roberts, 2019) to perform principle coordinates analysis. We also used the diversity() function in the vegan package to calculate Shannon diversity indices for each site on each monitoring date, and exponentiated the Shannon indices to calculate "true diversity", hereafter simply referred to as diversity (Hsieh et al., 2016). Diversity is an index that takes species abundances into account, in addition to absolute numbers of species.

Flower visitation

Flower visits (total visits to all inflorescences on an individual plant) were observed at the same two *E. tiehmii* sites and two non- *E. tiehmii* sites near the beginning (May 12, 2020) and peak (June 8-9, 2020) of the flowering season. Fifteen plants per site were observed at one time point on 5/12/2020 (between 9:00 am-11:15 am) and at two time points on 6/9/2020 (between 10:00 am-12:00 pm and 2:00 pm-4:00 pm). In *E. tiehmii* sites, the *E. tiehmii* plant with at least 5 open inflorescences nearest to each sampling bowl location was chosen for observation, and we noted the number of inflorescences on each plant. In non-tiehmii sites, the clump of flowers nearest to each bowl location that was of comparable size to an average *E. tiehmii* plant (approx. 15 cm in diameter) was chosen for observation. An additional 5 bowls were chosen randomly for additional observation, selecting the next-closest plant or flower-clump to the bowl marker locations. For all sites, each plant or flower clump was observed for 1 minute, and the number and category of visitor was recorded. These categories included easily identified types of potential pollinators: bees, wasps, flies, beetles, lepidoptera, and "other".

We first summarized flower visits by site and date in tabular form. Next, we asked how flower visits differed between monitoring dates, between flower species (*E. tiehmii*/non- *E. tiehmii*), and among pollinator types, using generalized linear models with zero-inflated negative binomial regression. Negative binomial regression accounts for the unique distribution of count data (i.e. there were many visitors of some types and few to no visitors of other types), while the zero-inflation model structure allowed us to model the processes that produced positive counts and zero counts separately to reduce over-dispersion in model residuals. Poisson distributions and zero-inflated Poisson distributions were also tested; however, likelihood ratio tests and comparisons of residual dispersion indicated that the models built with zero-inflated, negative binomial regression were most appropriate for our data. Potential predictive variables included pollinator type, site, flower species (*E. tiehmii* or other), and observation date, and our response variable was the count of visits made per minute. Akaike information criterion values (indicators of model fit) were used for model selection.

Lastly, we used negative binomial regression to ask whether, within *E. tiehmii* sites, the number of inflorescences was correlated with the total number of pollinator visits per minute. Predictive variables included number of inflorescences and site, and our response variable was the count of flower visits in one minute.

Pollination and seed production

To test whether plants are capable of self-pollination, white organza mesh bags (www.uline.com) were placed over two unopened inflorescences on each of 15 plants in two sub-populations, ERTI1 and ERTI6A, near the

beginning of the flowering season, May 22, 2020. Unopened flowers were staked with a toothpick for support and mesh bags were tied around both the stakes and flowers to preclude insect visits. We marked two additional unopened flowers at the same phenological stage on the same plant, using ties but no bags, and allowed these two flowers to be freely visited by pollinators. On June 8th, 2020, two additional bags were placed over these previously marked flowers. All bags were harvested on June 23, 2020 to allow seeds time to develop. A small number of bags (7) had come un-tied, were blown off, or otherwise separated from their plant of origin, and these bags were excluded from further analysis. Seeds were carefully cleaned, counted and, where present, stored in paper coin envelopes.

To examine the effect of pollination on seed production, we again used a generalized linear model with negative binomial regression. Potential explanatory variables included: pollinator exclusion/open pollination, the individual plant from which samples were collected (plant number), and the site, either ERTI1 or ERTI6A. Site and plant number were found to be unpredictive variables, and were thus excluded from our final model.

Finally, we used data on the spatial density of mature plants in each site and the average number of flower stalks per plant to calculate seed production per square meter for each of these sites.

1.3 Results

Arthropod diversity and abundance

Our pitfall traps collected a total of 3,617 invertebrate specimens from 14 orders, 90 families, and 177 species (Table 1). 1,898 specimens from 12 orders, 70 families, and 129 species were found in *E. tiehmii* (ERTI) sites, and we found 79 specimens from 17 families and 47 species that occurred only in *E. tiehmii* sites (Table 1). In non- *E. tiehmii* (NT) sites, we collected 1,719 specimens from 12 orders,73 families, and 130 species; this included 40 specimens from 15 families and 48 species that occurred only in these sites.

Of all specimens collected, the majority of individuals were collected in ERTI6A, followed by NT.1, NT.6A, and ERTI1 (Table 1, Fig. 1A). The abundance of arthropods was higher in the May sampling dates, at all sites, with 64% of all arthropods (2330 total) collected in May (Fig. 1B). This higher abundance in May was observed at all sites: the proportion of specimens collected in May out of the total collected at each site was 75%, 72%, 51%, and 62% in ERTI1, ERTI6A, NT.1, NT.6A respectively (Fig. 1B).

In addition to changes in abundance between sampling dates, arthropod community composition also varied greatly between May and June (Fig. 2). There was differentiation in arthropod communities among each of our sites at each monitoring date, but there was no greater similarity between *E. tiehmii* sites than non-*E. tiehmii* sites (Fig. 2; no obvious groupings of the two *E. tiehmii* or non-*Tiehmii* sites at either time period). Rather, each site was differentiated from the others: only 11% (May) and 13.5 % (June) of species were shared among all four sites at the same time period (Fig. 3). Further, as much as 16% of species were unique to individual sites within a single time period (ranging from 6.25-16%), indicating that each site hosted a significant amount of unique local arthropod species that were not found in other sites (Fig. 3). This was true in both May and June.

Just as the abundance of arthropods differed among sites, so too did the total number of species (richness) and diversity (which considers abundance as well as the number of species). The greatest number of species collected in May were at NT.1, and in June, the number of species was highest at ERTI6A (Fig. 4A). Though the total number of species at ERTI6A remained the same between May and June, diversity more than doubled at ERTI6A over time, increasing from 8.65 to 20.22 between the first and second monitoring dates (Fig. 4B). This is because in the first time period, the community at ERTI6A was dominated by a few species (59.8% of specimens in the sample were a species of “common false chinch bug” in the Lygaeidae, while the next most common

specimens- 4.49%- were sweat bees in the Halictidae), whereas the relative abundance of species was more evenly distributed among all species present in June (7 different species made up the first 54.4% of all specimens sampled this round, including flies, bees, wasps, and beetles in the Tachinidae, Halictidae, Sphecidae, Milichiidae, and Melyridae). During the same period, the opposite pattern was observed at the other sites, and there were greater decreases in diversity at the non-*E. tiehmii* sites: between May and June, diversity at NT.6A dropped from 23.59 to 15.01, at NT.1 from 25.85 to 7.88, while at ERTI 1 diversity only dropped from 13.82 to 12.53.

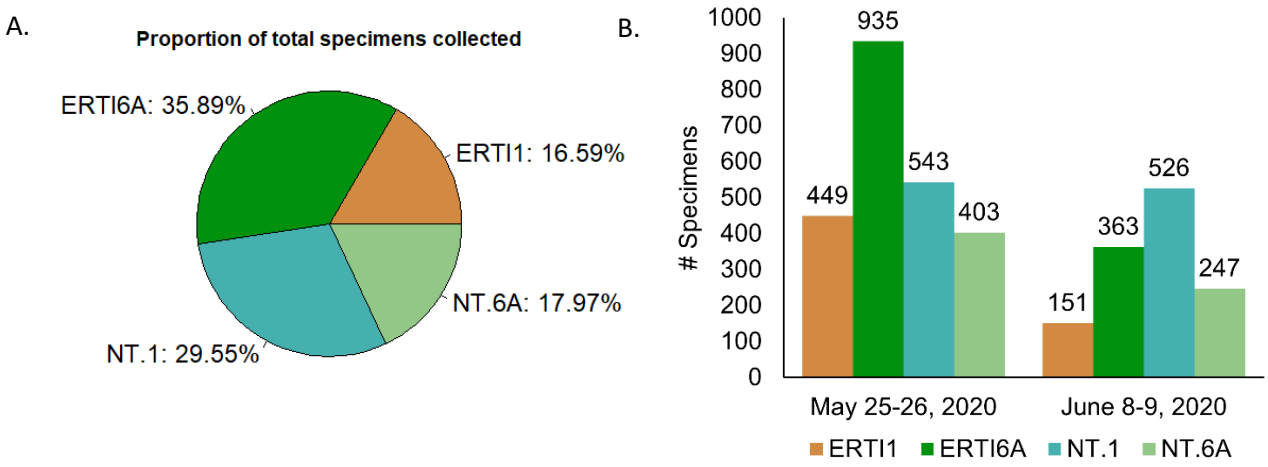

Figure 1: (A) The overall proportion of arthropod specimens collected in pitfall traps at two *E. tiehmii* sites (ERTI1, ERTI6A) and two adjacent Non-*E. tiehmii* sites (NT.1, NT.6A). The total number of specimens collected was 3,617. (B) The number of arthropod specimens collected in pitfall traps in each site, on each sampling date.

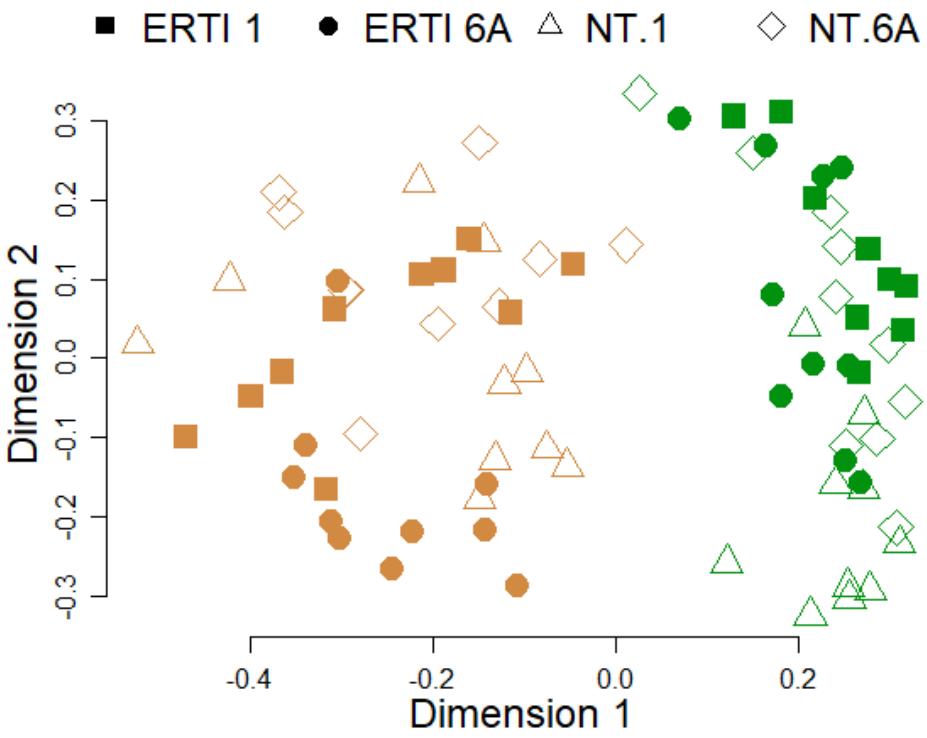

Figure 2: Plot of the first two dimensions of a principal coordinates analysis of species composition and abundance from pitfall traps placed in two *E. tiehmii* sites (ERTI1, ERTI6A) and two adjacent Non-*E. tiehmii* sites (NT.1, NT6A), at two time periods. Solid makers represent *E. tiehmii* sites, open markers are non-*E. tiehmii* sites. Shapes represent the four individual sites. Colors represent sampling events, with brown indicating samples collected at the beginning of the flowering season (May 25-26, 2020) and green indicating samples collected at the peak of the flowering season (June 8-9, 2020).

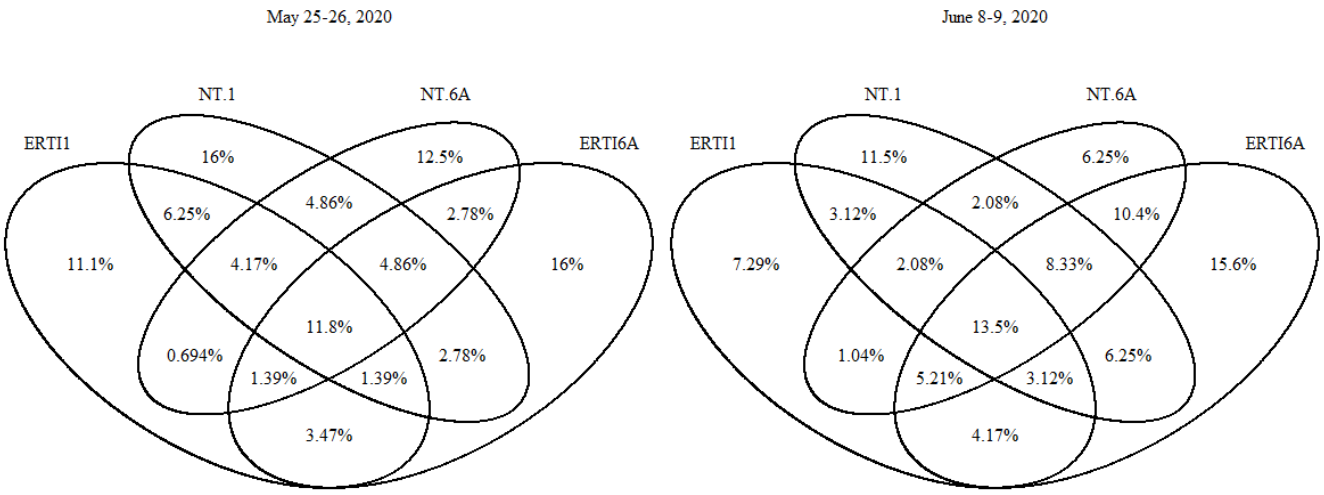

Figure 3: Venn diagrams of the proportions of pollinator species collected from our pitfall traps that were unique to and shared between each of two *E. tiehmii* sites (ERTI1, ERTI6A) and two adjacent Non-*E. tiehmii* sites (NT.1, NT.6A), in May and June. Values in overlapping regions show the percent of species shared among sites. For example, in May, 11.1% of all species were found only at ERTI1, 6.25% of species were found at both ERTI1 and NT.1; 16% of species were found only at ERTI6A, 4.17% of species were found at ERTI1, NT.1, and NT.6a, etc. Note: the area of different sections are not proportional to the values inside; ellipses are oriented vertically for readability.

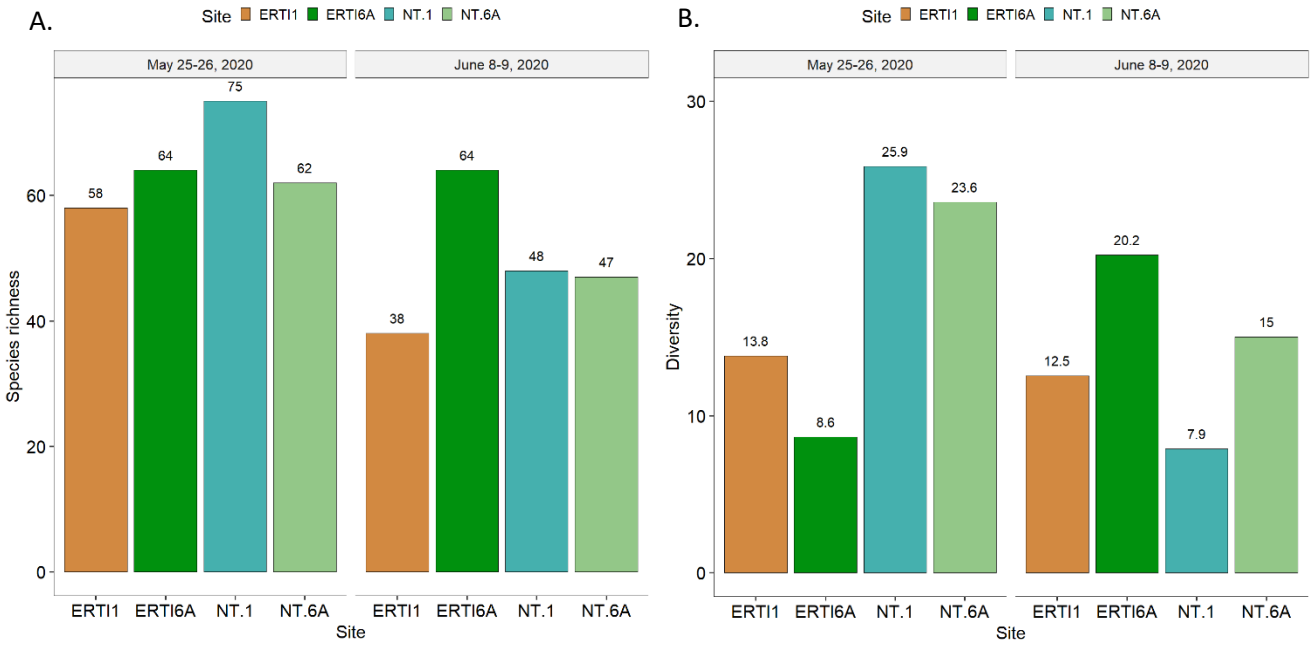

Figure 4: (A) The number of species (species richness) present in pitfall traps at each of two *E. tiehmii* sites (ERTI1, ERTI6A) and two adjacent Non-*E. tiehmii* sites (NT.1, NT.6A) in May and June. Species composition changed dramatically over time, with largely different community assemblages present on different sampling dates; however, overall numbers of species present remained constant at ERTI6A. (B) Diversity in each site, in

May and June. Diversity in ERTI6A more than doubled between May and June, and diversity at ERTI1 only fell by 1.3 points, while diversity at both non-*E. tiehmii* sites dropped substantially between sampling dates.

Table 1: Table of arthropod specimen abundance by family and order. Values are the total number of specimens at each site, for a given order or family, followed by the percent of the total specimens made up by that taxonomic group, within each site.

Site: **Total specimens collected:**	**ERTI1** ***N* = 600**	**ERTI 6A** ***N* = 1298**	**NT.1** ***N* = 1069**	**NT.6A** ***N* = 650**	**Total** ***N* = 3617**
Order					
Araneae	4 (0.7%)	2 (0.2%)	3 (0.3%)	2 (0.3%)	11 (0.3%)
Coleoptera	30 (5.0%)	51 (3.9%)	77 (7.2%)	35 (5.4%)	193 (5.3%)
Diptera	106 (17.7%)	160 (12.3%)	117 (10.9%)	116 (17.9%)	499 (13.8%)
Entomobryomorpha	1 (0.2%)	25 (1.9%)	0 (0%)	0 (0%)	26 (0.7%)
Hemiptera	223 (37.2%)	543 (41.8%)	323 (30.2%)	129 (19.9%)	1218 (33.7%)
Hymenoptera	109 (18.2%)	204 (15.7%)	203 (19.0%)	171 (26.3%)	687 (19.0%)
Lepidoptera	5 (0.8%)	6 (0.5%)	8 (0.8%)	5 (0.8%)	24 (0.7%)
Mantodea	0 (0%)	0 (0%)	1 (0.1%)	1 (0.2%)	2 (0.1%)
Microcorypia	0 (0%)	14 (1.1%)	0 (0%)	0 (0%)	14 (0.4%)
Neuroptera	0 (0%)	0 (0%)	0 (0%)	1 (0.2%)	1 (0%)
Orthoptera	0 (0%)	2 (0.2%)	6 (0.6%)	0 (0%)	8 (0.2%)
Symphypleona	12 (2.0%)	33 (2.5%)	26 (2.4%)	26 (4.0%)	97 (2.7%)
Thysanoptera	110 (18.3%)	247 (19.0%)	304 (28.4%)	145 (22.3%)	806 (22.3%)
Trombidiformes	0 (0%)	11 (0.9%)	1 (0.1%)	19 (2.9%)	31 (0.9%)
Family					
Acrididae	0 (1.0%)	2 (0.2%)	6 (0.6%)	0 (0%)	8 (0.2%)
Agromyzidae	0 (0%)	1 (0.1%)	0 (0%)	1 (0.2%)	2 (0.1%)
Andrenidae	0 (0%)	4 (0.3%)	0 (0%)	0 (0%)	4 (0.1%)
Anthomyiidae	1 (0.2%)	0 (0%)	1 (0.1%)	0 (0%)	2 (0.1%)
Anthophora	0 (0%)	0 (0%)	7 (0.7%)	6 (0.9%)	13 (0.4%)
Aphididae	1 (0.2%)	0 (0%)	0 (0%)	0 (0%)	1 (0%)
Aphilinidae	1 (0.2%)	12 (0.9%)	17 (1.6%)	9 (1.4%)	39 (1.1%)
Apidae	27 (4.5%)	32 (2.5%)	51 (4.8%)	27 (4.2%)	137 (3.8%)
Berytidae	0 (0%)	0 (0%)	1 (0.1%)	0 (0%)	1 (0%)
Bethylidae	3 (0.5%)	5 (0.4%)	2 (0.2%)	3 (0.5%)	13 (0.4%)
Bombyliidae	2 (0.3%)	5 (0.4%)	3 (0.3%)	3 (0.5%)	13 (0.4%)
Braconidae	2 (0.3%)	1 (0.1%)	3 (0.3%)	1 (0.2%)	7 (0.2%)
Buprestidae	2 (0.3%)	10 (0.8%)	6 (0.6%)	3 (0.5%)	21 (0.6%)
Cecidomyiidae	2 (0.3%)	5 (0.4%)	2 (0.2%)	20 (3.1%)	29 (0.8%)
Ceraphronidae	1 (0.2%)	1 (0.1%)	1 (0.1%)	2 (0.3%)	5 (0.1%)
Cercopidae	0 (0%)	0 (0%)	0 (0%)	3 (0.5%)	3 (0.1%)
Chalcididae	0 (0%)	2 (0.2%)	0 (0%)	1 (0.2%)	3 (0.1%)
Chamaemyiidae	1 (0.2%)	0 (0%)	1 (0.1%)	0 (0%)	2 (0.1%)
Chiromelidae	2 (0.3%)	0 (0%)	0 (0%)	0 (0%)	2 (0.1%)

Chironomidae	1 (0.2%)	0 (0%)	0 (0%)	0 (0%)	1 (0%)
Chloropidae	0 (0%)	0 (0%)	1 (0.1%)	5 (0.8%)	6 (0.2%)
Chrysididae	0 (0%)	0 (0%)	1 (0.1%)	0 (0%)	1 (0%)
Chrysomelidae	1 (0.2%)	0 (0%)	2 (0.2%)	0 (0%)	3 (0.1%)
Cicadellidae	29 (4.8%)	46 (3.5%)	233 (21.8%)	38 (5.9%)	346 (9.6%)
Cixiidae	0 (0%)	0 (0%)	2 (0.2%)	0 (0%)	2 (0.1%)
Cleridae	0 (0%)	9 (0.7%)	14 (1.3%)	1 (0.2%)	24 (0.7%)
Coccinellidae	3 (0.5%)	0 (0%)	1 (0.1%)	0 (0%)	4 (0.1%)
Colletidae	0 (0%)	1 (0.1%)	2 (0.2%)	3 (0.5%)	6 (0.2%)
Coniopterygidae	0 (0%)	0 (0%)	0 (0%)	1 (0.2%)	1 (0%)
Conopidae	0 (0%)	5 (0.4%)	0 (0%)	3 (0.5%)	8 (0.2%)
Crabronidae	1 (0.2%)	1 (0.1%)	4 (0.4%)	2 (0.3%)	8 (0.2%)
Dictyopharidae	11 (1.8%)	5 (0.4%)	10 (0.9%)	2 (0.3%)	28 (0.8%)
Dryinidae	1 (0.2%)	0 (0%)	1 (0.1%)	0 (0%)	2 (0.1%)
Empididae	17 (2.8%)	7 (0.5%)	2 (0.2%)	1 (0.2%)	27 (0.8%)
Encyrtidae	1 (0.2%)	1 (0.1%)	3 (0.3%)	3 (0.5%)	8 (0.2%)
Eulophidae	3 (0.5%)	3 (0.2%)	1 (0.1%)	4 (0.6%)	11 (0.3%)
Eupelmidae	0 (0%)	2 (0.2%)	1 (0.1%)	0 (0%)	3 (0.1%)
Figitidae	4 (0.7%)	0 (0%)	0 (0%)	0 (0%)	4 (0.1%)
Formicidae	1 (0.2%)	17 (1.3%)	9 (0.8%)	10 (1.5%)	37 (1.0%)
Geocoridae	0 (0%)	0 (0%)	3 (0.3%)	0 (0%)	3 (0.1%)
Halictidae	41 (6.8%)	61 (4.7%)	59 (5.5%)	66 (10.2%)	227 (6.3%)
Heleomyzidae	9 (1.5%)	3 (0.2%)	8 (0.8%)	4 (0.6%)	24 (0.7%)
Hesperiidae	0 (0%)	0 (0%)	0 (0%)	2 (0.3%)	2 (0.1%)
Ichneumonidae	1 (0.2%)	3 (0.2%)	2 (0.2%)	0 (0%)	6 (0.2%)
Latridiidae	0 (0%)	1 (0.1%)	0 (0%)	1 (0.2%)	2 (0.1%)
Leucostoma	1 (0.2%)	0 (0%)	0 (0%)	0 (0%)	1 (0%)
Lygaeidae	178 (29.7%)	483 (37.2%)	59 (5.5%)	64 (9.9%)	784 (21.7%)
Mantidae	0 (0%)	0 (0%)	1 (0.1%)	1 (0.2%)	2 (0.1%)
Megachilidae	0 (0%)	1 (0.1%)	6 (0.6%)	3 (0.5%)	10 (0.3%)
Meinertellidae	0 (0%)	14 (1.1%)	0 (0%)	0 (0%)	14 (0.4%)
Melittidae	3 (0.5%)	3 (0.2%)	6 (0.6%)	3 (0.5%)	15 (0.4%)
Meloidae	4 (0.7%)	0 (0%)	3 (0.3%)	1 (0.2%)	8 (0.2%)
Melyridae	16 (2.7%)	20 (1.5%)	51 (4.8%)	29 (4.5%)	116 (3.2%)
Milichiidae	36 (6.0%)	18 (1.4%)	17 (1.6%)	9 (1.4%)	80 (2.2%)
Miridae	2 (0.3%)	5 (0.4%)	14 (1.3%)	10 (1.5%)	31 (0.9%)
Unknown mites	0 (0%)	11 (0.9%)	1 (0.1%)	19 (2.9%)	31 (0.9%)
Unknown moth	5 (0.8%)	5 (0.4%)	7 (0.7%)	2 (0.3%)	19 (0.5%)
Unknown moth 2	0 (0%)	1 (0.1%)	0 (0%)	0 (0%)	1 (0%)
Mutillidae	0 (0%)	0 (0%)	3 (0.3%)	0 (0%)	3 (0.1%)
Mymaridae	5 (0.8%)	2 (0.2%)	6 (0.6%)	1 (0.2%)	14 (0.4%)
Mythicomyiidae	5 (0.8%)	7 (0.5%)	40 (3.7%)	6 (0.9%)	58 (1.6%)
Nephus	1 (0.2%)	0 (0%)	0 (0%)	0 (0%)	1 (0%)
Nymphalidae	0 (0%)	0 (0%)	1 (0.1%)	1 (0.2%)	2 (0.1%)
Pentatomidae	0 (0%)	0 (0%)	0 (0%)	12 (1.9%)	12 (0.3%)

Phoridae	0 (0%)	4 (0.3%)	1 (0.1%)	0 (0%)	5 (0.1%)
Pipinculidae	0 (0%)	1 (0.1%)	0 (0%)	0 (0%)	1 (0%)
Platygastridae	8 (1.3%)	16 (1.2%)	4 (0.4%)	12 (1.9%)	40 (1.1%)
Pompilidae	0 (0%)	3 (0.2%)	2 (0.2%)	2 (0.3%)	7 (0.2%)
Psilidae	0 (0%)	0 (0%)	0 (0%)	1 (0.2%)	1 (0%)
Psyllidae	2 (0.3%)	4 (0.3%)	0 (0%)	0 (0%)	6 (0.2%)
Pteromalidae	1 (0.2%)	5 (0.4%)	2 (0.2%)	5 (0.8%)	13 (0.4%)
Richardiidae	0 (0%)	1 (0.1%)	0 (0%)	0 (0%)	1 (0%)
Scarabaeidae	0 (0%)	10 (0.8%)	0 (0%)	0 (0%)	10 (0.3%)
Sciaridae	6 (1.0%)	2 (0.2%)	5 (0.5%)	8 (1.2%)	21 (0.6%)
Scphecidae	0 (0%)	1 (0.1%)	1 (0.1%)	0 (0%)	2 (0.1%)
Simuliidae	0 (0%)	0 (0%)	0 (0%)	1 (0.2%)	1 (0%)
Sphecidae	4 (0.7%)	24 (1.9%)	8 (0.8%)	6 (0.9%)	42 (1.2%)
Unknown spider	4 (0.7%)	2 (0.2%)	3 (0.3%)	2 (0.3%)	11 (0.3%)
Staphylinidae	1 (0.2%)	1 (0.1%)	0 (0%)	0 (0%)	2 (0.1%)
Tachinidae	25 (4.2%)	101 (7.8%)	36 (3.4%)	52 (8.0%)	214 (5.9%)
Tephritidae	0 (0%)	0 (0%)	0 (0%)	2 (0.3%)	2 (0.1%)
Unknown thrips	110 (18.3%)	247 (19.0%)	304 (28.4%)	145 (22.3%)	806 (22.3%)
Tiphiidae	1 (0.2%)	1 (0.1%)	0 (0%)	0 (0%)	2 (0.1%)
Torymidae	0 (0%)	0 (0%)	1 (0.1%)	0 (0%)	1 (0%)
Unknown Entomobryomorpha	1 (0.2%)	25 (1.9%)	0 (0%)	0 (0%)	26 (0.7%)
Unknown Hemiptera	0 (0%)	0 (0%)	1 (0.1%)	0 (0%)	1 (0%)
Unknown Hymenoptera	0 (0%)	2 (0.2%)	0 (0%)	0 (0%)	2 (0.1%)
Unknown Symphypleona	12 (2.0%)	33 (2.5%)	26 (2.4%)	25 (3.9%)	96 (2.7%)
Unknown Symphypleona 2	0 (0%)	0 (0%)	0 (0%)	1 (0.2%)	1 (0%)
Vespidae	0 (0%)	0 (0%)	0 (0%)	2 (0.3%)	2 (0.1%)

[1]Numbers in parentheses are the percentage of specimens observed in that category out of all specimens collected at that site.

Pollinator visitation rates

A total of 467 visits were recorded to flowers in four sites (Table 2). Generally, beetles and "others" were the most numerous visitors recorded in both *E. tiehmii* and non-*E. tiehmii* sites, followed by wasps and flies (Fig. 5a, Fig. 6). Considering only flying insects, which are typically considered pollinators, and excluding one outlier observation of 48 beetles on a single *Stanleya pinnata* plant on 5/12, total visits to flowers in *E. tiehmii* sites were 14% higher than in non-*E. tiehmii* sites, and there were a greater number of visits by wasps at *E. tiehmii* sites and bees at non-*E. tiehmii* sites (Fig. 3b, 5b).

Visitation rates varied between *E. tiehmii* and non-*E. tiehmii* flowers, between sampling dates, and among pollinator groups. On 5/12/20, the number of visits per minute to *E. tiehmii* and non-*E. tiehmii* flowers did not differ ($p=0.56$); however, on 6/9/20, the rate of visits to non-*E. tiehmii* flowers was 146% higher than that of visits to *E. tiehmii* flowers ($p<0.01$), driven mainly by an increase in the presence of beetles and "others" in non

E. tiehmii sites (Fig. 6, Fig. 7). The "others" in non-*E. tiehmii* sites were primarily ants found on *Stanleya pinnata*. Within *E. tiehmii* sites, the dominant visitors on 5/12 were also "others" (primarily small white spiders) and wasps, followed closely by beetles, while the dominant visitors on 6/9 were "others" and beetles, followed by wasps. The frequency of visits by wasps was slightly lower in both *E. tiehmii* and non-*E. tiehmii* sites during the second observation period on 6/9/20 (Fig. 7).

Within *E. tiehmii* sites, the number of visits per minute increased by 5.8% with each additional inflorescence present, and the overall number of visits was 50% higher in ERTI1 than in ERTI6A (p=0.003, Pseudo-R^2= 0.14).

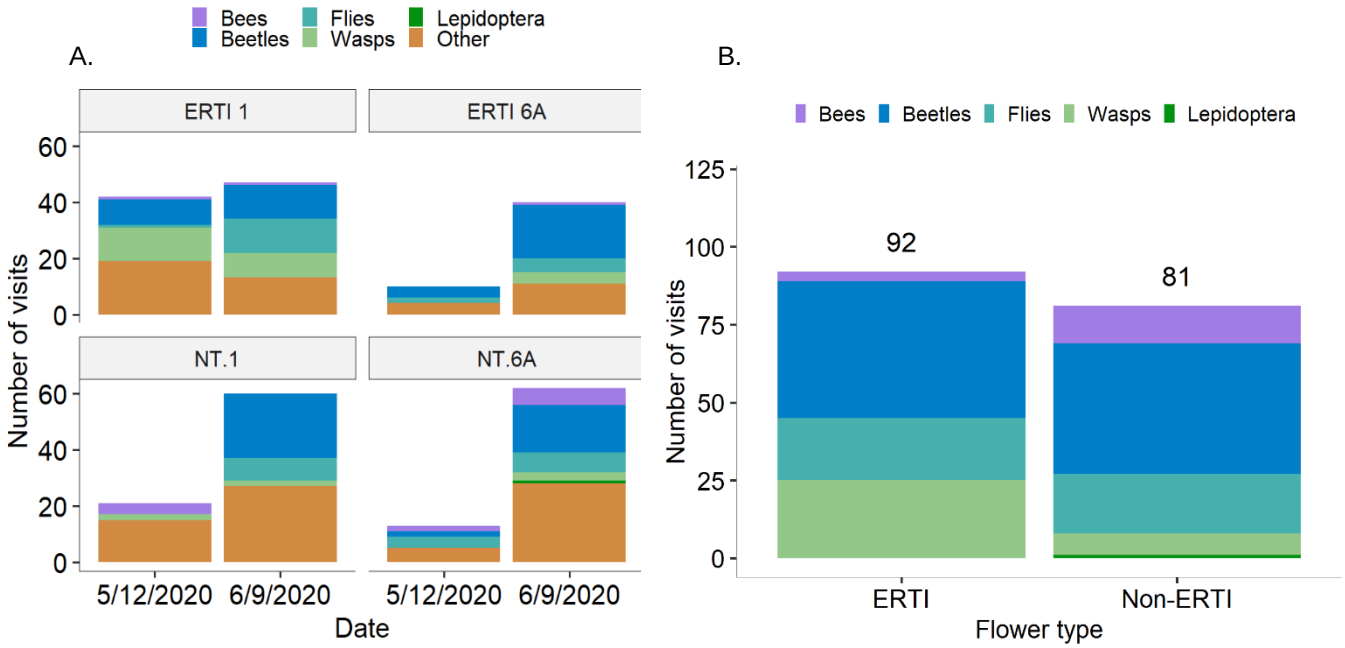

Figure 5: (A) Flower visits in *E. tiehmii* (ERTI) and adjacent non-*E. tiehmii* (NT) sites at the beginning and middle of the flowering season. (B) Total visits to *E. tiehmii* and non-*E. tiehmii* flowers as a group. These figures exclude one outlier observation of 48 beetles on one *Stanleya pinnata* plant at NT.6A on 6/9/2020.

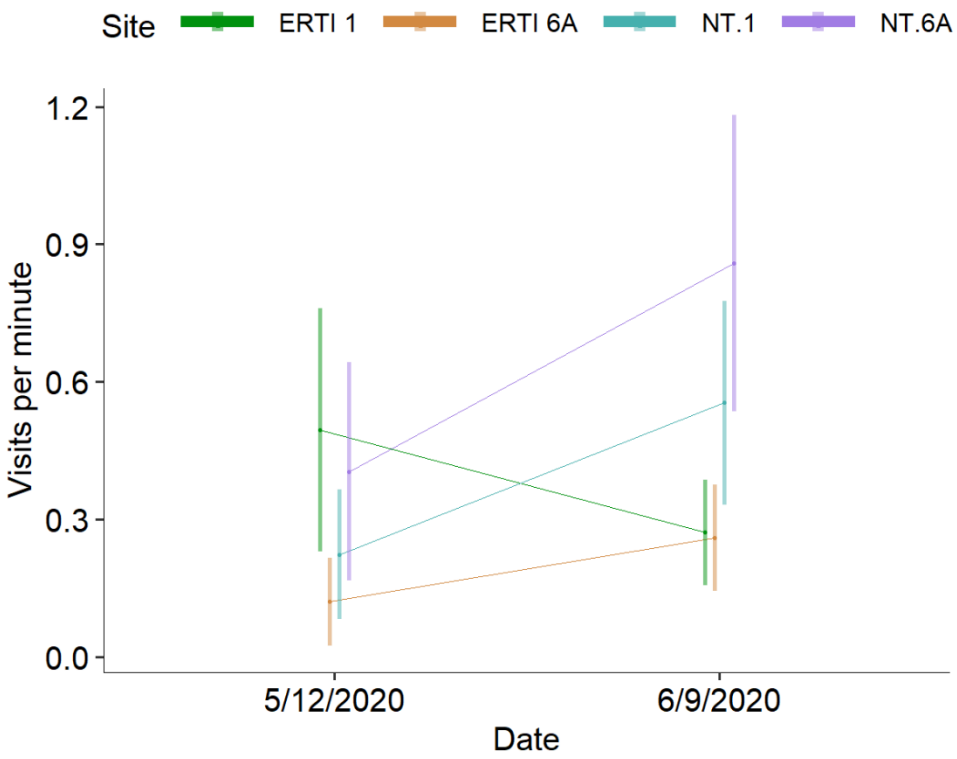

Figure 6: Interaction plot based on estimates from our generalized linear models of the average rate of flower visits by potential pollinators at adjacent *E. tiehmii* (ERTI) and Non- *E. tiehmii* (NT) sites on two monitoring dates.

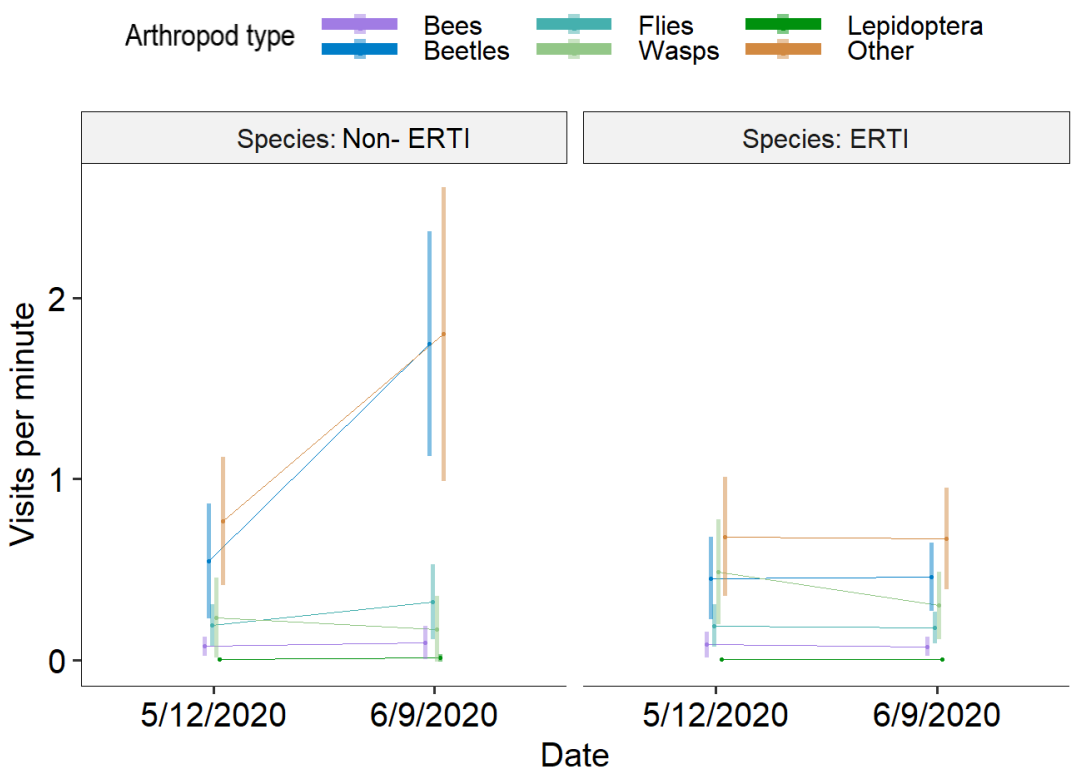

Figure 7: Interaction plot based on estimates from our generalized linear models of the rate of visits by potential pollinators across two monitoring dates, grouped by visits within *E. tiehmii* (ERTI) and non- *E. tiehmii* (other) sites. In non- *E. tiehmii* sites, visits on both dates were most frequently made by beetles and "other" species, and the rate of visits by both categories of visitors increased significantly between monitoring dates. In *E. tiehmii* sites, the dominant visitors were "other" species, wasps, and beetles; the visit frequency of "other" and beetles remained constant across monitoring dates, while the visit frequency by wasps declined slightly towards the end of the flowering season.

Table 2: Summary of visits to flowers in *E. tiehmii* (ERTI) and adjacent non- *E. tiehmii* (NT) sites.

	ERTI 1		ERTI 6A		NT.1		NT.6A		Overall	
Pollinator type[a]	**5/12/20 (*N* = 15)**	**6/9/20 (*N* = 30)**	**5/12/20 (*N* = 14)**	**6/9/20 (*N* = 30)**	**5/12/20 (*N* = 15)**	**6/9/20 (*N* = 30)**	**5/12/20 (*N* = 15)**	**6/9/20 (*N* = 30)**	**5/12/20 (*N* = 59)**	**6/9/20 (*N* = 120)**
Bees										
Sum	1	1	0	1	4	0	2	6	7	8
Mean (SD)	0.07 (0.3)	0.03 (0.2)	0 (0)	0.03 (0.2)	0.3 (0.5)	0 (0)	0.1 (0.5)	0.2 (0.8)	0.1 (0.4)	0.07 (0.4)
Min, Max	0, 1	0, 1	0, 0	0, 1	0, 1	0, 0	0, 2	0, 4	0, 2	0, 4
Wasps										
Sum	20	9	0	10	2	2	0	8	20	30
Mean (SD)	1 (2)	0.3 (0.6)	0 (0)	0.3 (1)	0.1 (0.4)	0.07 (0.4)	0 (0)	0.3 (1)	0.3 (1)	0.2 (0.9)
Min, Max	0, 6	0, 2	0, 0	0, 6	0, 1	0, 2	0, 0	0, 5	0, 6	0, 6
Flies										
Sum	1	10	2	5	0	8	4	7	7	30
Mean (SD)	0.07 (0.3)	0.4 (0.6)	0.1 (0.4)	0.2 (0.6)	0 (0)	0.3 (0.7)	0.3 (0.8)	0.2 (0.8)	0.1 (0.5)	0.3 (0.7)
Min, Max	0, 1	0, 2	0, 1	0, 3	0, 0	0, 3	0, 3	0, 4	0, 3	0, 4
Beetles										
Sum	9	10	4	20	0	40	2	100	20	200
Mean (SD)	0.6 (1)	0.4 (0.6)	0.3 (0.6)	0.6 (1)	0 (0)	1 (2)	0.1 (0.4)	3 (9)	0.3 (0.7)	1 (5)
Min, Max	0, 4	0, 2	0, 2	0, 4	0, 0	0, 7	0, 1	0, 100	0, 4	0, 100
Lepidoptera										
Sum	0	0	0	0	0	0	0	1	0	1
Mean (SD)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.03 (0.2)	0 (0)	0.008 (0.09)
Min, Max	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 1	0, 0	0, 1
Other										
Sum	20	20	4	10	20	100	30	40	100	100
Mean (SD)	1 (3)	0.6 (1)	0.3 (0.6)	0.4 (0.8)	1 (0.8)	2 (3)	2 (3)	1 (3)	1 (2)	0.9 (2)
Min, Max	0, 10	0, 5	0, 2	0, 2	0, 2	0, 10	0, 8	0, 20	0, 10	0, 20

[a] Values are the total, mean and standard deviation (SD), minimum (min) and maximum (max) number of flower visits made by a variety of arthropods to *E. tiehmii* (ERTI) and *non- E. tiehmii* (NT) flowers at adjacent sites. Flowering plants observed at non-ERTI sites included *Chaenactis douglasii, Stanleya pinnata, Mentzelia albicaulis, Eriogonum ovalifolium,* and *Krascheninnikovia lanata.* In *E. tiehmii* sites, visits were recorded at 14-15

E. tiehmii plants with at least five open inflorescences, and at non- *E. tiehmii* sites, visits were recorded to fifteen clusters of flowers of other species that were arrayed in displays approximately 15 cm in diameter. Observations were performed between 9:00 am-11:15 am on 5/12/2020, and between 10:00 am-12:00 pm and 2:00 pm-4:00 pm on 6/9/20. Categories of visitors recorded included bees, wasps, flies, beetles, lepidoptera, and "other." "Other" species recorded included spiders, ants, and thrips, which were numerous and covered flowerheads, especially of *Chaenactis douglasii*.

Pollination and seed production

Overall, we found 265 seeds produced by 59 inflorescences. Un-bagged, open pollinated inflorescences produced an average of 7.3 times as many seeds as bagged inflorescences ($p < 0.001$, Pseudo-$R^2 = 0.548$) when controlling for the effects of individual fecundity (Fig. 8). Mean seed production in un-bagged inflorescences was 9.5 seeds (SD= 9.4), and mean seed production in bagged inflorescences was 1.3 (SD= 2.3) (Table 3). There was no significant difference in seed production per inflorescence between sites ($p>0.1$).

A demographic study performed in mid- June 2020 estimated that the average density of flowers at ERTI6A is 50.57 flower stalks/m2, and 5.51 flower stalks/m2 at ERTI1 (see Activity 4). Therefore, average seed production in open-pollinated plants at ERTI6A is roughly 455 seeds/m2, and average seed production in pollinated plants at ERTI1 is 56 seeds/m2 (Table 3).

Table 3: Seed production among open-pollinated and bagged *E. tiehmii* flowers.

	ERTI1		ERTI6A		Overall	
Open-pollinated?[1]	**No (*N* = 13)**	**Yes (*N* = 9)**	**No (*N* = 23)**	**Yes (*N* = 14)**	**No (*N* = 36)**	**Yes (*N* = 23)**
Seed count						
Total	24	92	23	126	47	218
Mean (SD)	1.9 (2.7)	10.2 (12.2)	1.0 (2.0)	9.00 (7.58)	1.31 (2.27)	9.48 (9.39)

[1] Pollinators were excluded from bagged flowers and allowed to visit open-pollinated flowers. To accomplish this, white organza mesh bags were placed over two unopened inflorescences on each of 15 plants in two sub-populations, ERTI1 and ERTI6A, near the beginning of the flowering season, May 22, 2020. On June 8th, 2020, two additional bags were placed over previously opened, marked flowers that had had the opportunity to be visited by pollinators. All bags were harvested on June 23, 2020 to allow seeds time to develop.

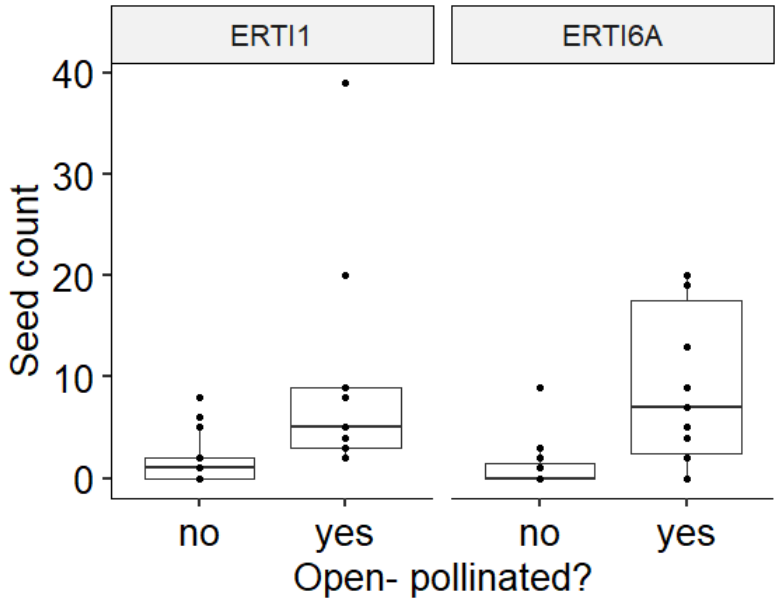

Figure 8: The number of seeds produced in open- pollinated and bagged *E. tiehmii* (ERTI) flowers at two sites. Pollinators were excluded from bagged flowers and allowed to visit open-pollinated flowers. To accomplish this, white organza mesh bags were placed over two unopened flowers on each of 15 plants in two sub-populations, ERTI1 and ERTI6A, near the beginning of the flowering season, May 22, 2020. On June 8th, 2020, two additional bags were placed over previously opened, marked flowers that had had the opportunity to be visited by pollinators. All bags were harvested on June 23, 2020 to allow seeds time to develop.

1.4 Discussion

Arthropod abundance and diversity within the surveyed communities was high and, notably, diversity was distributed across surveyed sites, with each of the four locations housing a substantial proportion (6.25-16%) of unique species not found at any other site. Only 11.8-13.5% of arthropod species were shared between all four sites at any time. The diversity in *E. tiehmii* sites was remarkably high for a site dominated by a single plant species. For instance, pollinator observations on *E. crosbyae* flowers identified visitors from 4 orders and 11 families (Kaye, 1990), and a study of diversity of beneficial insects in Oregon collected specimens from 22 orders visiting 10 species of *Eriogonum* (James et al., 2014). In contrast, we collected 1,898 specimens from 12 orders, 70 families, and 129 species in two *E. tiehmii* sites, and 79 specimens from 17 families and 47 morphospecies that occurred only in *E. tiehmii* sites. There were similar numbers of unique taxa found only at non- *E. tiehmii* sites, indicating that this area supports a diverse array of arthropods with unique habitat preferences.

We also documented high rates of turnover in arthropod communities between monitoring dates; this change in arthropod communities over time was greater than differences observed among sites at a single time point. The abundance of arthropods was highest or second- highest at ERTI6A at both time periods, and the number of species collected at ERTI6A was consistent between both monitoring dates (64 species). Diversity was initially highest at the two non- *E. tiehmii* sites, and decreased at three of the four sites between May and June, but there was a notable increase (+245%) in diversity in ERTI6A between May and June. The most species collected at a single location shifted from 75 in NT.1 in May (11 higher than ERTI 6A, the next-highest) to 64 at ERTI6A in June (16 higher than NT.1, the next-highest). Arthropods in both *E. tiehmii* and non- *E. tiehmii* habitat were similarly abundant and diverse, and the presence of similar volumes of insects, species richness, and overall

diversity in both habitat types suggests that *E. tiehmii* substantially contributes to the diversity and abundance of local arthropod communities throughout their flowering season.

The patterns in abundance of pollinators were different from the overall abundance of all arthropods. For example, while the greatest abundance of all arthropods were observed at ERTI6A, the greatest number of pollinator visitors within *E. tiehmii* sites were observed at ERTI1, where visits were consistently high in both May and June. This could be related to phenological differences between sites, with flowers in ERTI6A coming into full bloom slightly later than those in ERTI1, or even to overall plant cover, which is lower at ERTI1 and could make *E. tiehmii* flowers stand out better to insect visitors (Lázaro et al., 2013). Including all categories of flower visitors, overall visits were higher in non- *E. tiehmii* sites than in *E. tiehmii* sites, driven mainly by high abundance of ants found on *Stanleya pinnata* flowers, especially during the second observation period. Excluding one outlier beetle observation and looking only at flying insects, which are typically considered pollinators, total visits to *E. tiehmii* flowers were 14% higher than to flowers in non- *E. tiehmii* sites (Fig. 3b). This, combined with the generally lower density of flowering plants within *E. tiehmii* sites than in nearby sites (pers. obs.), implies that *E. tiehmii* flowers are highly attractive to pollinators.

This year was characterized by low seed set in *E. tiehmii* plants overall, possibly due to spring frosts (Ed Kleiner, pers. comm.), but overall, we found 265 seeds produced by 59 inflorescences, with significantly higher numbers of seeds produced in open-pollinated flowers. Our results indicate that, while *E. tiehmii* plants may be able to produce some seeds when pollinators are excluded (through wind pollination or selfing), open flowers that were visited by pollinators substantially increased seed production. It is also possible that very small insects (such as thrips, which were abundant at these sites) could have penetrated the bags and pollinated bagged flowers. The differences we observed here between open and bagged flowers may be even greater in years when more flowers are able to set seed, as the seed set of open pollinated flowers could have been reduced by climatic conditions. Consistent production of seeds is a common adaptation of desert plants in highly variable climates, because it increases the likelihood of establishment in rare wet years (Jordan and Nobel, 1979), though viability of seed over time in the soil seedbank is unknown for *E. tiehmii*. From our observations of pollinator visits, the most important pollinators for *E. tiehmii* are likely to be wasps, beetles, and flies. The increase in seed set when pollinators have open access to flowers strongly suggests that presence of an intact pollinator community is important for maintaining population viability in *E. tiehmii*, as insects significantly increased plant fecundity, even in an unfavorable year for seed production.

Activity 2: Plant/soil relationships

2.1 Introduction

Soil specialists are plant species that occur primarily or exclusively on patches of "challenging" soils that differ from the surrounding soil matrix. Challenging soils are those that would be difficult substrates for the majority of plants due to chemical or physical properties that reduce or preclude growth. Examples include serpentine soils and soils developed over limestone, shale, gypsum, and hydrothermally altered rock outcrops (Keener, 1983; Harrison et al., 2009). These soils may be shallow, have extreme pH, low water holding capacities, high proportions of clay or sand, accumulations of salts, heavy metals or other toxic elements, and/or nutrient deficiencies, among other characteristics (Palacio et al., 2007; Anacker, 2014; Boisson et al., 2017). The differences between challenging soils and the surrounding environment are most dramatic in dry climates, where slow rock weathering and lack of water may further exacerbate nutrient deficiencies (DeSiervo, 2015).

Plants that are soil specialists can evolve a range of adaptations to grow on challenging soils, which may include slow growth, high root allocation, reduced seed dispersal (which can be favored if seeds do poorly when dispersed beyond the boundaries of challenging soils), early flowering phenology, compact growth forms, enhanced root exudates or obligate mycorrhizal associations (to aid in nutrient acquisition), and the ability to preferentially take up, store, and compartmentalize nutrients and toxins (Brady et al., 2005; Escudero et al., 2015). Strong evolutionary pressures combined with relative isolation encourages speciation, and as a result, soil specialists can make up a disproportionately high amount of regional biodiversity relative to the amount of land area they occupy (Safford et al., 2005). However, many of these species are also highly vulnerable to habitat loss from land use changes and climate change because, once their habitats are destroyed or local climatic conditions shift beyond their range of tolerance, these highly specialized plants may have no other suitable habitat available within their dispersal capacity (Harrison et al., 2009). Therefore, an understanding of soil specialists' habitat requirements and the impacts of environmental variation on plant growth across the life history of plants is crucial for successful conservation (Lazarus, 2010; Lazarus et al., 2011). This information can help in the identification of critical habitat, and in understanding the range of conditions in which species can be expected to survive, both important considerations for managers considering whether to allow impacts to these species or anticipating the effects of climate change on small populations (Hall et al., 2004).

The adaptations that allow plants to survive on challenging soils may have tradeoffs that reduce performance in other situations, such as the inability to significantly improve growth in more fertile environments, which makes them poor competitors (Maestri et al., 2010; Sianta and Kay, 2019), or even the inability to grow normally on any soils other than their own (Rajakaruna and Bohm, 1999; Küpper et al., 2001). Plant growth responses in soils with varying properties can be used as indicators of the presence and strength of soil specialization in a given species (Veblen and Young, 2009; McClinton, 2019). Better performance in challenging soils than in more fertile soils, even without competition, may indicate that species belong to a "specialist" model of soil specialization, where species are specifically adapted to harsh soils, rather than a "refuge" model, wherein species tolerate stress as an escape from competition but might still prefer more fertile environments given the chance (Palacio et al., 2007; Veblen and Young, 2009; Madawala Weerasinghe et al., 2010; Escudero et al., 2015). "Specialist" model species would be expected to have a narrower range of conditions they can tolerate and would show better growth in soils from occupied than unoccupied sites, even when those soils have challenging characteristics.

E. tiehmii is a known soil specialist with a very restricted distribution. It grows in clay-rich soils developed over a variety of interbedded sedimentary rocks, and occurs only in a series of small outcrops, each separated by

several hundred meters, near Rhyolite Ridge, Nevada (Tiehm, 1994). These soils vary in texture and appearance, but the degree of variation in soil chemistry and texture among occupied sites is currently unknown, as is the range of soil conditions that are within the growth tolerance of *E. tiehmii*. We aimed to fill these knowledge gaps, asking: (1) what are the defining/average soil characteristics of *E. tiehmii* habitat, (2) how do *E. tiehmii* habitat soil properties differ from surrounding soils, and (3) how does variation in soil properties affect seedling growth? To answer these questions, we analyzed chemical and physical soil properties in samples collected from 21 different sites that had been identified as occupied or unoccupied *E. tiehmii* habitat, focusing on unoccupied habitats within or close to the boundaries of occupied sites. We also planted seeds into each soil type in a common-garden greenhouse experiment, and monitored emergence and survival, and eventually harvested, dried, and weighed above and below-ground biomass. From these observations and experiments, we can better understand the range of soil characteristics that define *E. tiehmii* habitat.

2.2 Methods

Soil and seed collection, soil sample preparation for analysis by A&L Western Labs

We collected soil samples from 21 different sites near Rhyolite Ridge, Nevada, aiming to sample as much soil variation as possible in the geographically restricted area around *E. tiehmii* populations (See Appendix 1 for a map of sampling locations, and Appendix 2 for sampling location coordinates, site occupation designations, and reasoning behind individual sample site choices). We included soils from all eight known *E. tiehmii* sub-populations, from nearby un-occupied habitat developed from the same parent materials, surrounding alluvium, occupied and un-occupied trenches where past disturbance had altered surface soils, from un-occupied sites that were identified as potential habitat in habitat models provided by the environmental consulting company EM Strategies, or areas that were considered to be potential habitat by expert opinion during field surveys. The prefix “ERTI” indicates sites currently occupied by *E. tiehmii*, and the prefix “PTS” indicates “potential sites” identified by habitat models and/or expert opinion. Some of the sampling locations were specific locations within the boundaries of a larger sub-population. Specifically, the two sites ERTI 1- orange and Trench 1 are occupied areas within the boundaries of larger sub-populations (ERTI1 and ERTI6, respectively), but they have unique characteristics, including a history of disturbance or a very different soil color than the surrounding area, so we sampled them separately.

At each of the 21 sites, we collected ~17 liters of soil from open spaces between plants in the top 10 cm of the soil profile, chosen to reflect the root zone conditions of seedlings. Samples were collected using hand trowels, and care was taken not to disturb any *E. tiehmii* plants. Upon returning to the University of Nevada, Reno campus, soil was homogenized by rolling in a tarp. Three sub-samples of the final composite were removed, sifted to <2mm, and approximately 1 liter of soil was shipped to A&L Western Labs (www.al-labs-west.com) for analysis. Soil properties analyzed included pH, texture, organic matter, cation exchange capacity, essential macro and micro-nutrients (including boron), salinity, and other elements (See Appendix 3 for all soil variables). We also report values from two other soils types, as a comparison for this study: in our previous work, we measured the same soil properties of typical habitat of *E. crosbyae,* as well as soil properties in a more typical Great Basin soil from Washoe Valley, Nevada, which is a substrate we frequently use to grow native plants, including other *Eriogonum* species.

E. tiehmii seeds used in the soil preference experiment were field collected by Comstock Seed (Gardnerville, NV) in July 2019 and stored at the Rae Selling Berry Seed Bank (Portland, OR) until use.

Greenhouse soil preference experiment installation, monitoring, and harvesting

Experimental methods broadly followed those of McClinton *in press.* First, each field soil was sieved to 1.3 cm to remove large rocks, then combined in a 50/50 mixture with decomposed granite, using tarps to ensure thorough soil mixing. Field soils can become highly compacted during transport and sieving, creating unrealistic and very dense soil conditions that are inhospitable for plant growth. Adding decomposed granite improves soil texture and drainage, without changing nutrient composition, and approximates soil structure found in field conditions. Our replications were limited by seed availability, and we planted 29 (9 soil types) or 28 (12 soil type) replicates of each soil type, randomly selecting which soil types received one fewer replicate, for a total of 600 pots. Pots were filled with soil, arranged in an alternating, sequential grid by soil type across three tables in the greenhouse, and watered for one week prior to planting on March 2-4, 2020. (Fig. 9). This pre-planting watering ensured that all soils were fully moistened, important because some field soils can initially be hydrophobic, and repel, rather than absorb, water.

Figure 9: The layout of soil testing in the UNR greenhouse. Soils were alternated sequentially across the benches, ensuring that pots were distributed evenly across any variations in microclimatic light and temperature conditions in the greenhouse.

Two seeds of *E. tiehmii* were planted per pot. Seeds from five of the *E. tiehmii* sub-populations were available for planting, and we planted seeds from each sub-population in each soil type, with replicates dependent on seed availability. For each soil type, three replicate pots were planted with seeds from sub-populations 1, 3, 4, and 6, and sixteen or seventeen were planted with seeds from sub-population 2. All pots were watered every 2-3 days as needed for the duration of the experiment, with special attention to maintaining surface moisture during germination. Light and temperature conditions were set to follow the gradation from cooler spring to warmer summer conditions in the Great Basin, with full sunlight and temperatures maintained between 2.2°C (36°F) - 12.8°C (55°F) from March 2- March 15, increased to a maximum of 15.6°C (60F) from March 15 until April 15, then raised to between 4.4°C (40°F) - 21°C (70°F). Temperatures were increased the final time June 15, and set to a range of 12.8°C (55°F) - 3.9°C (75°F). We monitored seedling emergence and noted the death of any seedlings on a weekly basis. Seedlings were harvested August 10-13, 2020. During harvest, soil was gently washed away from the roots, then above and belowground biomass was separated, stored in separate coin envelopes, dried in a drying oven to a constant mass, and weighed.

Data analysis

To ask whether growth responses in the greenhouse or soil variables differed between soils from occupied sites and those from unoccupied sites, we used both two-sided Welch's t-tests (which do not assume equal variance among sample groups) and non-parametric Wilcoxon rank sum tests (which are less powerful for detecting differences among groups than parametric tests, but do not assume a normal distribution or equal variance), to compare all response variables in occupied vs. unoccupied sites (Appendix 3). Because these tests include many individual comparisons, we note whether differences were significant in individual t-tests at the traditional p <0.05 level, but we also note differences where p-values have been corrected using the Bonferroni method for multiple comparisons (p_{adj}). Bonferroni correction involves multiplying individual p-values by the number of

comparisons being made (42 soil variables). This is a more conservative method of comparison that attempts to adjust for the likelihood of finding differences among large numbers of comparisons simply by chance.

Then, we took an exploratory approach to determine which soil variables had the greatest effects on *E. tiehmii* site occupation in the field and days until emergence, days lived, total biomass and root mass ratio in the greenhouse. Forty- two soil variables were measured and could be used as potential predictors (Appendix 3); to reduce multicollinearity among them we removed one of each pair of soil variables with Pearson correlations of 60% or higher (Appendix 4). Note that in some cases, these variables were extremely correlated (e.g. 0.98 correlation between saturated paste sulfur (S.SP) and soluble salts (Sol_Salts.SP)), which means that variables could have been used almost interchangeably. Each growth or occupancy response was modeled separately, and for each we chose which correlated variables to remove by comparing deviances of univariate generalized linear models and keeping the variable from each pair with the lowest deviance, which means it had the greatest explanatory power in the model.

We then visualized soil variation across all sampling sites with principle components analysis, using the subset of uncorrelated variables that best predicted site occupation by *E. tiehmii* to avoid over-emphasizing highly correlated variables.

For all analyses, soil properties from all three samples analyzed per site were averaged, and averaged soil variables were scaled to a mean of 0 and standard deviation of 1 to remove differences in measurement scale. Generalized linear models, fit using maximum likelihood, were created for each analysis by selecting the error distribution based on the response variables and model fit diagnostics. Selections were as follows: a quasi-poisson distribution with a log link for days until emergence and days lived, lognormal distribution for total biomass (log of total biomass, model fit using a gaussian distribution with an identity link), and gaussian distribution with an identity link for root mass ratio in seedlings grown in the greenhouse. We included plant age (days) and number of plants per pot at harvest (a very small number of pots had two surviving plants) as potential predictors for total biomass and root mass ratio. Final models were fit using a genetic algorithm in the R package “glmulti”, which adaptively tests models with different predictors until a minimum information criterion value (here, AICc) is reached. Coefficient estimates, which are an indication of the strength and direction of the effect of each predictor on the response variable, were averaged across the set of models that yield 95% of total evidence weight, generally models within 2 AIC units of the top model. The residual distributions and dispersion in top generalized linear models were tested with the “DHARMa” package in R, and 10-fold cross-validation was performed to check for model over-fitting.

We also examined plant performance in individual soil types and in occupied and unoccupied soils. To examine the relationship between emergence and survival, we created linear models with percent survival as the response variable and percent emergence as the predictive variable for occupied and unoccupied soils. Percent emergence was calculated as the percentage of all seeds planted that emerged, and percent survival was calculated as the percentage of seedlings that emerged that also survived until harvest. Percent survival and percent emergence were averaged for each of our 21 soil types. Then, we created a “plant growth index”, intended to represent overall plant success in each soil type by multiplying average percent emergence and percent survival with average total biomass. We created bar charts to visualize plant success in soils from different sites, and created generalized linear models to compare the plant growth index, percent emergence, and percent survival in occupied and unoccupied soils.

Lastly, we created linear models to ask whether there were any correlations among days to emergence, days lived, total biomass, and RMR.

2.3 Results

Soil properties of *E. tiehmii* habitat

Occupied *E. tiehmii* habitat soils were characterized by a variety of textures, and include clay soils, sandy clay loams, sandy loams, and loams. Relative to occupied *E. crosbyae* soils, *E. tiehmii* soils were, on average, higher in NO3-N, lower in P, lower in K, higher in Ca, lower in Mg, lower in S, higher in B, lower in CEC, and higher in pH (Table 4). Relative to the more typical Great Basin soil from Washoe Valley, *E. tiehmii* sites had, on average, lower nitrate nitrogen, extremely low phosphorus, higher potassium, calcium, magnesium, sulfur, CEC, pH, and extremely high boron (Table 4).

Table 4: Comparison of selected soil properties for soils collected from occupied *E. tiehmii* and *E. crosbyae* habitat and from a more typical Great Basin soil from Washoe Valley, NV. Values are means (± 1 standard deviation) for the two *Eriogonum* soils, and single values for the Washoe soil.

Soil property[a]	***E. tiehmii* soils (*N* = 10)**	***E. crosbyae* soils (*N* = 25)**[b]	**Washoe soil (*N* = 1)**[b]
NO_3-N (ppm)	12.31 (±12.04)	5.20 (±3.32)	69
P (ppm)	6.89 (±3.41)	18.72 (±10.16)	196
K (ppm)	454.27 (±138.88)	853.48 (±1061.75)	163
Ca (ppm)	3224.47 (±431.59)	2232.20 (±763.27)	1196
Mg (ppm)	325.64 (±124.61)	445.72 (±206.84)	103
SO_4-S (ppm)	173.61 (±320.68)	373.84 (± 1455.44)	17
B (ppm)	38.26 (±37.07)	0.42 (±0.18)	0.4
CEC (meq/100g)	22.22 (±3.2)	23.62 (± 18.51)	9
pH	8.2 (±0.26)	6.45 (± 0.66)	5.8

[a] All elemental concentrations refer to extractable values, methods according to those listed in Appendix 3, except for P concentrations. In *E. tiehmii* soils, P was extracted using the Olsen method for calcareous soils, while the Bray-P method was used in the acidic *E. crosbyae* and Washoe soils.
[b] Data on *E. crosbyae* and Washoe soils is from McClinton et al, *in press*.

In addition to considering average differences among occupied and unoccupied sites, we used principal components analysis to visualize which soil variables explained the most variation among our sample sites at Rhyolite Ridge. The first three principal components axes explained 48.8% of the variation between sites. The clearest differentiation between *E. tiehmii* and other sites is seen along principal component two (16.62% variation), which is dominated by the saturated paste concentrations of boron, copper, and bicarbonate (Fig. 10A). Among occupied sites, ERTI1 was the most extreme along principal component 2, while ERTI8, a site with only a few plants, was the least extreme, and there was some overlap between occupied and unoccupied sites at the lower range of values along principal component 2 (Fig. 10B). The sub-areas sampled within ERTI1 (ERTI1-orange), and ERTI6 (Trench 1) were different than the main population, as it appeared to be in the field. Occupied sites were found across almost the entire range of conditions represented by principal component 1, which was most strongly associated with percent clay and nitrate- nitrogen. Some of the unoccupied sites had soil texture and chemistry characteristics within the envelope of conditions of occupied sites in this principal component analysis, namely PTS-G, Alluvium 1 and 2, PTS-B, and PTS-D.

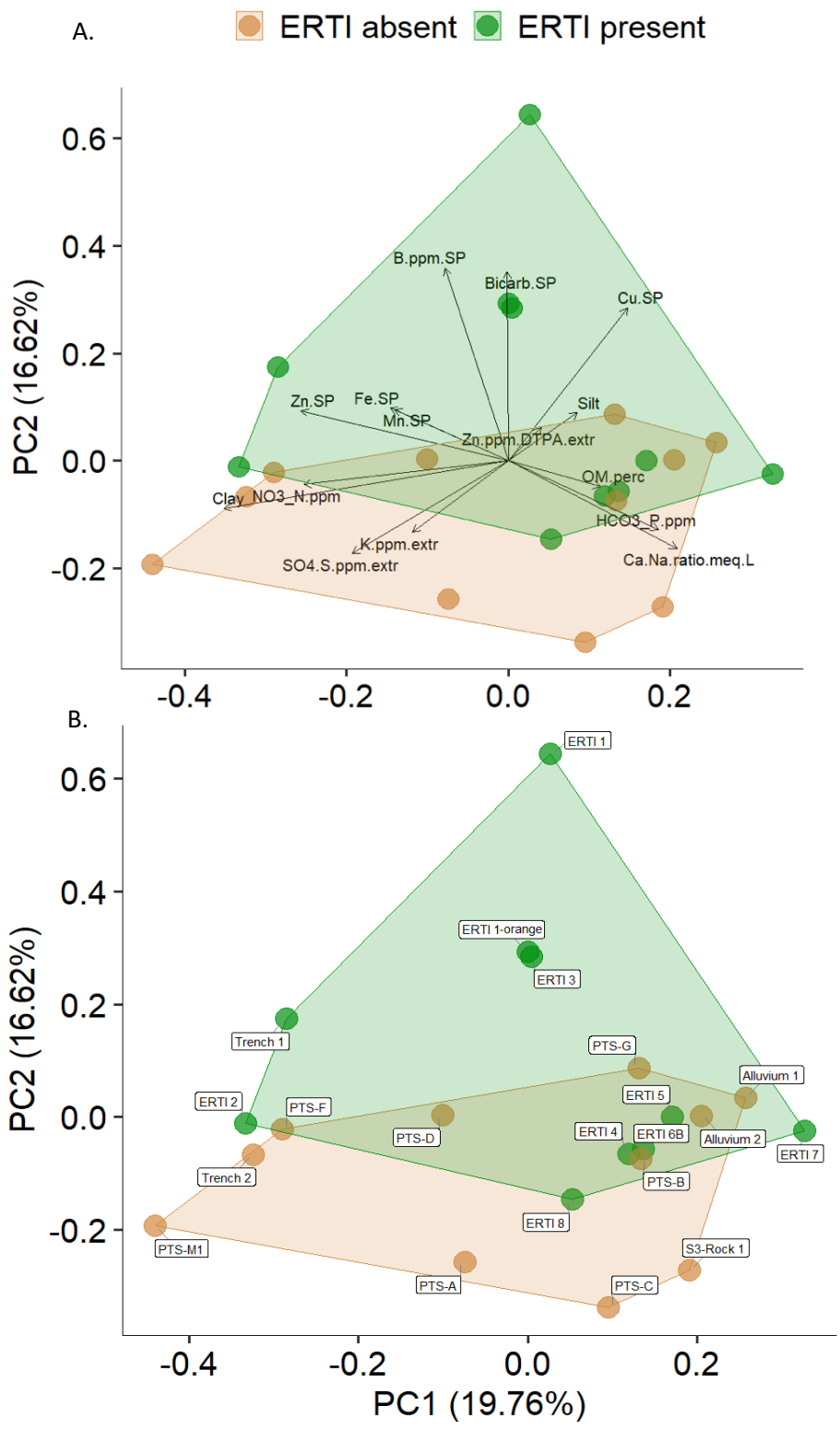

Figure 10: (A) Biplot of component scores for the first and second axes of a principal components analysis of variation in soil properties among sampling sites, with variable loadings overlaid as arrows. (B) Biplot of the same component scores, with sites labeled. In these figures, Here, *E. tiehmii* and non- *E. tiehmii* sites are moderately separated along principal component two. Silt and organic matter (OM) are in units of percent, "extr" refers to extractable amounts of each variable using the method most appropriate for that soil character, and "SP" refers to saturated paste (water) extraction amounts. See Appendix 1 for abbreviations and soil analysis methods.

Considering Rhyolite Ridge soils only (i.e. no Washoe or *E. crosbyae* soils), Wilcoxon rank sum tests also revealed differences in soil chemistry and texture among soils that were occupied and unoccupied by *E. tiehmii*. Overall, occupied soils had less potassium (extractable, saturated paste, and percent cation saturation), less extractable zinc and sulfate-sulfur, and less saturated paste magnesium; values were, on average, higher in occupied soils for extractable boron, percent silt, bicarbonate, and pH (p<0.05, Fig. 11, Appendix 3). There was variation in some of these soil characteristics among occupied and unoccupied sites. For example, while extractable boron was, on average, higher in occupied sites, there were both occupied and unoccupied sites with either high or low values of boron, with no intermediate values observed in our samples (Fig. 11). On average, occupied soils also tended to be lower in CEC, aluminum, iron, extractable phosphorus, sodium, and sulfur, and higher in silt, pH, saturated paste phosphorus, and bicarbonate than surrounding unoccupied soils (see Appendix 3 for all test statistics with and without p-value corrections, and Appendix 5 for means and standard deviations of soil variables by site occupation).

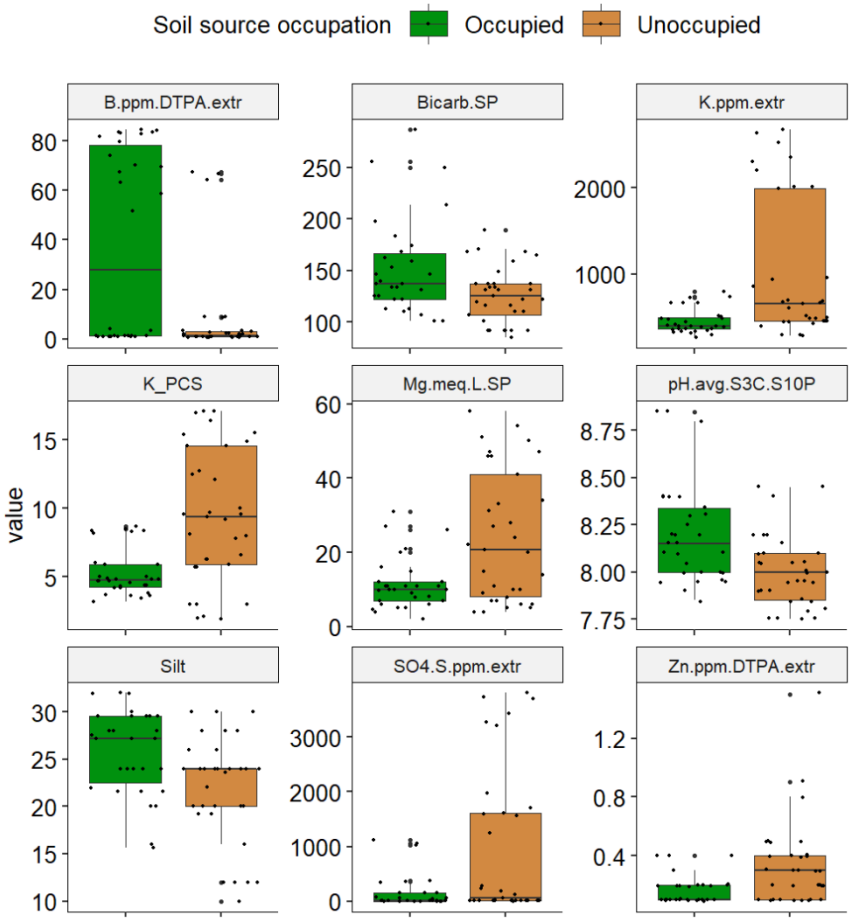

Figure 11: Soil variables that were shown to be significantly different (p< 0.05) between sites that were occupied and unoccupied by *E. tiehmii* in the wild in Wilcoxon rank sum tests. Occupied sites are in green (bars on the left) and unoccupied sites are in tan (bars on the right). Saturated paste potassium was also lower in occupied sites than in unoccupied sites, but not shown here; all test statistics and p-values for parametric and non-parametric tests are in Appendix 3. Soil variables that were significantly different using the more conservative Bonferroni-corrected p-values were extractable and percent cation saturation potassium. The suffix "extr" refers to a chemically extractable amount and "SP" refers to saturated paste (water) extraction amounts. DTPA indicates

that the DTPA-Sorbitol extraction method was used to measure the element concentration. Units are: B.ppm.DTPA.extr (ppm), Bicarb.SP (ppm), K.ppm.extr (ppm), K_PCS (% Cation saturation), Mg.meq.L.SP (ppm), pH.avg.S3C.S10P (pH), Silt (%), SO4.S.ppm.extr (ppm), Zn.ppm.DTPA.extr (ppm). See Appendix 3 for additional soil analysis methods.

Soil properties and greenhouse plant performance

Overall, 24.5% (293/1197) of all seeds planted emerged during the soil preference experiment. Emergence differed among sites (Fig. 13A), with the unoccupied sites S3 Rock and PTS-A having particularly high emergence, followed by ERTI1. However, there was no significant difference in emergence between occupied and unoccupied sites overall. Survival also differed among sites (Fig. 13B), and there was again no difference in survival among occupied and unoccupied sites. The top sites for survival were different than those that were top for emergence, with two unoccupied sites (PTS-G and PTS-B) having the highest survival, followed by ERTI5.

There was a strong positive association between emergence and survival in occupied sites, with survival increasing 2.5% per 1% increase in emergence ($p= 0.01$, $R^2= 0.59$). In other words, in occupied sites, soils that were more favorable for seedling emergence were also more favorable for seedling survival (Fig. 12). However, there was no significant relationship between emergence and survival in unoccupied sites, indicating a disconnect in this group between soils that were favorable for different life stages. For example, unoccupied sites like Trench 2 had low emergence but high survival, while S3-Rock 1 had the opposite pattern, high emergence but low survival.

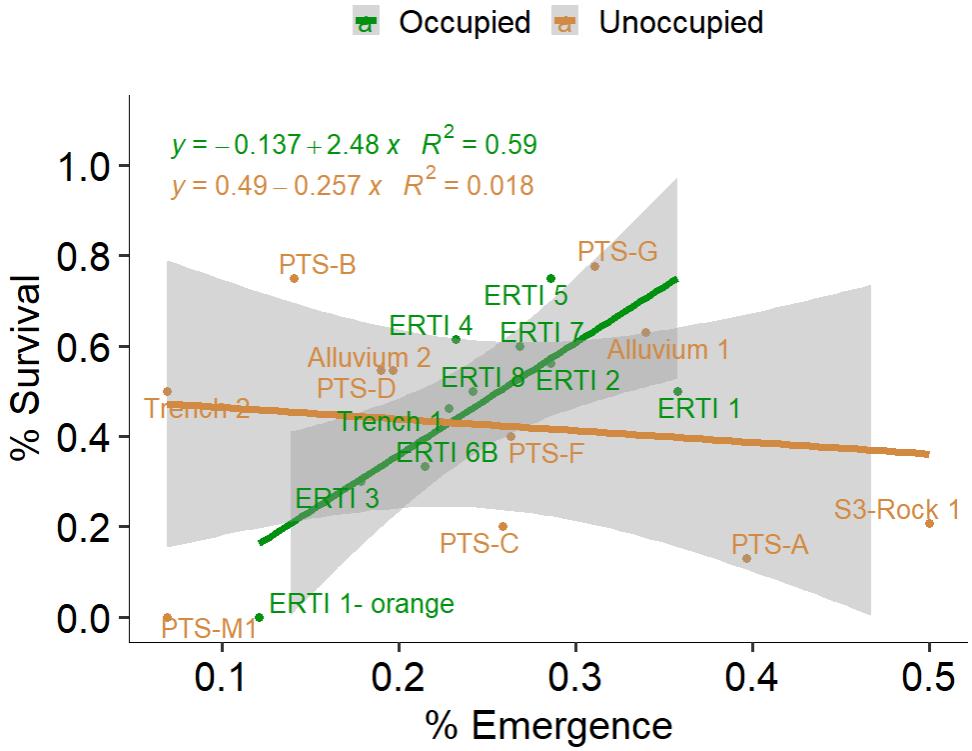

Figure 12: Relationships between *E. tiehmii* emergence and survival until harvest in soils from occupied (green) and unoccupied (orange) sites, overlaid with linear models of the correlation between the responses and the confidence intervals around those estimates (shading). Emergence and survival were strongly positively correlated in occupied soils *(p= 0.01, R^2= 0.59)*, and slightly negatively correlated in unoccupied soils (p>0.05).

In addition to differences in emergence and survival, there were differences among populations in plant size, with large differences in plant growth in currently occupied vs. unoccupied soils (Fig. 13C). Total biomass of plants grown in occupied soils was 53% higher than for seedlings grown in soil from unoccupied sites (p<0.05, Appendix 3). The highest average total biomass we measured in soils from an occupied site was 235.0 mg (ERTI 5), and the highest in an unoccupied site was 146.4 mg (PTS-B). These differences were also seen in root size and allocation: on average, root weight was 81% higher and RMR was 19% higher in occupied sites (p<0.05, Appendix 3). The highest average root weight we measured in soils from an occupied site was 172.7mg (also ERTI 5), while the highest in an unoccupied site was 96.9mg (also PTS-B).

Individual sites also differed in the "plant growth index" (created by multiplying emergence x survival x biomass to get an all-in-one measure of seedling performance), but there was no significant difference in this index between occupied and unoccupied sites (Fig. 13D). The highest index value was in ERTI5, followed by ERTI2. Two unoccupied sites, Alluvium 1 and PTS-G, were also among the top sites with this metric, though for different reasons: performance in Alluvium 1 was in the top ~20% for all three metrics (#4 in emergence and survival, #5 for biomass), giving it an overall higher rank than other unoccupied sites, while PTS-G ranked high because of high survival (#1) but was weaker in emergence (#5) and in the lower third for biomass (#13). There were two occupied soils where seedlings performed relatively poorly in the greenhouse (ERTI6B and ERTI3, Fig. 13), due to lower emergence and survival in these soils.

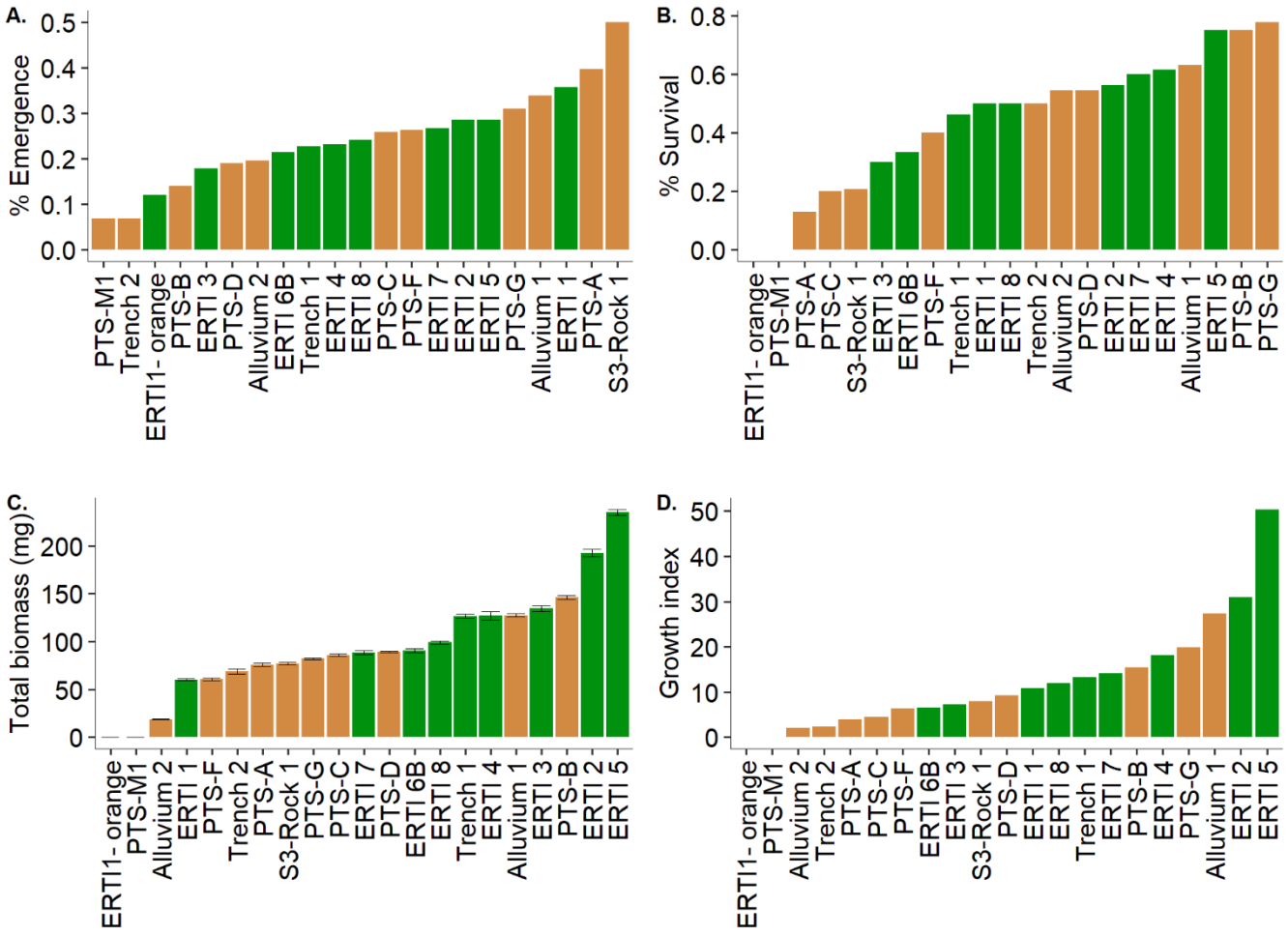

Figure 13: Means of *E. tiehmii* seedling growth in soils from each sampling site. Seedlings were grown in the greenhouse at the University of Nevada, Reno. Green bars indicate soils from occupied sites in the wild, and brown bars indicate soils from unoccupied sites. The growth index (D) was calculated by multiplying total percent emergence (A), total percentage of seedlings that emerged that survived until harvest (B), and average total biomass (C) of seedlings grown in each soil type. ERTI1- orange and PTS-M1 had 0% survival and no biomass to measure, and therefore they had growth indices of 0. Error bars in panel (C) are the standard errors of the mean.

Soil chemical and physical properties partially explained differences in plant growth in the greenhouse. Seeds emerged more quickly on soils with high sand, aluminum, and manganese (Figure 14). Seedlings grown in soils with high sulfur tended to die sooner than seedlings grown in other soils. Plants had higher total biomass when grown in soils with higher organic matter and nitrate- nitrogen, and were smaller when grown in soils with higher zinc, calcium, iron, and silt. Plants allocated less resources to roots when grown in soils with high zinc, iron, and sulfate-sulfur, and increased root allocation in soils with higher N:P ratios, organic matter, and copper (Fig. 14, Appendix 6).

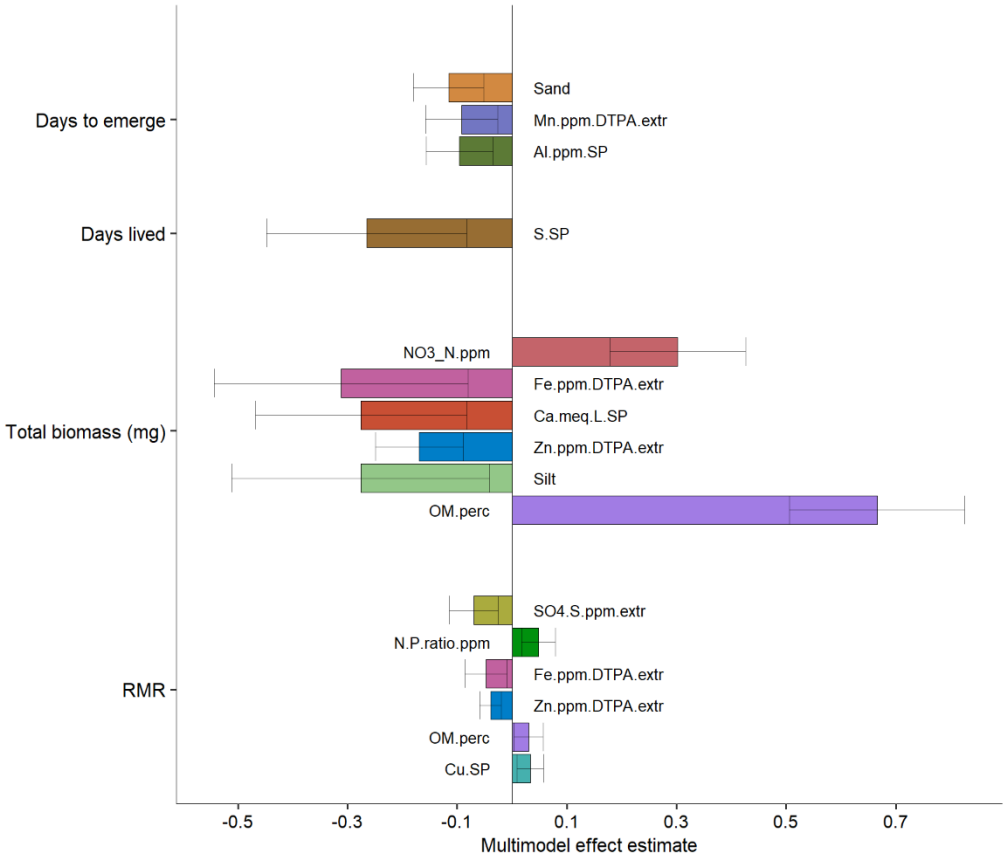

Figure 14: Model-averaged estimates of the effects of soil variables and plant age on *E. tiehmii* growth responses in the greenhouse at the University of Nevada, Reno. Coefficient estimates for total biomass, days to emerge, and days lived are in units of percent change in the response variable (decimal format), and estimates for effects of soil variables on RMR (root: total biomass ratio) are unit-less, the same as RMR. Estimates are per one-SD increase in each soil variable, and 1-day increase in plant age. Example: For every 1-SD increase in extractable zinc (0.22ppm, Appendix 6), total biomass decreased by 17%. For individual model coefficients of the top (within 2AIC units) models for each response, see Appendix 8.

Lastly, there was a positive association between root allocation and total biomass; for each 0.12 unit increase in RMR, total biomass increased by 34mg ($p<0.05$n $R^2_{adj}= 0.24$). There was no significant association between RMR and days lived, nor between days to emergence and days lived, total, biomass, or RMR.

2.4 Discussion

Our analysis of soil chemistry confirmed that *E. tiehmii* habitat soils are challenging in comparison to surrounding unoccupied soils, to *E. crosbyae* soils, and to the more typical Great Basin soil from Washoe Valley that we tested during our work with *E. crosbyae*. *E. tiehmii* soils feature extremely low phosphorus, low nitrogen, high boron, and high pH, all of which would negatively affect the growth of most plants (Barker and Pilbeam, 2015). Further, within the relatively small area sampled at Rhyolite Ridge, we observed significant differences in a variety of soil characteristics between occupied and un-occupied sites, including potassium, zinc, sulfur, and magnesium, which were on average lower in occupied sites than in un-occupied sites, and boron, silt, bicarbonate, and pH, which were, on average higher, though there was variation among sites. Differences

between occupied and unoccupied sites were also evident in our principal components analysis, which showed moderate separation between occupied and unoccupied sites along principal component 2 (with occupied sites associated with positive loadings of boron, bicarbonate, and copper) indicating that there is a unique envelope of soil conditions in which *E. tiehmii* can thrive. Further analysis could consider the influence of the underlying geology on predicting site occupation, as well as hypothesize the physiological mechanisms constraining or promoting the growth of *E. tiehmii* on particular soil types.

Our soil preference experiment suggested a high degree of soil specialization in *E. tiehmii*, confirming what could be assumed from its extremely small natural range (Tiehm, 1994; Program, 2001). Unlike our results for *E. crosbyae*, which suggested more of a "refuge" model of specialization for that species (i.e. we observed no differences in performance when grown in occupied vs. unoccupied field soils, and a strong growth response to fertile soil; McClinton, 2019), for *E. tiehmii,* we identified greenhouse plant growth responses (biomass, root allocation) that differed between sites that are occupied or not occupied in the wild. Of special note is that for *E. tiehmii*, seedlings grown in soils collected from existing *E. tiehmii* habitat developed higher total biomass overall than seedlings grown in soils from surrounding unoccupied areas, even those that are comparatively more fertile, such as the alluvial and S3-rock soils, which were generally lower in clay and copper and higher in organic matter and phosphorus. The lack of growth response to more fertile soils, combined with the strong positive association between emergence and survival only in occupied soils, is consistent with a "specialist" model of soil specialization for *E. tiehmii*.

Variation in soil properties were associated with significant differences in plant growth in the greenhouse, and it was notable that the relative performance of plants in different soils varied depending on the life stage of the seedlings. This type of response has been observed in other soil specialists, including *E. crosbyae* (McClinton, 2019), gypsum specialists in Spain (Sánchez et al., 2017) and copper endemics in the D.R. Congo (Boisson et al., 2017). For *E. tiehmii,* soil properties that were associated with earlier emergence were unpredictive of how long seedlings survived, or of their total biomass and root allocation. However, some soil properties had negative effects across multiple life stages; for instance, increasing sulfur concentrations were associated with shorter lifespans and lower root allocation. The observation that root allocation increased as plants grew larger has been seen in other plant species growing in low-nutrient conditions, and could indicate an evolutionary strategy for increasing biomass in challenging soils (Shipley and Meziane, 2002; Husáková et al., 2016).

While some unoccupied sites were individually favorable for emergence, survival, or seedling growth, there were no unoccupied soils that were favorable for all life history stages for *E. tiehmii.* For example, seedlings emerged and survived in the unoccupied PTS-G and Alluvium 1 at rates comparable to those observed in occupied sites, but biomass in those soils were lower than in the best occupied sites. In another example, seedlings grown in the unoccupied site PTS-B were the largest of the unoccupied sites, and also had some of the highest survival of all soil types, but in contrast, the seedling emergence rates were among the lowest observed. All of the unoccupied sites that we sampled were within a distance that could reasonably have been colonized by seeds from existing populations, and it is possible that conditions unfavorable for emergence, survival, or growth at the seedling stages have precluded the formation of additional *E. tiehmii* populations within its existing range. The unoccupied locations that supported more positive responses for at least some life stages were also those that were most similar in soil properties to occupied sites, indicating that the envelope of chemical and physical properties that we observed (Fig. 10B) has some predictive power, in terms of *E. tiehmii* response to unoccupied sites.

In our greenhouse study, seedlings also performed relatively poorly at some stages in soils from two occupied sites, ERTI 6B and ERTI 3, which both support thriving populations in the wild. This serves to emphasize the limits of using the greenhouse environment, which is artificial by nature, to fully understand plant-environment interactions in the wild. While the early life history stages we studied here are often limiting for populations of arid plants (James et al., 2019), additional demographic research would be needed to determine the limiting life stages in particular populations. For example, at ERTI 6B and ERTI 3, the relatively high density of plants and presumably seed production may mean that population persistence is more affected by how well *E. tiehmii* plants perform in these soils at later life history stages (e.g. juvenile survivorship and flowering). Findings in a greenhouse are only a guide for how seeds and seedlings may perform under more natural conditions, where performance would likely be affected by factors such as reduced water availability, the presence of competition from other plants, and other biotic interactions.

In summary, this work demonstrated measurable impacts of soil variation on measures of plant growth at different life history stages that might directly impact population establishment and persistence in the wild. Our results suggest that even with the relatively small area of Rhyolite Ridge, populations are persisting in occupied habitats through a variety of strategies (high emergence, high survival, or high seedling biomass), and that occupied habitats are those that have conditions that are sufficiently favorable to promote persistence despite differences in performance across life history stages. Our tests identified several unoccupied locations at Rhyolite Ridge that appeared to meet some, but not all, requirements for seeds and seedlings, but it is possible that other locations exist where conditions are more similar to currently occupied habitats; soil chemistry and texture analyses could be used to identify such sites. Like many other soil specialists, colonization of unoccupied but suitable habitat patches by *E. tiehmii* may be limited by dispersal, and further research into dispersal mechanisms and how habitat connectivity is impacting population dynamics in this species would also be important to identify whether there is potential for it to survive anywhere else. Finally, understanding how plant competition affects population persistence would be key for understanding if any favorable but unoccupied sites would be favorable for plant growth in the wild.

Activity 3: Seedling transplant experiment

3.1 Introduction

The need for biodiversity conservation, especially of rare and threatened species, is pressing. Approximately 39% of all vascular plant species on earth are currently at risk of extinction, due primarily to exploitation, land use changes, and/or climate change (Nic Lughadha et al., 2020). In addition to inherent aesthetic values, many rare species are vital to the functioning of their ecosystems, providing resources upon which life depends, and have potential economic value as sources of food crops, medicine, renewable energy, and bioremediation, among many other benefits (Phillips and Meelleur, 1998; Whiting et al., 2004; Dee et al., 2019). Rare plants are also ideal test systems for evolutionary and ecological research that helps us understand the origins and distribution of biological diversity (Linhart and Grant, 1996; Rajakaruna and Harrison, 2011; Strauss and Boyd, 2011; Rajakaruna et al., 2014; Rajakaruna, 2018). The goals of rare plant research are to fully understand the ecology of these species, in order to preserve "resilient, self-sustaining populations that have sufficient genetic resources to undergo adaptive evolutionary change" (Guerrant Jr and Kaye, 2007). Thus, the study of rare plants can serve applied conservation objectives as well as contribute to our understanding of the evolution of earth's diversity.

The most effective plant conservation involves maintaining intact, healthy populations of plants within their native range, maintained without human intervention (Eriksson et al., 1993; Christensen et al., 1996). However, some circumstances, such as rapid habitat loss, novel disturbances, or anthropogenic climate change, may make this impossible, necessitating further action to ensure species' survival (Maschinski and Haskins, 2012; Maschinski and Albrecht, 2017). The fields of ecological restoration and rare plant translocation have developed because of this need; they are relatively young and frequently rely on incomplete knowledge of complex biological and ecological interactions (Naeem, 2016). Not all species are candidates for restoration or reintroduction, either within their native range or in new locations. This may be due to factors such as limited availability of suitable habitat or propagule material, sensitivity to disturbance, and/or lack of the associated biological community that enables survival, among other challenges (Maschinski and Albrecht, 2017). For rare plants that are experiencing habitat loss, research is required to ascertain whether restoration or reintroduction are possible conservation approaches for threatened populations, considering factors such as the species' ecological interactions, life history, climate niche, and substrate requirements, as well as understanding best practices for propagation and effective methods for population establishment, (Schemske et al., 1994; Guerrant Jr and Kaye, 2007; Albrecht and Long, 2019).

When appropriate sites are available for restoration or translocation, studies have shown that directly transplanting seedlings can result in much higher survival than seeding, and that the benefits of using scare seed efficiently can outweigh the additional costs for labor and supplies associated with transplants (Wallin et al., 2009; Reckinger et al., 2010). Seedling transplant has proved promising in the conservation of *E. crosbyae*, another Great Basin soil specialist with a limited, but broader, distribution than *E. tiehmii*. In that example, the Leger Lab at the University of Nevada, Reno worked with the Bureau of Land Management to install a transplant experiment with seedlings propagated from a soon-to-be extirpated population of *E. crosbyae* into a new, carefully chosen site within the Black Rock Desert High Rock Canyon National Conservation Area— a location that is within the broader range of this species, and where it can be protected in perpetuity. We used a method involving planting seedlings next to clay terra cotta pots buried in the soil that were routinely filled with water, which allowed for the even and slow delivery of water to the roots of these seedlings during the warm high-desert summer. After their first year, overall survival of the *E. crosbyae* plants was >60%, and the transplanted

seedlings have begun to flower, indicating potential for the establishment of a self-sustaining population (McClinton et al, Unpublished Data; Fig. 17).

Increasing threats from mineral extraction, OHV use, and climate change have generated interest in understanding whether *E. tiehmii* might benefit from similar active conservation methods. *E. tiehmii* has a small range, limited global population (Tiehm, 1994; Morefield, 1995), and low fecundity in some years, due to both low seed production and low seed viability (personal observation). Its range is situated near the western edge of the Great Basin and is within the rain-shadow of both the Sierra Nevada Mountains and the White Mountains of California, leading to high variability in the amount and timing of annual precipitation (Swetnam and Baisan, 2003). High inter-annual variability in weather combined with low fecundity means that wild-collected seeds are scarce and precious, and that seed supply would likely be insufficient for the repeated seeding efforts that would be required to establish or significantly augment a resilient, self-sustaining population, or for the experiments needed to understand the ecology of this plant. Therefore, part of our research focused on testing the feasibility of propagating *E. tiehmii* seedlings in a greenhouse setting, in and testing transplant methods in the field, and understanding the effects of site variation on transplant seedling survival in unoccupied sites within the boundary of its native range. We specifically asked: 1) can *E. tiehmii* be grown in a greenhouse, and what conditions does it need in order to produce hardy transplant seedlings, 2) is the terra cotta pot transplant method we used for *E. crosbyae* also effective for establishment of *E. tiehmii* transplants, and 3) how did survival vary between transplant sites?

3.2 Methods

Greenhouse propagation of transplant seedlings

We planted a total of 3,276 seeds from five *E. tiehmii* sub- populations in white SC7 “cone-tainers” (3.8 cm diameter, 14 cm depth) on January 7-8, 2020. Seeds were field collected by Comstock Seed (Gardnerville, NV) in July 2019 and stored at the Rae Selling Berry Seed Bank (Portland, OR) until use. Two seeds were planted per pot; pots were filled with a 50/50 mix of field soil collected from un-occupied areas of ERTI6, and washed 0.95 cm (3/8”) decomposed granite. Soil from sub-population ERTI6 was chosen for this test because it is a large site that supports a high density of *E. tiehmii* plants and has lower levels of challenging soil components than other sites, which we expected to be conducive to greenhouse propagation efforts. Prior to mixing, field soil was sifted to < 1.3 cm, and field soil and decomposed granite were then homogenized using a tarp-rolling method.

Seedlings were watered every 2-3 times a week, as needed, and monitored for emergence weekly from January 7- February 11, at which point seedling emergence declined dramatically. After that, pots were monitored monthly until transplanting. Greenhouse conditions were set to mirror seasonal conditions in the Great Basin, within the boundaries of a minimum nighttime temperature of 2.2°C (36°F) and maximum daytime temperature of 12.8°C (55°F) from January 7- March 15, and maximum of 15.6°C (60F) from March 15 until they were moved outside into a sand-bed for hardening off on April 13. Seedlings were hardened off by placing them outside into a sand bed (prevents roots from freezing but allows seedlings to be exposed to cold temperatures) for two weeks prior to transplanting in the field.

Transplant experiment installation and monitoring

Three unoccupied sites were chosen for transplanting, based on soil type and accessibility (Appendix 1; Table 5). Site choice was made after field soil collection but before soil chemistry and texture analyses were completed, and at a time when we had seedling emergence, but not survival or biomass data from Activity 2. Therefore, transplant sites were selected based on the pre-existing habitat suitability models, seedling emergence information from Activity 2, and accessibility for experiment installation and repeated watering. A total of 958

surviving seedlings were transplanted into the wild on April 27-29, 2020. Prior to planting, 287 terra cotta pots (20.3 cm/8" diameter and height) were buried in the three transplant sites. The holes in the bottoms of the pots were plugged with a waterproof, adhesive silicone sealant, and the rims were painted white with an eco-friendly paint to help reduce evaporation. Pots were spaced in a grid approximately 1.2 meters (~4 feet) apart, and buried with just the rim remaining aboveground. Seedlings were then planted in the soil approximately 2.5 cm from the outside edge of each pot at 3 sites, with 3 container plugs planted at each pot. Some plugs had 2 seedlings in them, and we did not separate them in the field, as our goal was to minimize root disturbance. In these cases, each seedling was monitored separately. Seedlings were watered in, and the terra cotta pots buried beside them were filled with water, then each pot was covered with aluminum lids weighted down by rocks. Three small holes were punched in the top of each lid using nails, which allows any rainwater to collect inside the pots. The pots were filled with water bi-weekly, and seedlings were monitored monthly from April 29- June 8, then monitored and watered biweekly until July 6, 2020. At each monitoring, we noted whether plants were green, present but brown, or absent.

After a major herbivory event in July, a variety of herbivory exclosures were installed over surviving seedlings July 12, 2020, pinned to the ground surface by U-shaped stakes. These exclosures included hamster cages with bars >2.5 cm apart, open-topped metal cones made of 0.6cm mesh, large fine-meshed metal strainers (20cm diameter), small fine-meshed metal strainers (15.9cm diameter), and small fine-meshed plastic strainers (13.3cm diameter). These were intended to be a rapid test of whether and which types cages could effectively reduce seedling herbivory, as well as get information on the general size of the herbivore, by varying mesh size. Final monitoring took place on July 21, 2020.

Table 5: Transplant site locations, rationale for site choice and other site characteristics, and planting information. All sites were between 0.6-1 km from existing sub-populations (~0.6 km, ~0.8 km, and ~0.95 km from the closest sub-population, which is ERTI1), and were selected by considering soil and vegetation characteristics, emergence in our soil preference trial, and accessibility for planting and watering.

Site	Latitude/Longitude (CRS: WGS84)	Rationale for site choice, other site characteristics	# Seedlings planted	Number of terra-cotta water pots	Average # seedlings planted per pot
PTS-C	37.82373, -117.84908	Identified as potential habitat by habitat model, moderate vegetation cover. Some areas of clay, steeper slope, SW aspect	325	100	3.25
PTS-A	37.82458, -117.84815	Lithologic unit same as largest *E. tiehmii* sub-populations. Moderate slope, N aspect	338	100	3.38
S3-Rock	37.82458, -117.84815	A soil type with high seedling emergence in greenhouse trials. Gentle slope, S aspect	295	87	3.39

Data analysis

Summary statistics were calculated using R and Excel, and we calculated percent daily mortality for each site as : (1- # Seedlings at end/# Seedlings at start)*100. We estimated mortality rates using the counts of green plants, rather than plant presence, because while the leaves of dying seedlings quickly turned brown, remnant stems and leaves seemed to be able to persist in the dry climate near Rhyolite Ridge for an indeterminate amount of time. We did not expect these plants to resprout, so they were not counted in our survival estimates. We used analysis of variance with Tukey's Honestly Significant Difference for multiple comparisons to ask how survival prior to the herbivory event differed between sites.

3.3 Results

We found that *E. tiehmii* seedlings can be grown in a greenhouse environment, and that seedlings propagated with our methods were hardy enough to survive initial out-planting. Overall, 32% of all seeds planted in the greenhouse emerged (1,057 seeds, Table 6), and of those, 958 (90.6%) were mature enough (>2 leaves) for transplanting to the wild by the end of April (Table 5). There were differences in emergence among sub-populations: seeds from ERTI4 had the highest emergence at 48%, and seeds from ERTI2 had the lowest at 27.8% (Table 5). Fifty-nine seedlings that were too small at the time of transplanting were still alive at the UNR greenhouse as of 10/21/2020.

Table 6: Summary of *E. tiehmii* transplant seedling emergence in the greenhouse. Seeds were planted into a 50/50 mix of field soil from ERTI6 and washed decomposed granite.

Sub-Population	Total seeds planted	Total emergence	% Emergence
ERTI1	710	205	28.9
ERTI2	1,002	279	27.8
ERTI3	718	253	35.2
ERTI4	204	98	48.0
ERTI6	642	246	38.3
TOTAL	**3,276**	**1,057**	**32.3**

There were differences in transplant survival among sites at some, but not all, time points. Seedlings from all three sites perished at a similar rate between installation at the end of April and the first monitoring event on 5/11/2020 (Fig. 15A), likely representing accidental seedling damage, installation too far from the edge of the water pots, or inhospitable conditions at the installation site. The percent daily mortality at PTS-C was the highest, at 0.6% per day. This was 4 times higher than the daily mortality rate at PTS-A (0.15% per day). The daily mortality rate at S3-Rock was 0. 28%, which was moderate (1.87 times higher than the rate at PTS-A). Cumulative survival probability (Fig. 15B) was consistently the lowest in PTS-C prior to the herbivory event, and cumulative survival probability was the highest and most constant at PTS-A. After the initial transplant shock, seedling mortality rates slowed at PTS-A and S3-Rock between 5/11 and 6/22, but continued to decline at PTS-C. On 6/22, the greatest survival was at the PTS-A site (83.1%), followed by S3-Rock (70.8%) and PTS-C (47.4%).

There was a major herbivory event between 6/22 and our last census on 7/6/2020. At that point, survival was low at all sites, though S3-Rock had the greatest number of remaining plants (40).

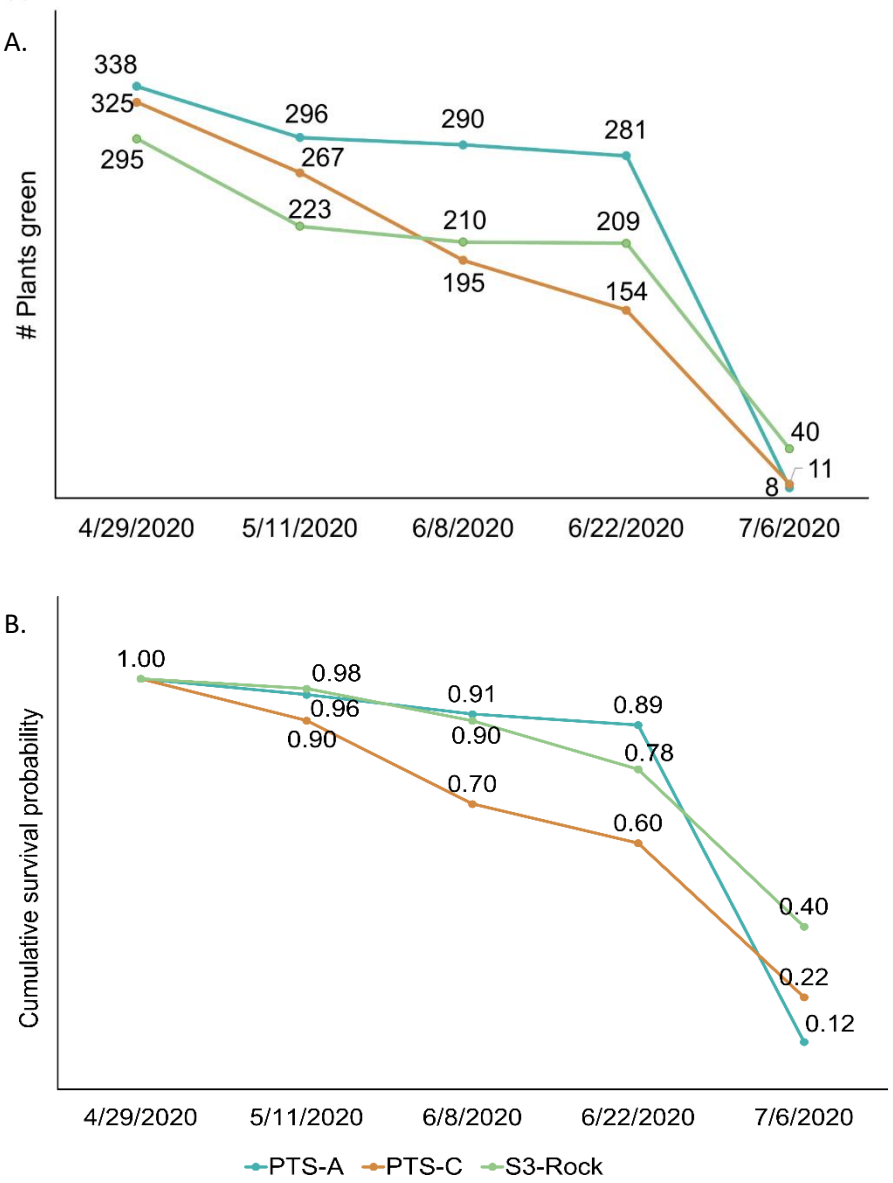

Figure 15: (A) Number of green *E. tiehmii* seedlings present in each of three field transplant sites at each monitoring date through 7/6/2020. (B) Cumulative survival probability at each site over time; the probability that an individual from each site will still be alive at each time point. Multiplying the points in panel B by 100 There was a major herbivory event during the 14-day period between monitoring events on 6/22/20 and 7/6/20 where 585 plants were lost.

After the herbivory event and subsequent installation of a variety of exclosures, 59.1% (13/22) of seedlings that were protected in some manner survived until 7/21/2021, our last visit to monitor these plants. No uncovered seedlings or seedlings under hamster cages survived the same period (Figure 16). Sample sizes were very low, so results should be interpreted with caution, but survival was highest under small plastic strainers, followed by small and large metal strainers. Hamster cages with larger openings were ineffective at excluding herbivores.

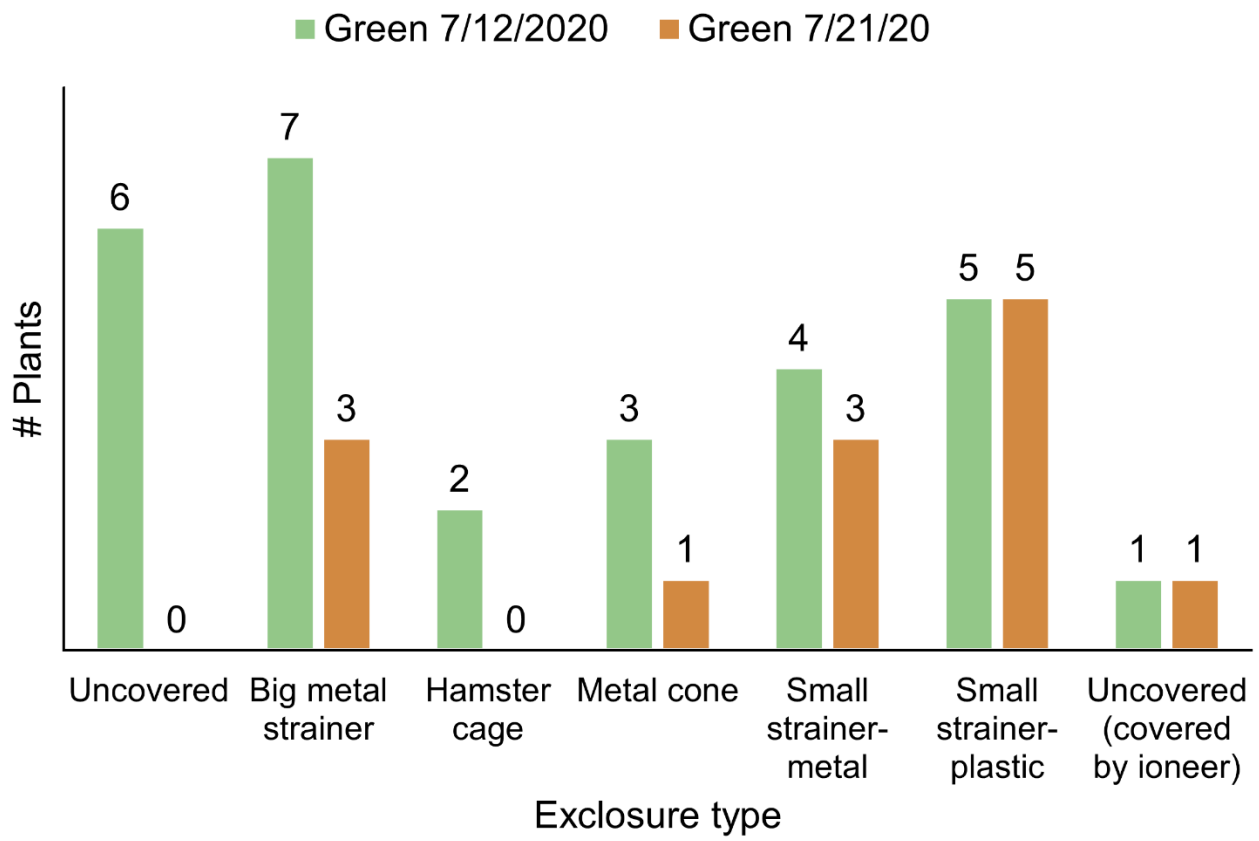

Figure 16: *E. tiehmii* survival under a variety of different herbivore exclosures. Green plants were covered on 7/12/2020 with a variety of exclosure types, and resurveyed for survival on 7/21/2020, with sample sizes (numbers above the green bars) driven by seedling and exclosure availability.

3.4 Discussion

Overall, *E. tiehmii* seeds had higher than expected germination in the greenhouse (we expected viability of ~16% based on seed viability tests performed by the Nevada Dept. of Agriculture; Kris Kuyper, pers. comm.), and propagation of seedings in field soils produced a robust set of seedlings. Survival within the *E. tiehmii* transplant sites over the first two months was encouraging, and comparable to that obtained at our *E. crosbyae* transplant sites over the same period. After two months of growth in the wild, and before the major herbivory event, 62.2% of all the seedlings planted at our *E. tiehmii* sites were still present and green, compared with 65.2% that were present and green at the *E. crosbyae* site over the same amount of time. However, this early success was superseded by a major herbivory event that occurred between June 22 and July 6, 2020. Small holes were dug into the slopes around the bases of our terra cotta pots, and plants were either totally excavated or their stems were severed, resulting in the loss of 585 plants. In our previous work with *E. crosbyae,* none of the seedlings in our transplant experiment were lost to herbivory, and so installing herbivore exclosures over seedlings was not part of our experimental protocol. A very small trial at the end of this transplant experiment indicated that seedlings may be protected for short periods of time with relatively simple cages. A similar major herbivory event occurred at the natural *E. tiehmii* sub-populations in late August or early September of 2020; thousands of mature plants were excavated in a manner consistent with the digging of small rodents, and roots were chewed and often completely severed (McClinton, unpublished observations). Based on these two events, it is possible

that biological interactions with herbivores are a major factor in the ecology of this plant, and devising ways to mitigate their impacts will be key for any planting efforts.

Even though herbivores ultimately reduced differences in performance among transplant sites, there were differences in plant performance among sites before the herbivory event that can give insights into how differing environmental conditions affect transplants. After the first monitoring, survival declined the most quickly at PTS- C, which also had the steepest average slope and most westerly aspect of all three sites. The more extreme slope and west-facing aspect gave this site the greatest potential for water runoff away from seedlings and the most exposure to heat from the afternoon sun. In contrast, survival was highest at PTS-A, with over 80% survival of juvenile plants after two months, where seedlings were planted into a north-facing aspect with a more moderate slope. Average clay content and estimated plant-available soil water capacity was also slightly higher at PTS-A than at PTS-C. PTS-A was the same site that had the second highest emergence in our greenhouse study; however, it also had the third lowest seedling survival of the 21 soil types we tested in the greenhouse, highlighting the need for additional work on seedling growth in these soils under field conditions. Soil collection was performed prior to transplant site choice, so we do not have soil data for our exact S3-Rock transplant site, but another similar site we sampled (near sub-population ERTI 3) was much sandier in texture and had a lower plant-available water capacity than PTS-A or PTS-C. High early rates of attrition in PTS-C, which was selected with a habitat model, suggest that not all locations identified as potentially suitable habitat are capable of supporting *E. tiehmii*, and that slope, aspect, and basic soil properties are all important considerations for transplant survival. Finally, we note that, due to the simultaneous nature of activities 2 and 3, our site choice was not fully informed by our current understanding of ideal chemical and physical properties of *E. tiehmii* habitats. By chance, the sites we selected were outside of the envelope of existing habitat conditions (Fig. 10), and it is possible that success would be greater, for either biological or physical reasons, at sites more similar to existing habitat, such as PTS-G.

Eriogonum seedlings have fragile roots, and propagation in field soil made the installation of plugs challenging, which is one reason some growers use more cohesive, high-organic matter growth mediums. However, high early survival after the first monitoring event suggests that the benefits of growing hardier seedlings in field soil outweighed the drawbacks of plug fragility. Our soil preference experiment showed that seedlings grown in soil collected from sites inhabited by *E. tiehmii* in the wild developed higher root allocations and total biomass than those grown in soils from unoccupied sites, which would likely increase transplant success. Further, it is possible that there would be less moisture evaporation from plugs composed of field soils, which can blend more fully into the surrounding matrix, unlike standard potting mixtures that are typically high in peat and organic matter. Due to sensitivity of this plant to soil conditions and the known association between high root allocation and survival in native plants (Leger and Baughman, 2015), using *E. tiehmii* habitat soils for propagation is recommended over attempting to use a more conventional greenhouse growth medium.

We covered our transplant pots with aluminum lids, even though full terra cotta lids are available. The aluminum lids have the advantages of allowing for rainwater collection during storm events, which could provide a long-term benefit to plants. However, they did not appear to be durable enough to prevent rodent access at this site, as rodents chewed large holes through many of the aluminum lids placed over our terra-cotta water pots. In contrast, at our *E. crosbyae* transplant site, aluminum lids had no damage after two seasons, including the 2020 season (McClinton and Leger, pers. obs). At that site, aluminum lids were in place for two growing seasons, after which we filled the pots with large gravel (Fig. 17) and removed the lids. We made this change to avoid removing the pots altogether, which could have damaged any transplant roots that grew around the pot, while still allowing for rainwater collection and preventing access to the pot interior by rodents,

reptiles, and large invertebrates. Although we have not tested the effectiveness of whether pots filled with large gravel would still hold enough water to benefit seedlings, such a method could be considered instead of using aluminum or terra cotta lids in areas where rodents pressure is high.

Fig. 17: Successful transplants of a different species, *E. crosbyae,* after two growing seasons, showing the large gravel-filled pots. Gravel was installed to permanently prevent access to the pot interior by rodents, reptiles, and large invertebrates while still allowing for rainwater collection.

To get some basic information about herbivore size and test practical ways to exclude them, a variety of exclosures were placed over surviving plants soon after we observed the herbivory event. All methods involving a fine wire mesh seemed effective at preventing further seedling losses to herbivores, at least over a short period of time, and placing these exclosures on seedlings at the time of transplant may result in higher survival. Larger mesh cages did not prevent herbivory, and preliminary herbivore observations by Susan Fox at Wildlife Resource Consultants LLC identified a variety of small rodents in the vicinity of the transplant sites that may have contributed, including deer mice (*Peromyscus maniculatus*), Merriam's kangaroo rat (*Dipodomys merriami*), northern grasshopper mouse (*Onychomys leucogaster*), brush mouse (*Peromyscus boylii*), and the white-tailed antelope ground squirrel (*Ammospermophilus leucurus*).

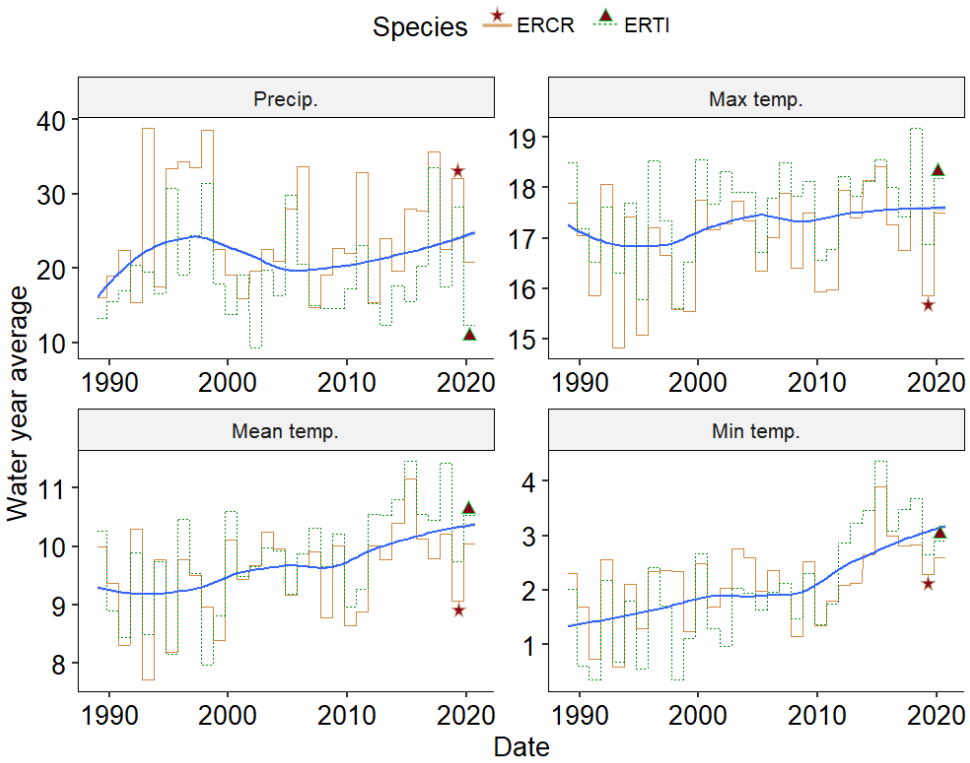

Figure 18. Water- year averages for total precipitation (cm), maximum, minimum, and mean temperatures (°C) for the 30 years prior to planting for *E. crosbyae* and *E. tiehmii*. Averages for *E. crosbyae* are shown as an orange line, and averages for *E. tiehmii* are shown as a dotted green line. The loess- smoothed trendline is in blue, and shows a clear upward trend in mean and minimum temperatures at both sites since 1989. The transplant experiment planting date for *E. crosbyae* is indicated by stars, and the planting date for *E. tiehmii* is indicated by triangles. Example: E. crosbyae was planted in the 2019 water year, when average precipitation was much higher than the 30-year trend. Conversely, when *E. tiehmii* was planted in the 2020 water year, precipitation was far below the 30-year average. Water years are calculated as average conditions between October and September; e.g. the 2019 water year includes data between October 2018 and September 2019.

Although we do not have data on herbivore populations or composition at our *E. crosbyae* site, the lack of herbivory there and extensive herbivory at our *E. tiehmii* sites may be partly due to differences in climatic conditions surrounding our transplant events. Climatic conditions at our *E. crosbyae* transplant site during the 2019 hydrologic year (Oct. 2018-Sept. 2019) were much more mild than those present at the *E. tiehmii* transplant sites during the 2020 hydrologic year (Oct. 2019- Sept 2020) (Fig. 18). Rhyolite ridge experienced precipitation far below the 30-year average during 2020, accompanied by higher maximum and mean temperatures than the average, and higher minimum temperatures than those experienced by the *E. crosbyae* transplants. In contrast, our *E. crosbyae* transplants were installed during a year with higher than average precipitation, and lower than average maximum, mean, and minimum temperatures. The conditions present around Rhyolite Ridge in 2020 were comparatively harsh, and followed a year with lower temperatures and higher precipitation that would have encouraged overall plant growth and likely boosted local herbivore populations. High herbivore abundance combined with unusual heat and drought in 2020 may have contributed to the large herbivore impacts at both the transplant sites and the natural populations.

Climate change is likely to continue causing rising temperatures and higher than normal variability in interannual precipitation in the Great Basin (Wagner, 2003), and this may also result in changes to herbivore communities (Previtali et al., 2009). In light of this, if this experiment were to be repeated in the future, we would recommend that several methodological changes be made: 1) install plastic or wire mesh domes over seedlings at the time of planting to prevent access by herbivores and partially shade seedlings, and 2) replace the aluminum lids with terra cotta lids or fill pots with large gravel at the time of planting. Using terra cotta lids would have the downside of preventing these pots from collecting rainwater, and gravel would require that pots be filled more frequently, but both efforts would likely prevent herbivores from accessing this resource.

Activity 4: Demography

4.1 Introduction

Understanding the mechanisms controlling changes in the abundance of plants is critical for developing effective conservation and management strategies for rare or threatened species (Heywood and Iriondo, 2003). Declining abundance can be driven by both declining recruitment of new plants and increasing mortality of existing ones. In addition, because individual-level reproductive output and survival are often controlled by plant size, individuals may contribute differently to overall population-level reproduction and mortality depending on their size. Thus, changes in population-level abundance can also depend on population size structure and individual-growth rates.

Structured population models (matrix or integral projection models) are the primary tool for understanding how individual reproduction, survival, and growth lead to changes in population abundance when demographic rates vary as a function of plant size (Caswell and Caswell, 2001; Morris and Doak, 2002). In their simplest forms these models break populations down into size classes where rates of recruitment, reproductive output, survival, and growth to a new size class are estimated using field measurements of tagged individuals within each size class. These estimated demographic rates can then be used to forecast changes in population size and structure (i.e. the number of individuals in each size class) using additional count data to validate forecasts and estimate process uncertainty due to demographic processes omitted because they are difficult or impossible to directly measure in the field (e.g. seed bank dynamics) (Plard et al., 2019).

In addition to being tools to forecast future population trends and risks, demographic models can identify key life stages driving population trend, help set targets for management strategies, and quantify the mechanisms that enable population stability for rare plants (Dibner et al., 2019). Finally, demographic models can be valuable tools to estimate life history parameters that would take many years or decades to directly measure, including average lifespan and time to first reproduction, by using data on multiple individuals over a smaller number of years (Cochran and Ellner, 1992).

In the early summer of 2020, we began field monitoring of demographic rates and abundance of extant population of *Eriogonum tiehmii* (ERTI)*,* following work initiated by EM Strategies, with the ultimate goal of developing integrated, structured population models to forecast population trends, determine the sensitivity of population growth to variation in demographic rates, and estimate life history parameters. The methods, results, and discussion below summarize the first year of this work.

4.2 Methods

In mid-June 2020, we relocated monitoring transects established by EM Strategies in *E. tiehmii* sub-populations 1, 2, 3, 4, 6 (A &B) in the spring of 2019 (See Appendix 1 for map). Transect lengths varied based on sub-population area, but all were one-meter width. A subset of transects established in 2019, including in sub-pops. 1,2, and 3 contained *E. tiehmii* plants that were individually tagged with unique identification numbers for demographic measurements. In addition, we added two demographic monitoring transects in sub-pop. 6A by locating and tagging all *E. tiehmii* plants within a portion of two existing transects.

For each tagged plant in the demography transects, we quantified individual size by assuming each plant is an ellipse and measuring the major axis and perpendicular minor axis across the plant and then visually estimating percent of the full ellipse that was missing. We quantified reproductive output by counting the number of inflorescences present on each plant. Finally, in transects with plants that were tagged in 2019, we searched for, tagged, and measured new recruits and plants that were previously missed across the entire transect and noted

previously tagged plants that died between 2019 and 2020 censuses. We modified size measurement approaches in 2020 from those used by EM Strategies in 2019, thus 2019 size is not directly comparable to 2020 size to estimate growth.

On the remaining non-demography monitoring transects, we slowly walked the entire transect and counted the number of *E. tiehmii* individuals present. This abundance data acts as an additional source of data to develop integrated population models using both individual-level demographic data and observed changes in population-level abundance. However, it is important to note that population counts do contain observation uncertainty, which is ubiquitous in count data (e.g. errors due to plants that are missed or small variation in transect tape placement) (Clark and Bjornstad, 2004). Thus, changes in observed counts from 2019 to 2020 represent both real changes in abundance due to inter-annual variability or long-term trends, as well as variation due to observation uncertainty. Typically, several years of count monitoring or marked individuals are needed to sperate the effects of observation uncertainty from ecological processes. Finally, all transect counts took place in mid-June, before the late-summer ERTI morality event.

Table 7: Number of demography and abundance count transects in each *E. tiehmii* (ERTI) subpopulation, performed in June 2020.

Sub-pop.	Transect type and number	
	Demography	Count
ERTI1	4	2
ERTI2	4	5
ERTI3	5	0
ERTI4	0	5
ERTI6A	2	5
ERTI6B	0	5

4.3 Results

Abundance

Relative to 2019 counts, we found approximately 100 fewer plants in sub-populations 6B and 4, and about 100 more plants in sub-population 2 (Fig. 19). We also found more modest increases and decreases in plant counts in sub-population 1, 3, and 6A (Fig. 19). Of the individuals tagged in 2019 and relocated in 2020, 1.4 % (4 of 281 individuals) died between the 2019 and 2020 census. 3 of the 4 dead individuals were in sub-population 3.

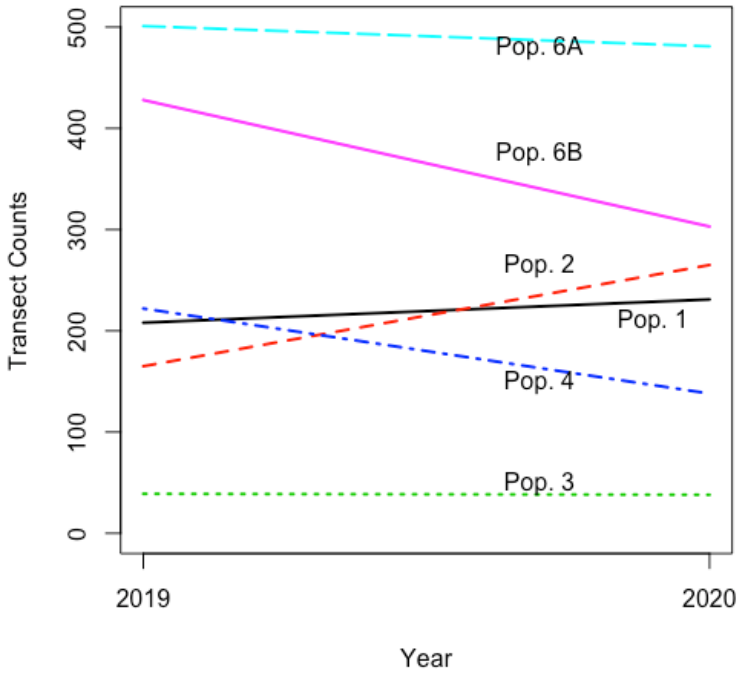

Figure 19: Total abundance of individuals in count and demography transects in 2019 and 2020.

Population size structure and cover

Using data on 2020 plant size from demography transects, we found that sub-pops. 1 and 6A contained a greater proportion of larger individuals ~10-100 cm^2 (Fig. 20). While sub-pops. 2 and 3 contain a comparatively greater proportion of smaller individuals.

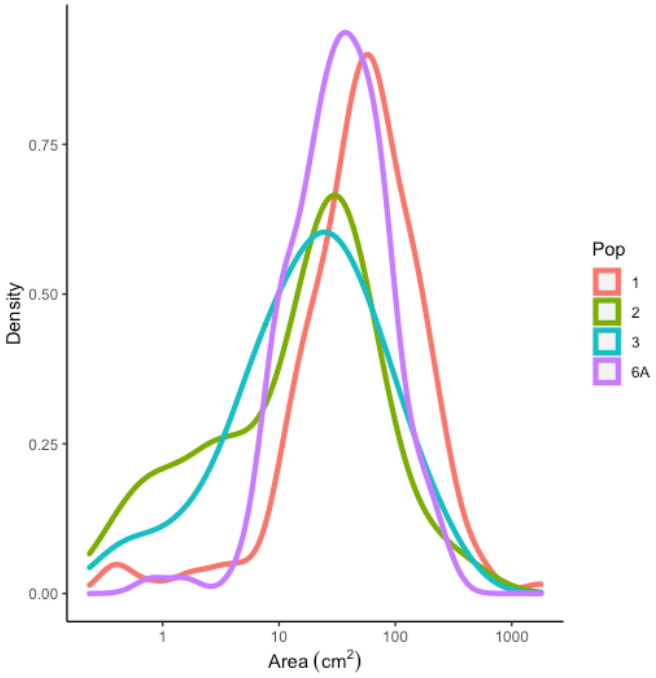

Figure 20. Distributions of individual plant sizes of populations 1, 2, 3, and 6A in 2020.

Reproductive output

Using Poisson generalized linear models fit to each population, we found that reproductive output (number of inflorescences) increased notably with size. No individuals smaller than ~6 cm^2 were found with inflorescences, while larger individuals (e.g. 100 cm^2) produced ~10-15 inflorescences on average (Fig. 21). Sub-pops. 1, 3, and 6A had nearly the same individual reproductive output as a function of plant area, while individuals in sub-pop. 2 produced, on average, fewer inflorescences at the same plant area.

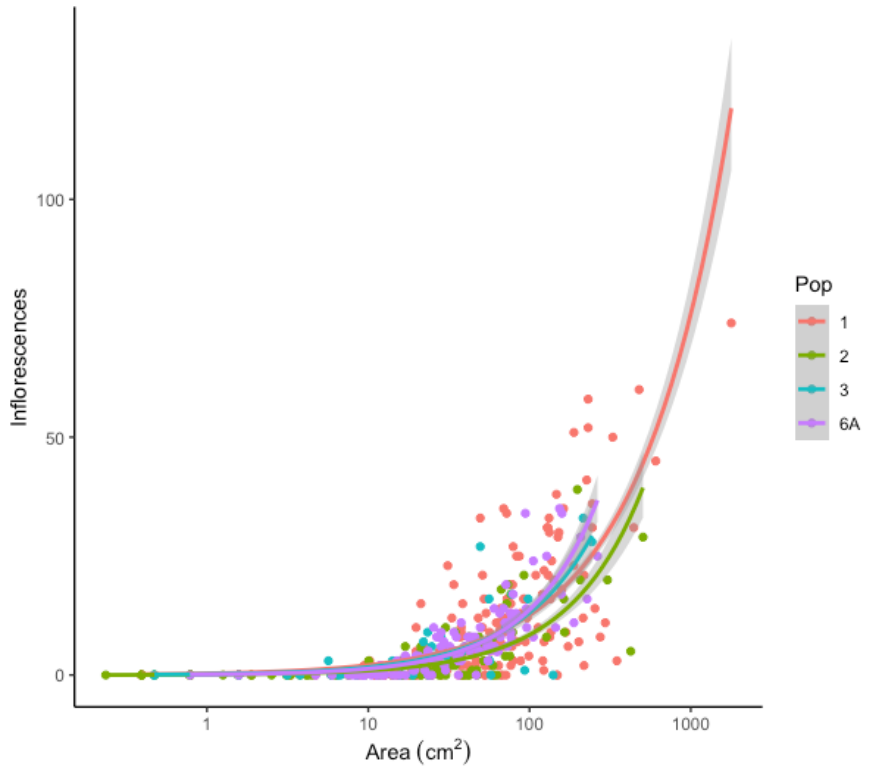

Figure 21. Reproductive output (inflorescences) in populations 1, 2, 3, 6A as a function of plant area in 2020. Lines represent mean from a Poisson GLM fir to each population with shaded 95% confidence interval

4.4 Discussion

We found both increases and decreases in ERTI abundance in different subpopulations from 2019 to 2020. Notably fewer individuals were counted in sub-populations 6B and 4 in 2020 than 2019, while we noted some increases in abundance in populations in sub-populations 1 and 2. While very little death was observed, sub-population 3 had the greatest proportion (3/4) of dead plants. Changes in observed counts from 2019 to 2020 represent both real changes in abundance due to inter-annual variability or long-term trends and variation due to observation uncertainty (Clark and Bjornstad, 2004). While neither sub-populations 6B nor 4 include transects with marked plants, the rates of population decline observed within these transects far exceed the mortality rate (1.4%) observed within the demography transects, suggesting that either observation uncertainty or large differences in population dynamics among sites could explain changes in abundance in 4 and 6B. Continued count monitoring and establishment of demography transects within sub-population 4 and 6B would help tease

apart the effects of site variability and observation uncertainty. While quite preliminary, it is interesting to note the correspondence between the results we observed here and the results of seed and seedling performance in the greenhouse in Activity 2. For example, ERTI 2, which showed population increases in the field, had the second highest growth index in the greenhouse, and ERTI3 and ERTI6B, which had the two lowest growth indexes of occupied sites in the greenhouse, showed either higher death (ERTI3) or population declines (ERTI6B) in extant populations.

Inflorescence production increased with plant size in all populations, indicating that larger individuals contribute more to reproduction within the population. Despite large average differences in reproductive output among individuals of different sizes within populations, there was very little difference in size-specific reproductive output among sub-populations, except for slightly lower inflorescence production rates for a given size in sub-pop. 2. However, individuals in sub-population 1 produced the most inflorescences per individual due to the higher abundance of large *E. tiehmii* individuals in sub-population 1.

Continued monitoring of demographic rates would provide the size-specific growth and survival rate data needed to parameterize structured population models. These models can be used to estimate and forecast population trajectories, analyze the sensitivity of population growth rate to changes in different demographic rates (e.g. mature plant survival), and estimate *E. tiehmii* life history parameters (e.g. lifespan, time to first reproduction).

Overall summary and conclusions

With this work, we filled multiple knowledge gaps about the ecology of *E. tiehmii*, including describing biotic and abiotic characteristics of occupied and unoccupied sites, understanding the role of pollinators in seed set, testing plant growth in a variety of field soils in the greenhouse and in field transplant settings, and the first observations of differences in population dynamics among *E. tiehmii* sub-populations.

We used a combination of pitfall traps, flower observation, and pollinator exclusion to assess the abundance and diversity of arthropod communities in *E. tiehmii* habitat, the most common visitors to *E. tiehmii* flowers, and the importance of pollination for seed set. We sampled arthropod diversity and flower visitation at two *E. tiehmii* sites (ERTI1 and ERTI6A), and two adjacent non-*E. tiehmii* sites (NT.1, NT.6A) at the beginning and peak of the flowering season, and tested the effects of pollinator exclusion on seed set.

We found an abundant and diverse arthropod community within and around both *E. tiehmii* and non- *E. tiehmii* sites. Each site contained unique species (6.25-16% of species were found only at one site), and there was high turnover in arthropod community composition over time as well as among sites. *E. tiehmii* sub-population 6 had the greatest biomass of arthropods collected out of any site on a single sampling date (172% greater than the next highest site), and diversity at that site increased over time, while it remained relatively steady at *E. tiehmii* sub-population 1, and declined at the two non-*E. tiehmii* sites. The total number of pollinator visits observed was higher at *E. tiehmii* sub-population 1 than at *E. tiehmii* sub-population 6, but sub-population 6 had the highest seed production/m^2. Open-pollination significantly increased *E. tiehmii* seed production, and the most important pollinators are likely beetles, wasps, and flies. The presence of similar volumes of insects, species richness, overall diversity, and flower visitation in both habitat types, despite differences in plant species richness and cover density between the two, suggests that *E. tiehmii* substantially contributes to supporting arthropod diversity and abundance in the Rhyolite Ridge area.

We analyzed soil samples collected from 21 occupied and unoccupied sites to assess the physical and chemical composition of *E. tiehmii* habitat soils, and set up a greenhouse soil preference experiment to test how seedlings respond to soil variation. Occupied sites were, overall, higher in boron, silt, bicarbonate, and pH, and lower in potassium, zinc, sulfur, and magnesium than unoccupied sites, but there was considerable variation and overlap between occupied and unoccupied sites for some soil characteristics. The soil preference experiment revealed that, on average, seedlings grown in soils from occupied sites had 81% higher root biomass, 19% higher root allocation, and 53% higher total biomass than seedlings grown in soils from unoccupied sites. There was also a significant positive association between emergence and survival in occupied soils, with no such trend in unoccupied soils.

Seedlings responded to different components of soil variation at different life stages: they were sensitive to sand, manganese, and aluminum during emergence, and to sulfur, phosphorus, zinc, organic matter, and copper during later survival and growth. Many of these soil variables were highly correlated with other unreported variables, and further physiological work could elucidate the mechanisms behind effects of particular soil properties on plant growth, as well as consider whether and how underlying geologic units predicted performance. We created a growth index to examine performance in each soil type overall, and found that while seedlings performed well at some life stages in unoccupied soils, none of the unoccupied soils we tested were as well-suited to growth across all life stages as the best occupied soils. Additional work is needed to determine how seedlings fare in these soils under more natural moisture conditions and in the presence of competition.

We tested the viability of greenhouse propagation and seedling transplants to a variety of potentially suitable habitat locations, using methods that were promising for *E. tiehmii*'s best-studied relative, *Eriogonum crosbyae*. We found that it is possible to propagate *E. tiehmii* seedlings in the greenhouse, and that growing them in field soils from occupied habitat promotes high root allocation, which was likely beneficial for transplant survival. Early transplant survival was promising, and overall comparable to that observed in our experiments with *E. crosbyae.* There were some early differences in transplant performance among sites, with seedlings planted on a sparsely vegetated, moderately-sloped, north-facing aspect in relatively higher-clay soils (PTS-A) performing best. Unfortunately, the experiment was cut short by a major herbivory event that resulted in the loss of 585 plants in a two week period between monitoring trips. Herbivory was never observed at our *E. crosbyae* transplant site; therefore, herbivore exclosures were not part of our experimental protocol. However, inherent differences in the ecosystems of these two sites and in the climatic conditions surrounding planting likely account for this change, and pose unique challenges for seedling transplants at this site. Although early transplant results were promising, longer-term monitoring and installation of herbivore exclosures at planting would be required to determine whether these methods truly have potential to establish self-sustaining populations, at the existing transplant sites or at new locations. Any search for potential habitat can now be guided by our increased understanding of the soil conditions that are conducive to plant growth at multiple life history stages.

We re-located 1-m wide monitoring transects in *E. tiehmii* habitat established by EM Strategies in Spring 2019 and monitored tagged plants for survival, size, and reproductive output. We also recorded total numbers of individuals present in designated count transects. We observed relatively larger changes in *E. tiehmii* plant abundance, both positive and negative, in sub-populations 2, 4, and 6B, and relatively smaller changes in sub-populations 1 and 6A. There were relatively greater proportions of larger plants in sub-populations 1 and 6A, and greater proportions of smaller plants in sub-populations 2 and 3. Reproductive output increased with plant size. Changes in counts between 2019 and 2020 could be due to both real changes in abundance as well as to observation uncertainty. A better understanding of this relationship, and data on size-specific growth and survival that is needed to create structured population models, could be acquired with continued monitoring and establishment of additional transects.

In conclusion, *E. tiehmii* substantially contributes to supporting the high abundance and diversity of arthropods and pollinators found in our sampling areas, with both sub-population 1 and sub-population 6 supporting high volumes of arthropods, species unique to those sites, and attracting high rates of pollinator visitation. Seedlings also demonstrated significant sensitivity to individual soil properties and growth trends that suggest a "specialist" model of soil specialization rather than a "refuge" model, indicating that they are not simply highly stress-tolerant, but that they are specifically adapted to grow best on their preferred soil types, which would be highly challenging for most plant species. This was borne out by the transplant experiment, where seedlings planted into a site whose properties most closely approximated their natural habitat had the highest early survival, but herbivore pressure precluded seedling survival at all sites. Overall, our work did not identify any unoccupied sites within the broader range of *E. tiehmii* that were ideal for growth across all life history stages, and herbivory posed unique challenges to the survival of seedling transplants, as well as to adult plants in this area. Additional demographic work would be required to untangle relationships between true changes in abundance and observational uncertainty, to estimate and forecast population trajectories (especially after the late-summer herbivory event), and to better understand different demographic rates and life history parameters in these sub-populations.

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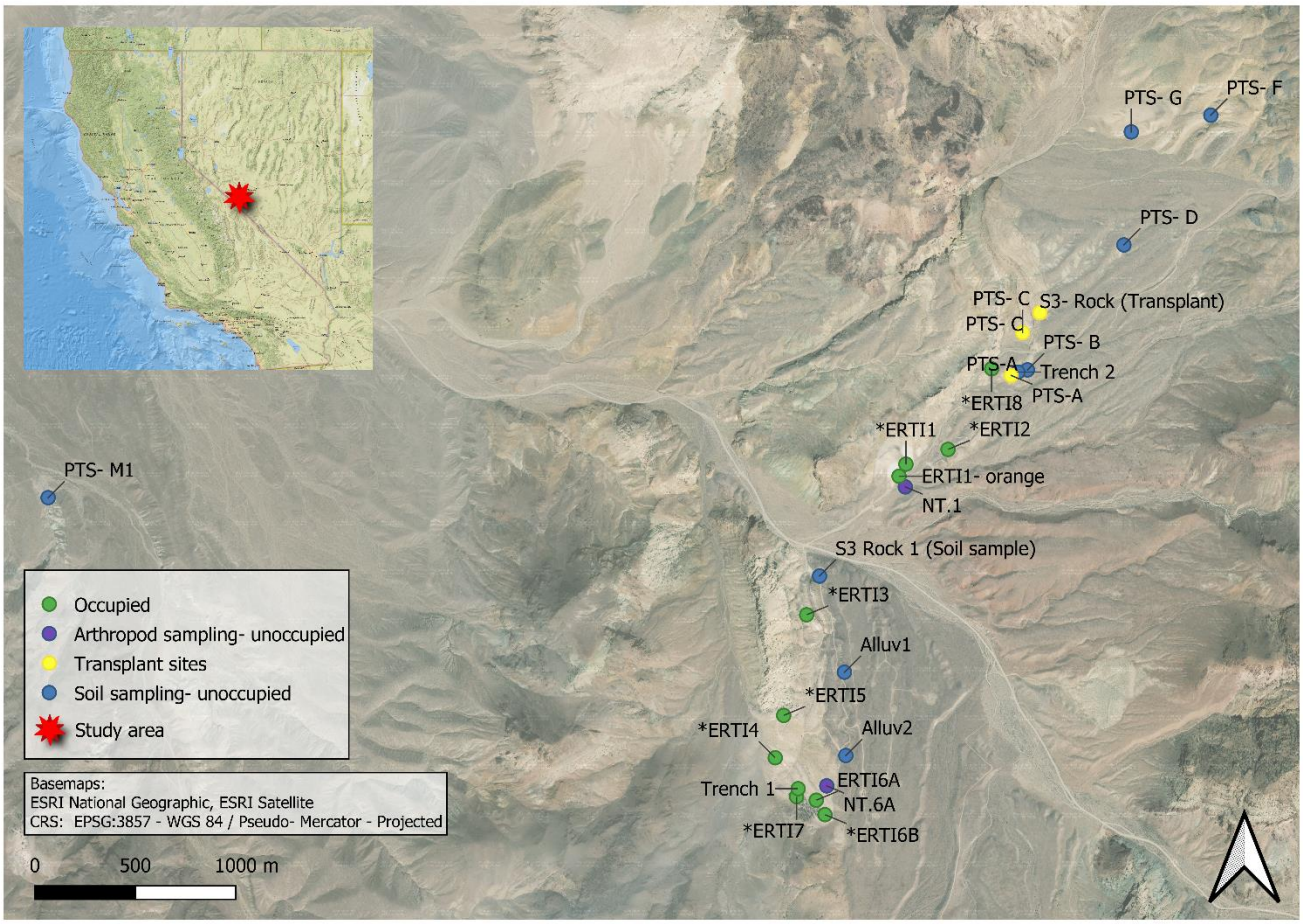
Appendices

Appendix 1: Map of arthropod sampling sites, soil sampling sites, transplant installation sites, and demography sampling sites.



Appendix 2: Table of soil sampling locations for soils used in the E. tiehmii *soil preference experiment.*

Site #	Site Name	Site Type	Latitude	Longitude	Additional information[a]	Other lithologic units present[a]
1	ERTI1	Occupied	37.81787	-117.85568	Lith. unit B5	M5, S5
2	ERTI2	Occupied	37.81852	-117.85330	Lith. unit M5	-
3	ERTI3	Occupied	37.80690	-117.86250	Lith. unit G6	B5, S5, L6
4	ERTI4	Occupied	37.80478	-117.86307	Lith. unit G5	M4, M5, B5, L6
5	ERTI5	Occupied	37.80667	-117.86259	Lith. unit M4	-
6	ERTI6B	Occupied	37.80224	-117.86026	Lith. unit B5[b]	S5, M5, G5, M4
7	ERTI7	Occupied	37.80304	-117.86186	Lith. unit L6	-
8	ERTI8	Occupied (very small population)	37.80224	-117.86026	Lith. unit L6	-
9	Trench1	Disturbed/Occupied	37.80340	-117.86177	Lith. units B5, M5, Disturbed site within ERTI6.	-
10	Trench2	Disturbed/Unoccupied	37.81785	-117.85434	Lith. unit M5, Disturbed site near ERTI1.	-
11	PTS-A	Disturbed/Unoccupied	37.82197	-117.84935	Disturbed site, in Lith. unit M5, farther from areas of activity	-
12	ERTI1-orange	Disturbed/Occupied	37.81733	-117.85607	Within boundaries of Lith. unit B5, ERTI1, very different soil color than rest of site	-
12	S3-Rock1	Unoccupied	37.80953	-117.85947	Lith. unit S3, may replicate overburden storage and quarry backfill	-
13	Alluv1	Unoccupied	37.80859	-117.85916	Lith. unit Qoa, may replicate overburden storage and quarry backfill, north of ERTI6.	-
14	Alluv2	Unoccupied	37.80488	-117.85908	Lith unit Qoa, may replicate alluvial backfill	-
15	PTS-M1	Unoccupied	37.81638	-117.90417	Accessible, identified as potential habitat by expert opinion, ioneer habitat area of interest. Lith. unit unknown.	?

Site #	Site Name	Site Type	Latitude	Longitude	Additional information[a]	Other lithologic units present[a]
16	PTS-B	Unoccupied	37.82207	-117.84881	Lith. unit M4; accessible, identified as potential habitat by habitat model, SE facing, minimal vegetation, near known range.	-
17	PTS-C	Unoccupied	37.82373	-117.84908	Lith unit G5, accessible, in ore zone, identified as potential habitat by habitat model, moderate vegetation, some clay.	-
18	PTS-D	Unoccupied	37.82764	-117.84336	Lith. unit S3, SE facing, slight vegetation, local minor gypsum.	-
19	PTS-F	Unoccupied	37.83342	-117.83844	Lith. unit S3, large area, identified as potential habitat by model and expert opinion, ioneer habitat area of interest.	-
20	PTS-G	Unoccupied	37.83299	-117.84287	Lith. unit S3, large area, identified as potential habitat by model and expert opinion, ioneer habitat area of interest.	-

[a] Lithhologic (Lith.) units B5, M5, G6, G5, M4, L6, S3, and Qoa refer to rock units present in *E. tiehmii* habitat, as mapped and named with internal alphanumeric codes, provided by John Reynolds at ioneer. Contact ioneer Ltd. for additional information; https://www.ioneer.com/contacts. We aimed to sample soils on each different lithology underlying various parts of *E. tiehmii* habitat, to ensure that we captured all variation in soil characteristics in occupied habitat.

[b] Aimed for S5, but missed in the field.

Appendix 3: *Table of average values, Welch's t-test results (including confidence intervals (Conf. Int.), t-test statistics (t.stat), degrees of freedom (DF), and p-values (p-val.)), and Wilcoxon rank sum test p-values for comparisons of plant growth and soil variables in soils from sites unoccupied (Unocc., N=11) or occupied (Occ., N= 10) by* E. tiehmii *with units, laboratory analysis methods for soil variables. Bolded p-values indicate significant differences in occupied and unoccupied sites, with and without Bonferroni-corrected p-values ($p_{adj.}$), accounting for multiple comparisons*

					t.test results					Wilcoxon rank sum test statistics	
Variable[a]	Unit	Method	Unocc.	Occ.	Conf. Int.[b]	t.stat	DF	*p*-val.	$p_{adj.}$	*p*-val.	P_{adj}
Days lived	days	NA	67.72	68.93	(-9.96, 12.38)	0.21	277	0.8311	1	-	-
Days until emergence	days	NA	24.85	26.07	(-1.55, 3.98)	0.86	282	0.3884	1	-	-
RMR	-	calculated	0.53	0.63	(0.07, 0.14)	5.43	120	**<0.0001**	**<0.0001**	-	-
Root weight	mg	NA	48.93	88.76	(23.43, 56.24)	4.82	105	**<0.0001**	**<0.0001**	-	-
Shoot weight	mg	NA	39.43	46.35	(-0.33, 14.17)	1.89	117	**0.0612**	0.3672	-	-
Total biomass	mg	NA	88.36	135.12	(24.71, 68.81)	4.21	105	**0.0001**	**0.0006**	-	-
Al.ppm.SP	ppm	Saturated paste	1.09	0.54	(-1.5, 0.4)	-1.18	42	0.246	1	0.54	1
B.ppm.DTPA.extr	ppm	DTPA-Sorbitol	8.02	38	(14.51, 45.45)	3.91	42	**0.0003**	**0.0126**	**0.0033**	0.1386
B.ppm.SP	ppm	Saturated paste	4.65	37.93	(15.88, 50.69)	3.9	31	**0.0005**	**0.021**	0.4327	1
Bicarb.SP	meq /L	Saturated paste	126.15	152.33	(6.4, 45.96)	2.67	45	**0.0106**	0.4452	**0.0221**	0.9282
Ca.meq.L.SP	meq /L	Saturated paste	185.15	77.29	(-178.72, -37.01)	-3.05	55	**0.0035**	0.147	**0.0244**	1
Ca.Mg.ratio.meq.L	meq /L	Saturated paste	6.52	5.28	(-2.88, 0.4)	-1.51	61	0.1364	1	0.0632	1
Ca.Na.ratio.meq.L	meq /L	Saturated paste	1.31	0.81	(-1.13, 0.13)	-1.59	49	0.1189	1	0.2079	1
Ca.ppm.extr	ppm	1N Ammonium Acetate	3664.36	3196.23	(-980.58, 44.32)	-1.85	39	**0.0722**	1	0.1982	1
Ca_PCS	% Cation Sat.	Saturated paste	69.37	72.95	(-1.53, 8.69)	1.41	55	0.1656	1	0.4167	1
CEC	meq /100 g	Calculated	27.68	22	(-10.28, -1.09)	-2.51	37	**0.0168**	0.7056	0.3897	1
Cl.SP	ppm	Saturated paste	51.42	46.6	(-42.7, 33.05)	-0.26	53	0.7993	1	0.6291	1
Clay	%	Na Hexametaph	30.56	28.71	(-9.79, 6.09)	-0.47	60	0.6428	1	0.9616	1

					t.test results					Wilcoxon rank sum test statistics	
Variable[a]	Unit	Method	Unocc.	Occ.	Conf. Int.[b]	t.stat	DF	*p*-val.	$p_{adj.}$	*p*-val.	P_{adj}
Cu.ppm.DTPA.extr	ppm	osphate + Hydrometer DTPA-Sorbitol	0.9	0.61	(-0.81, 0.22)	-1.16	36	0.2554	1	0.6216	1
Cu.SP	ppm	Saturated paste	0.01	0.01	(0, 0)	0.8	60	0.426	1	0.2781	1
Est.PAW.mm.cm	mm/cm	Unk.	1.39	1.44	(-0.16, 0.27)	0.52	60	0.6039	1	0.504	1
Fe.ppm.DTPA.extr	ppm	DTPA-Sorbitol	2.39	2.27	(-0.65, 0.41)	-0.46	61	0.6505	1	0.6593	1
Fe.SP	ppm	Saturated paste	0.61	0.27	(-0.81, 0.14)	-1.43	42	0.1599	1	0.3004	1
HCO3_P.ppm	ppm	Olsen sodium bicarbonate	7.75	6.89	(-4.55, 2.83)	-0.47	57	0.6431	1	0.4912	1
K.ppm.extr	ppm	1N Ammonium Acetate	1024.74	450.86	(-869.46, -278.29)	-3.94	34	**0.0004**	**0.0168**	**0.0003**	**0.0126**
K.SP	ppm	Saturated paste	31.85	17.49	(-22.49, -6.22)	-3.57	39	**0.001**	**0.042**	**0.0125**	0.525
K_PCS	% Cation Sat.	Saturated paste	9.48	5.3	(-6.02, -2.34)	-4.59	40	**0**	**0**	**0.0005**	**0.021**
Mg.meq.L.SP	meq/L	Saturated paste	24.27	11.54	(-19.47, -5.99)	-3.81	43	**0.0004**	**0.0168**	**0.0122**	0.5124
Mg.ppm.extr	ppm	1N Ammonium Acetate	278.61	318.66	(-14.78, 94.89)	1.46	58	0.1492	1	0.0747	1
Mg_PCS	% Cation Sat.	Saturated paste	9.53	11.95	(0.41, 4.42)	2.41	58	**0.019**	0.798	**0.0416**	1
Mn.ppm.DTPA.extr	ppm	DTPA-Sorbitol	1.22	1.34	(-0.14, 0.39)	0.94	50	0.3505	1	0.6108	1
Mn.SP	ppm	Saturated paste	0.08	0.09	(-0.04, 0.07)	0.71	41	0.4787	1	0.5606	1
N.P.ratio.ppm	ppm	Calculated	2.87	2.3	(-2.19, 1.04)	-0.71	58	0.4817	1	0.9123	1
Na.meq.L.SP	meq/L	Saturated paste	367	160.81	(-434, 21.62)	-1.84	35	0.0746	1	0.4657	1
Na.Mg.ratio.meq.L	meq/L	Calculated	16.42	22.37	(-7.72, 19.62)	0.87	58	0.3872	1	0.3529	1
Na.ppm.extr	ppm	1N Ammonium Acetate	1032.37	522.8	(-1079.52, 60.37)	-1.81	41	0.0783	1	0.2505	1
Na_PCS	% Cation	Saturated paste	11.62	9.81	(-7.39, 3.78)	-0.65	59	0.5196	1	0.449	1

					t.test results					Wilcoxon rank sum test statistics	
Variable[a]	Unit	Method	Unocc.	Occ.	Conf. Int.[b]	t.stat	DF	*p*-val.	$p_{adj.}$	*p*-val.	P_{adj}
NO3_N.ppm	n Sat. ppm	2 N KCl/Cadmium reduction	13.58	12.15	(-7.85, 5.01)	-0.44	61	0.6599	1	0.6646	1
OM.perc	%	Loss on ignition	1.43	1.55	(-0.09, 0.34)	1.13	61	0.2615	1	0.1605	1
P.SP	ppm	Saturated paste	0.25	0.34	(-0.07, 0.24)	1.13	39	0.2659	1	0.8094	1
pH.avg.S3C.S10P	pH	Saturated paste	8.01	8.2	(0.08, 0.32)	3.32	52	**0.0016**	0.0672	**0.0021**	0.0882
S.SP	p	Saturated paste	350.08	101.08	(-427.25, -70.74)	-2.83	39	**0.0074**	0.3108	0.0781	1
Sand	%	Na Hexametaphosphate + Hydrometer	47.5	45.64	(-9.78, 6.05)	-0.47	59	0.6393	1	0.2499	1
Silt	%	Na Hexametaphosphate + Hydrometer	21.94	25.65	(1.18, 6.25)	2.93	60	**0.0048**	0.2016	**0.0112**	0.4704
SO4.S.ppm.extr	ppm	1N Ammonium Acetate	965.18	170.77	(-1292.77, -296.04)	-3.23	36	**0.0026**	0.1092	**0.0197**	0.8274
Sol_Salts.SP	ppm	Saturated paste	1.27	0.67	(-1.1, -0.09)	-2.38	45	**0.0217**	0.9114	0.2409	1
Zn.ppm.DTPA.extr	ppm	DTPA-Sorbitol	0.33	0.17	(-0.27, -0.06)	-3.14	40	**0.0032**	0.1344	**0.0018**	0.0756
Zn.SP	ppm	Saturated paste	0.01	0.01	(0, 0)	0.34	50	0.7318	1	0.8353	1

[a] Variable abbreviations stand for periodic elements (for example, K = potassium), except for the following: CEC (Cation exchange capacity), OM.perc (% soil organic matter), pH.avg.S3C.S10P (pH, with values for each soil averaged between two measurement instances), Sol_Salts.SP (soluble salts), and Est.PAW.mm.cm (Estimated plant-available water).

[b] Conf. int. refers to the confidence interval of the difference between occupied (Occ.) and unoccupied (Unocc.) group means.

Appendix 4: Table of positive and negative soil variable correlations 60% and above.

Var. 1[a]	Var. 2	Correlation
Al.ppm.SP	Fe.SP	0.991
S.SP	Sol_Salts.SP	0.984
NO3_N.ppm	N.P.ratio.ppm	0.975
Na.meq.L.SP	Na.ppm.extr	0.936
Na.ppm.extr	Na_PCS	0.926
Na.meq.L.SP	S.SP	0.918
Ca.meq.L.SP	Mg.meq.L.SP	0.907

Var. 1[a]	Var. 2	Correlation
S.SP	SO4.S.ppm.extr	0.901
K.SP	SO4.S.ppm.extr	0.900
Na.meq.L.SP	Sol_Salts.SP	0.882
Zn.ppm.DTPA.extr	Cu.ppm.DTPA.extr	0.882
SO4.S.ppm.extr	Sol_Salts.SP	0.874
Cl.SP	N.P.ratio.ppm	0.865
Cl.SP	NO3_N.ppm	0.864
Na_PCS	Na.Mg.ratio.meq.L	0.862
Sol_Salts.SP	Ca.Mg.ratio.meq.L	0.854
Ca.meq.L.SP	Ca.Mg.ratio.meq.L	0.844
Ca.meq.L.SP	SO4.S.ppm.extr	0.843
B.ppm.SP	B.ppm.DTPA.extr	0.835
pH.avg.S3C.S10P	Na.Mg.ratio.meq.L	0.833
K.SP	S.SP	0.831
CEC	SO4.S.ppm.extr	0.829
Est.PAW.mm.cm	N.P.ratio.ppm	0.821
Na.ppm.extr	CEC	0.818
Est.PAW.mm.cm	NO3_N.ppm	0.812
S.SP	CEC	0.812
S.SP	Ca.Mg.ratio.meq.L	0.811
Ca.meq.L.SP	K.SP	0.805
Ca.ppm.extr	CEC	0.805
K.ppm.extr	K_PCS	0.797
K.SP	Sol_Salts.SP	0.795
Ca.ppm.extr	SO4.S.ppm.extr	0.794
S.SP	Na.ppm.extr	0.792
Ca.meq.L.SP	Sol_Salts.SP	0.790
Na.meq.L.SP	CEC	0.789
Na.ppm.extr	Na.Mg.ratio.meq.L	0.788
Na.meq.L.SP	Na_PCS	0.786
CEC	Sol_Salts.SP	0.775
SO4.S.ppm.extr	Ca.Mg.ratio.meq.L	0.775
Na.ppm.extr	Sol_Salts.SP	0.762
Ca.meq.L.SP	S.SP	0.761
Fe.ppm.DTPA.extr	Clay	0.760
P.SP	pH.avg.S3C.S10P	0.756
Zn.SP	Mn.ppm.DTPA.extr	0.750
Cl.SP	Est.PAW.mm.cm	0.741
Na.meq.L.SP	SO4.S.ppm.extr	0.737
Na_PCS	N.P.ratio.ppm	0.736
Est.PAW.mm.cm	Clay	0.734
P.SP	Bicarb.SP	0.728
B.ppm.SP	pH.avg.S3C.S10P	0.725

Var. 1[a]	Var. 2	Correlation
K.SP	Mg.meq.L.SP	0.725
Na.meq.L.SP	Na.Mg.ratio.meq.L	0.722
K.SP	Ca.Mg.ratio.meq.L	0.720
Mg.meq.L.SP	SO4.S.ppm.extr	0.702
Na.ppm.extr	N.P.ratio.ppm	0.696
CEC	Na_PCS	0.695
Cl.SP	Mn.ppm.DTPA.extr	0.693
Na_PCS	NO3_N.ppm	0.689
Est.PAW.mm.cm	Na_PCS	0.688
NO3_N.ppm	Mn.ppm.DTPA.extr	0.681
Mg.ppm.extr	Mg_PCS	0.676
pH.avg.S3C.S10P	B.ppm.DTPA.extr	0.672
Est.PAW.mm.cm	CEC	0.672
K.ppm.extr	Na.ppm.extr	0.672
Ca.meq.L.SP	Ca.ppm.extr	0.666
K.SP	Na.meq.L.SP	0.664
pH.avg.S3C.S10P	Na_PCS	0.658
Est.PAW.mm.cm	Na.ppm.extr	0.652
P.SP	Na.Mg.ratio.meq.L	0.649
K.ppm.extr	CEC	0.646
Na.meq.L.SP	N.P.ratio.ppm	0.643
Bicarb.SP	pH.avg.S3C.S10P	0.643
K.SP	CEC	0.641
B.ppm.SP	P.SP	0.638
Mg.meq.L.SP	Sol_Salts.SP	0.637
Mn.ppm.DTPA.extr	B.ppm.DTPA.extr	0.629
Na.ppm.extr	NO3_N.ppm	0.626
Mg.meq.L.SP	S.SP	0.625
Na.meq.L.SP	Ca.Mg.ratio.meq.L	0.614
Na.ppm.extr	SO4.S.ppm.extr	0.611
S.SP	Na_PCS	0.610
Na_PCS	Sol_Salts.SP	0.607
K.ppm.extr	Mg_PCS	-0.595
CEC	Sand	-0.608
OM.perc	Na.Mg.ratio.meq.L	-0.610
Mg_PCS	Na_PCS	-0.613
Ca_PCS	NO3_N.ppm	-0.628
Ca_PCS	N.P.ratio.ppm	-0.652
Mg_PCS	Fe.ppm.DTPA.extr	-0.655
K.ppm.extr	Ca_PCS	-0.682
Na.meq.L.SP	Ca_PCS	-0.697
Fe.ppm.DTPA.extr	Sand	-0.718
Est.PAW.mm.cm	Sand	-0.733

Var. 1[a]	Var. 2	Correlation
Ca_PCS	Na.Mg.ratio.meq.L	-0.749
Na.ppm.extr	Ca_PCS	-0.869
Ca_PCS	Na_PCS	-0.903
Sand	Clay	-0.952

[a] See Appendix 2 for variable abbreviations and soil analysis methods.

Appendix 5: Table of means and standard deviations for E. tiehmii *seedling responses (top) and soil variables (bottom).* E. tiehmii *seedling responses are shown for seeds grown in soils from sites that are occupied or unoccupied by* E. tiehmii *in the wild. Seeds from sub-populations 1,2,3,4, and 6 were sown into 10 occupied soils and 11 unoccupied soils and grown in the greenhouse at the University of Nevada, Reno. There are stars by significant differences ($p < 0.05$)*

Variable[a]	Occupied Mean (SD)	Unoccupied Mean (SD)
Days lived	69.02 (50.14)	67.77 (46.15)
Days until Emergence	26.07 (11.91)	24.85 (11.97)
*Root weight (mg)	88.76 (59.52)	48.93 (32.36)
Shoot weight (mg)	46.35 (24.9)	39.43 (16.56)
*RMR	0.63 (0.1)	0.53 (0.12)
*Total biomass	135.12 (80.01)	88.36 (43.45)
Al.ppm.SP	0.53 (0.71)	1.09 (1.67)
*B.ppm.DTPA.extr	38.26 (37.07)	8.08 (18.65)
*B.ppm.SP	38.06 (44.65)	4.68 (8.99)
Bicarb.SP	152 (43.07)	126 (23.97)
Ca.meq.L.SP	77.2 (108.79)	186.32 (165.24)
Ca.Mg.ratio.meq.L	5.27 (3.08)	6.54 (3.2)
Ca.Na.ratio.meq.L	0.78 (0.8)	1.31 (1.56)
Ca.ppm.extr	3224.47 (431.59)	3675.98 (1357.29)
Ca_PCS	72.86 (7.64)	69.33 (11.98)
CEC	22.22 (3.2)	27.79 (12.37)
Cl.SP	46.36 (46.38)	51.71 (90.43)
Clay	28.93 (13.33)	30.64 (16.86)
Cu.ppm.DTPA.extr	0.61 (0.29)	0.91 (1.3)
Cu.SP	0.01 (0)	0.01 (0)
Est.PAW.mm.cm	1.44 (0.36)	1.39 (0.45)
Fe.ppm.DTPA.extr	2.28 (0.92)	2.39 (1.01)
Fe.SP	0.27 (0.33)	0.61 (0.86)
HCO3_P.ppm	6.89 (3.41)	7.73 (4.75)
*K.ppm.extr	454.27 (138.88)	1028.79 (811.78)
*K.SP	17.22 (6.21)	31.89 (21.07)
*K_PCS	5.31 (1.68)	9.47 (4.84)
*Mg.meq.L.SP	11.44 (6.77)	24.35 (17.28)
Mg.ppm.extr	325.64 (124.61)	278.85 (100.34)

Variable[a]	Occupied Mean (SD)	Unoccupied Mean (SD)
Mg_PCS	12.03 (4.25)	9.5 (3.65)
Mn.ppm.DTPA.extr	1.34 (0.51)	1.22 (0.34)
Mn.SP	0.09 (0.1)	0.08 (0.05)
N.P.ratio.ppm	2.33 (2.59)	2.88 (3.62)
Na.meq.L.SP	161.62 (120.39)	369.94 (619.68)
Na.Mg.ratio.meq.L	22.37 (23.04)	16.52 (24.62)
Na.ppm.extr	524.63 (547.89)	1041.18 (1492.87)
Na_PCS	9.83 (9.31)	11.7 (12.39)
NO3_N.ppm	12.31 (12.04)	13.62 (12.95)
OM.perc	1.56 (0.33)	1.43 (0.39)
P.SP	0.34 (0.27)	0.25 (0.13)
pH.avg.S3C.S10P	8.2 (0.26)	8.01 (0.18)
S.SP	103.53 (148.81)	353.09 (463.79)
Sand	45.85 (13.28)	47.45 (16.63)
Silt	25.22 (3.77)	21.91 (4.98)
SO4.S.ppm.extr	173.61 (320.68)	974.17 (1347.02)
Sol_Salts.SP	0.68 (0.56)	1.28 (1.25)
Zn.ppm.DTPA.extr	0.17 (0.09)	0.33 (0.27)
Zn.SP	0.01 (0.01)	0.01 (0)

[a] See Appendix 2 for variable abbreviations and soil analysis methods.

Appendix 6: Summary of significant model-averaged estimates for the effects of soil variation on E. tiehmii *seedling growth in the greenhouse. Significance was determined by whether 95% confidence intervals overlapped with zero.*

Growth response	Predictor (units, method)[a]	Correlation direction	Estimate	Estimate units	*p*-val, Pseudo-R^2 of top model[b]
Days to emergence	% Sand	-	-11.56	% change in response	p < 0.05, McFadden's Pseudo- R^2= 14.27%
	Al (ppm, SP)	-	-9.57	% change in response	
	Mn (ppm, DTPA-extr.)	-	-9.17	% change in response	
Days lived	S (ppm, SP)	-	-26.48	% change in response	p < 0.05, McFadden's Pseudo- R^2= 9.79%
Total biomass	NO3-N (ppm)	+	30.21	% change in response	p < 0.05, McFadden's Pseudo- R^2= 58.41%

Growth response	Predictor (units, method)[a]	Correlation direction	Estimate	Estimate units	*p*-val, Pseudo-R^2 of top model[b]
	% Silt	-	-27.58	% change in response	
	Zn (ppm, DTPA-extr.)	-	-16.91	% change in response	
	% Organic matter	+	66.60	% change in response	
	Fe (ppm, DTPA-extr.)	-	-31.16	% change in response	
	Ca (meq/L, SP)	-	-27.50	% change in response	
Root mass ratio	Zn (ppm, DTPA-extr.)	-	-0.047	Unit-less; same as RMR	$p < 0.05$, McFadden's Pseudo-R^2= 45.34%
	SO4-S (ppm, ammonium acetate-extr.)	-	-0.070	Unit-less; same as RMR	
	% Silt	+	0.034	Unit-less; same as RMR	
	N:P ratio	+	0.048	Unit-less; same as RMR	
	Fe (ppm, DTPA-extr.)	-	-0.047	Unit-less; same as RMR	
	Cu (ppm, SP)	+	0.033	Unit-less; same as RMR	

[a] See Appendix 2 for variable abbreviations and soil analysis methods.

[b] McFadden's pseudo- R^2 is a measure of model fit for generalized linear models, calculated as : R^2 = 1 - (model deviance/ intercept-only model deviance (McFadden, 1977).

Appendix 7: Table of ***overall*** *means and standard deviations for* E. tiehmii *plant growth and soil variables. Seeds from sub-populations 1,2,3,4, and 6 were sown into 10 occupied soils and 11 unoccupied soils and grown in the greenhouse at the University of Nevada, Reno.*

Variable[a]	Mean	SD
Days lived	67.82	48.69
Days until emergence	25.41	11.94
Root weight (mg)	69.45	52.1
Shoot weight (mg)	43	21.47

Variable[a]	Mean	SD
RMR	0.58	0.12
Total biomass	112.45	68.79
Al.ppm.SP	0.83	1.34
B.ppm.DTPA.extr	22.33	32.54
B.ppm.SP	20.43	35.50
Bicarb.SP	138.27	36.69
Ca.meq.L.SP	134.81	151.52
Ca.Mg.ratio.meq.L	5.94	3.21
Ca.Na.ratio.meq.L	1.06	1.28
Ca.ppm.extr	3462.86	1053.87
Ca_PCS	71.00	10.31
CEC	25.16	9.66
Cl.SP	49.18	73.05
Clay	29.83	15.32
Cu.ppm.DTPA.extr	0.77	0.97
Cu.SP	0.01	0.00
Est.PAW.mm.cm	1.42	0.41
Fe.ppm.DTPA.extr	2.34	0.97
Fe.SP	0.45	0.68
HCO3_P.ppm	7.33	4.19
K.ppm.extr	757.61	662.65
K.SP	24.97	17.49
K_PCS	7.51	4.24
Mg.meq.L.SP	18.26	14.86
Mg.ppm.extr	300.94	114.81
Mg_PCS	10.69	4.14
Mn.ppm.DTPA.extr	1.27	0.43
Mn.SP	0.08	0.08
N.P.ratio.ppm	2.62	3.18
Na.meq.L.SP	271.61	469.31
Na.Mg.ratio.meq.L	19.28	24.06
Na.ppm.extr	797.36	1176.41
Na_PCS	10.82	11.08
NO3_N.ppm	13.00	12.54
OM.perc	1.49	0.37
P.SP	0.29	0.21
pH.avg.S3C.S10P	8.10	0.24
S.SP	235.29	373.45
Sand	46.69	15.16
Silt	23.47	4.75

Variable[a]	Mean	SD
SO4.S.ppm.extr	596.30	1079.65
Sol_Salts.SP	0.99	1.03
Zn.ppm.DTPA.extr	0.25	0.22
Zn.SP	0.01	0.01

[a] See Appendix 2 for variable abbreviations and soil analysis methods.

Appendix 8: Coefficient estimates of soil variables that were significant after model averaging (Fig. 14) from all individual top models (within 2AIC units) of days to emergence, days lived, total biomass, and RMR in the greenhouse.

Response	Mod. #	Al.ppm.SP[a]	Mn.ppm.DTPA.extr	S.SP	Sand	Ca.meq.L.SP	Fe.ppm.DTPA.extr	NO3_N.ppm	OM.perc	Silt	Zn.ppm.DTPA.extr	Cu.ppm.DTPA.extr	N.P.ratio.ppm	SO4.S.ppm.extr
Days to emerge	1	-0.14	-0.08	NA	-0.13	NA	NA	NA	NA	NA	NA	NA	NA	NA
Days to emerge	2	-0.10	-0.06	NA	-0.13	NA	NA	NA	NA	0.06	NA	NA	NA	NA
Days to emerge	3	-0.14	-0.09	NA	-0.13	NA	NA	NA	NA	NA	NA	NA	NA	NA
Days to emerge	4	-0.13	-0.07	NA	-0.15	NA	NA	NA	NA	NA	NA	NA	NA	NA
Days to emerge	5	-0.14	-0.12	NA	-0.16	NA	NA	NA	NA	NA	NA	NA	NA	NA
Days to emerge	6	-0.13	-0.14	NA	-0.15	NA	NA	NA	NA	NA	NA	NA	NA	NA
Days to emerge	7	-0.16	-0.10	NA	-0.15	NA	NA	NA	NA	NA	NA	NA	NA	NA
Days to emerge	8	-0.14	-0.13	NA	-0.17	NA	NA	NA	NA	NA	0.02	NA	NA	NA
Days to emerge	9	-0.14	-0.12	NA	-0.14	NA	NA	NA	NA	NA	NA	NA	NA	NA
Days to emerge	10	-0.14	-0.10	NA	-0.14	NA	NA	NA	NA	NA	0.02	NA	NA	NA
Days to emerge	11	-0.13	-0.10	NA	-0.12	NA	NA	NA	NA	NA	NA	NA	NA	NA
Days to emerge	12	-0.14	-0.08	NA	-0.13	NA	NA	NA	NA	NA	0.01	NA	NA	NA
Days to emerge	13	-0.10	-0.06	NA	-0.13	NA	NA	NA	NA	0.06	0.01	NA	NA	NA
Days to emerge	14	-0.15	-0.08	NA	-0.14	NA	NA	NA	NA	NA	NA	NA	NA	NA
Days to emerge	15	-0.12	-0.11	NA	-0.14	NA	NA	NA	NA	0.04	NA	NA	NA	NA
Days to emerge	16	-0.13	-0.08	NA	-0.16	NA	NA	NA	NA	NA	0.01	NA	NA	NA
Days to emerge	17	-0.14	-0.14	NA	-0.15	NA	NA	NA	NA	NA	0.02	NA	NA	NA

Days to emerge	18	-0.15	-0.18	NA	-0.19	NA	NA	NA	NA	NA	0.04	NA	NA	NA
Days to emerge	19	-0.10	-0.06	NA	-0.12	NA	NA	NA	NA	0.06	NA	NA	NA	NA
Days to emerge	20	NA	NA	NA	NA	NA	NA	NA	NA	0.11	NA	NA	NA	NA
Days to emerge	21	-0.12	-0.08	NA	-0.14	NA	NA	NA	NA	NA	NA	NA	NA	NA
Days to emerge	22	-0.15	-0.12	NA	-0.15	NA	NA	NA	NA	NA	0.02	NA	NA	NA
Days to emerge	23	-0.13	-0.13	NA	-0.11	NA	NA	NA	NA	NA	NA	NA	NA	NA
Days to emerge	24	-0.11	-0.05	NA	-0.13	NA	NA	NA	-0.03	0.07	NA	NA	NA	NA
Days lived	1	NA	NA	-0.34	NA	NA	NA	NA	NA	NA	NA	0.08	NA	NA
Days lived	2	NA	NA	-0.32	NA	NA	NA	NA	NA	NA	NA	0.08	NA	NA
Days lived	3	NA	NA	-0.41	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Days lived	4	NA	NA	-0.31	NA	NA	NA	NA	NA	-0.05	NA	0.07	NA	NA
Days lived	5	NA	NA	-0.23	NA	NA	NA	NA	NA	-0.08	NA	NA	NA	NA
Days lived	6	NA	NA	-0.40	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Days lived	7	NA	NA	-0.35	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Days lived	8	NA	NA	-0.18	NA	NA	NA	NA	NA	-0.09	NA	0.07	NA	NA
Days lived	9	NA	NA	-0.43	NA	NA	NA	NA	NA	-0.02	NA	NA	NA	NA
Days lived	10	NA	NA	-0.34	NA	NA	NA	NA	NA	NA	NA	0.08	NA	NA
Days lived	11	NA	NA	-0.29	NA	NA	NA	NA	NA	NA	NA	0.07	NA	NA
Total biomass	1	NA	NA	NA	NA	-0.25	-0.35	0.25	0.53	-0.33	-0.19	NA	NA	NA
Total biomass	2	NA	NA	NA	NA	-0.32	-0.42	0.29	0.56	-0.43	-0.20	NA	NA	NA
Total biomass	3	NA	NA	NA	NA	-0.32	-0.42	0.28	0.56	-0.41	-0.18	NA	NA	NA
Total biomass	4	NA	NA	NA	NA	-0.25	-0.34	0.25	0.52	-0.32	-0.19	NA	NA	NA
Total biomass	5	-0.05	NA	NA	NA	-0.25	-0.30	0.22	0.50	-0.31	-0.20	NA	NA	NA
Total biomass	6	NA	NA	NA	NA	-0.25	-0.35	0.25	0.52	-0.33	-0.20	NA	NA	NA
RMR	1	NA	NA	NA	NA	NA	-0.05	NA	0.03	NA	-0.04	NA	0.05	-0.08
RMR	2	NA	NA	NA	NA	NA	-0.05	NA	0.03	NA	-0.04	NA	0.05	-0.08
RMR	3	NA	NA	NA	NA	NA	-0.05	NA	0.03	NA	-0.04	NA	0.04	-0.08
RMR	4	NA	NA	NA	NA	NA	-0.05	NA	0.03	NA	-0.04	NA	0.05	-0.08
RMR	5	NA	NA	NA	NA	NA	-0.05	NA	0.03	NA	-0.04	NA	0.05	-0.08
RMR	6	NA	NA	NA	NA	NA	-0.05	NA	0.03	NA	-0.04	NA	0.05	-0.08
RMR	7	NA	NA	NA	NA	NA	-0.04	NA	0.03	NA	-0.04	NA	0.05	-0.09

[a] See Appendix 2 for variable abbreviations and soil analysis methods.