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Plant-pollinator associations in an eastern serpentine savannah and the effects of overbrowsing

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Plant-pollinator associations in an eastern serpentine savannah and the effects of overbrowsing

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at Virginia Commonwealth University

By

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Chapter 1: Native plant response to deer overbrowsing in a serpentine savannah

Abstract

Plants are particularly vulnerable to physical disturbance in low productivity areas, due to a high cost of replacing lost plant tissue. In the eastern United States, serpentine grasslands are fragmented ecosystems with high concentrations of rare endemic plant species, low concentrations of soil nutrients, and uncontrolled deer overpopulation. This study assessed functional responses of native angiosperms in a rare eastern serpentine savannah to selective deer browsing. Plant count, flower count, floral area, vegetative area, and plant height of 10 serpentine plant species were compared inside and outside of deer exclusion structures throughout the growing season of 2019 (April-October). Throughout the growing season and across the plant community, deer presence consistently reduced values for all plant response traits measured. Species most impacted by deer herbivory included *Oenothera biennis, Solidago nemoralis, Sabatia angularis, Liatris spicata*, and serpentine endemic *Symphyotrichum depauperatum.* Eastern serpentine grasslands could lose biodiversity and be permanently degraded by continued intense browsing pressure. We recommend that management programs should be implemented to monitor rare serpentine flowering plant species, and that hunting be considered to limit the deer population in areas of high plant conservation priority.

Introduction

Severe ecosystem disturbances including species invasion, anthropogenic land use change, and extinction of carnivores have resulted in dramatic changes in ecosystem composition and losses of global biodiversity (Myers et al., 2000, Rambo & Faeth, 2003, Raghubanshi 2009, Strong & Frank, 2010). Especially when disturbances directly impact the abundance and distribution of vegetation, their effects can propagate across foodwebs and cascade through ecosystems (Nakamura, 2000, Strong & Frank, 2010, Ripple & Bestcha, 2011). As a result, acute or chronic disturbance can alter species interactions, reduce foodweb stability and resilience, and ultimately lead to lowered biodiversity (Raghubanshi 2009, Strong & Frank, 2010). Understanding both the scale and cascading impacts of disturbance across ecosystems, and exploring their mitigation is imperative to prevent the loss of unique species (Raghubanshi 2009).

In the past few decades, white-tailed deer (*Odocoileus virginianus* Zimmerman, 1780) populations have expanded across the eastern United States, due to local extinction of natural predators, landscape and ecological changes, reduction in hunting pressure, and the ability of deer to adapt to human-modified ecosystems (Côté et al. 2004, Latham et al., 2005, Creacy, 2006, Rawinski, 2008, Strong & Frank, 2010). At high densities, deer disperse exotic species (Williams & Ward, 2006, Knight et al, 2009, Averill et al., 2017), increase the spread of infectious disease (Belay et al., 2004, CFSPH, 2006, Borer et al., 2009), and alter community dynamics and ecosystem processes through trampling and selective browsing (Latham et al., 2005, Rawinski, 2008, Averill et al., 2017). White-tailed deer are considered a keystone species, because their browsing has such strong direct and indirect impacts on the ecosystems they live in (Rooney, 2001, Rooney & Waller, 2003).

At high deer density, browsing limits floral biodiversity and promotes homogeneity (Miller et al., 1992, Rawinski, 2008). Browsing has an outsized effect on herbaceous plants, particularly when deer preferentially forage on reproductive plants (Augustine & Frelich, 1998, Ruhren & Handel, 2003, Geddes et al., 2006). During spring and summer months, deer feed primarily on herbaceous plants due to their digestibility and high nitrogen content (Miller et al., 1992, Geddes et al., 2006). Browse-intolerant herbaceous plants found in areas of high deer density are often smaller, have fewer flowering heads, and are less likely to reproduce, either due to direct consumption or chronic resource depletion (Englund & Mayer, 1986, Augustine & Frelich, 1998, Ruhren & Handel, 2003, Côté et al., 2004, Wang et al., 2008). Deer browsing, especially florivory, can reduce the density and diversity of herbaceous plants over time, causing local extinctions (Augustine and Frelich, 1998, Strong & Frank, 2010).

The effects of deer browsing on plant communities may be magnified in low productivity areas (Olff & Ritchie 1998; Proulx & Mazumder 1998, Bakker et al. 2006) where low soil nutrient availability increases the costs of replacing lost plant tissue (Janzen 1974). This may be especially true for serpentine barrens, which are globally rare, heavily fragmented habitats that occur on unique soils derived from ultramafic rock (Latham & McGeehin, 2012). These soils tend to be shallow and rocky, with low moisture, low soil nutrient availability, and high concentrations of heavy metals (Flinn et al., 2017). Due to the considerable obstacles for plant colonization, serpentine ecosystems are hotspots for rare and endemic plant species, with unique adaptations to the harsh conditions (Anacker, 2011, Kay et al., 2011, Wolf & Thorp, 2011).

While serpentine soils can be found around the globe, most ecological research about serpentine systems is restricted to the west coast of the United States (Anacker, 2011, Kay et al., 2011). Based on these studies, we know that compared to non-serpentine taxa, many serpentine species exhibit slow growth rates, high root:shoot ratios, small stature, small flower size, and mechanisms to prevent desiccation and regulate the uptake of heavy metals. (O'dell & Rajakaruna, 2011, Wolf & Thorpp, 2011) For example, the serpentine endemic *Cerastium arvense* Hollick & Briton (serpentine chickweed; Caryophyllaceae) prevents desiccation by producing a dense layer of hairs to reflect excess sunlight and to trap evaporating moisture (Prince et al., 2004). Another serpentine endemic, *Alyssum lesbiacum,* Candargy (Lesvos alyssum; Alyssae) hyperaccumulates nickel in storage tissues to prevent ion toxicity from nickel-rich serpentine soils (Kazakou et al., 2010). Unique adaptations to serpentine have also been observed in species that grow both on and off serpentine substrates. For example, *Leptosiphon parviflorus* Porter & Johnson (variable linanthus; Polemoniaceae) exhibits decreased plant height and earlier flowering date, and *Lasthenia californica* Lindl (California goldfield; Asteraceae) shows physiological tolerance to ionic stress when grown on serpentine soils, as compared to adjacent non-serpentine soils (Kay et al., 2011).

The unique adaptations that allow serpentine species to tolerate extreme soil conditions can be energetically and reproductively costly (Flinn et al., 2017). For example, secondary chemicals and heavy metals in nectar and fruits may negatively affect pollinators and other mutualist species, limiting pollination and seed dispersion for serpentine taxa (Strauss and Boyd, 2011). Plants in these sparse, low diversity communities may also be both more conspicuous to herbivores, and susceptible to their impacts. Due to the increased cost for regrowth in resource-poor environments, serpentine species have reduced resilience to herbivory (Strauss & Boyd, 2011, Flinn et al., 2017). Additionally, because calcium is required for effective plant immune response, low soil calcium levels cause serpentine species to be susceptible to diseases carried by herbivores (Huntly, 1991, Borer et al., 2009, Kay et al., 2011, Strauss and Boyd, 2011).

While serpentine systems and their responses to disturbance have been studied rigorously in California and Oregon where serpentine soils are abundant, smaller, more fragmented eastern serpentine systems have been largely unstudied (Flinn, et al., 2017). Remnants of an extensive prairie that covered most of the eastern United States 25,000 years ago, eastern serpentine grasslands are a vestige of a unique and ancient ecosystem (Prince, et. al, 2004). As the climate became cooler and wetter, and the prairies receded, serpentine areas remained open and grassy due to their low moisture retention and high heavy metal concentrations. Lightning induced fires and fires set by Native Americans to hunt deer maintained these grassy barrens for thousands of years until European colonization (Floyd, 2006, Prince, et. al, 2004, Tyndall, 1992). Although settlers used serpentine grasslands to graze livestock, these unique ecosystems began to decline due to encroachment of woody plants (Prince, et al., 2004, Floyd, 2006). Encroachment increased rapidly after livestock were removed in 1930, and over the course of the 20th century, a combination of residential development and mining of serpentine minerals, such as talc, asbestos, and chromite, decimated what was left of the serpentine barrens (Floyd, 2006, Prince, et. al, 2004).

Today, 96% of eastern serpentine barrens are found in the Northern Piedmont of Maryland and Pennsylvania, covering only 3,400 acres in total (about 5 square miles) (Latham & McGeehan, 2012). These ecosystems are ecologically distinct both from the habitats that surround them, and from their western counterparts, with flora and fauna most similar to that of Midwest prairies (Latham & Anderson, 2003, Latham & McGeehin, 2012). A newspaper article in the 1960s described a serpentine barren in Maryland as a "hunk of the American (Mid)west dropped into Maryland" (Modell, 1967). As such, eastern serpentine barrens are not only home to globally rare endemic serpentine species, but also to many plant species whose main ranges are in the Midwest, and are thus locally or regionally endangered (Flinn et al., 2017, Rajakaruna, 2009).

While no studies to date have examined the impact of high deer densities on eastern or western serpentine systems, a few studies have described the significant negative impacts of deer overbrowsing in Midwest prairies (Anderson, 2003, Latham & McGeehin, 2012). Although prairie grass species are well adapted to herbivory, direct consumption of flowering forbs by deer reduces both their vigor and reproductive success. Additionally, deer generally avoid consuming invasive non-native plants, allowing them to proliferate unchecked (Latham & McGeehin, 2012). These competitive disadvantages to native forbs have resulted in shifts in community composition, and an overall decline in prairie floral diversity (Anderson, 2003, Strong & Frank, 2010).

Eastern serpentine barrens are typically small fragments of prairie habitat, surrounded by dense pine forest. White-tailed deer thrive along the forest edges of these serpentine grasslands, consuming a combination of saplings, shrubs, and wildflowers (Anderson, 2003, Latham & McGeehin, 2017). Rare plants in these fragmented ecosystems are especially vulnerable to deer browsing effects, due to an elevated rate of encounter, and a limited ability for plants to disperse and recolonize between habitat fragments (Miller et al. 1992). Several endangered flowering forbs, including *Lobelia puberula* Turner (downy lobelia; Campanulaceae) and *Symphyotrichum ericoides* Nesom (white heath aster; Asteraceae) have been locally extirpated from eastern serpentine barrens in the past decades (Latham & McGeehin, 2012). While it is unclear if deer overbrowsing is responsible for these declines, it is clear that rare and endangered species that are highly preferred by deer are extremely vulnerable. The regeneration of many rare eastern serpentine species has been slowed or halted completely due to the onslaught of deer consumption (Latham & McGeehin, 2012). Due to a lack of knowledge about the specific dietary preferences and overall impacts of deer overbrowsing on eastern serpentine plant communities, Latham & McGeehin (2012) suggest that completely excluding deer and comparing vegetation response where deer are present and absent is the "only practical method" to monitor deer impacts on rare serpentine species.

This study used deer exclusion plots to assess the impacts of deer browsing on flowering plant community composition in a rare serpentine savannah in the eastern United States. Specific objectives included: 1) quantifying the community response of serpentine vegetation to deer browsing, 2) determining plant species that were most impacted by deer presence, and 3) quantifying how the serpentine vegetation response to deer browsing shifted over the course of the growing season. Excluded areas were predicted to have higher abundances of flowering species, greater numbers of flowers, larger vegetative and floral areas, and taller flowering plants. Additionally, species that were preferred by deer were predicted to show stronger negative responses to deer presence than species deer avoided. This research provides valuable information about the effects of deer overbrowsing on eastern serpentine species which can be directly applied to management and conservation decisions in serpentine grasslands and other rare ecosystems occurring across the eastern United States.

Methods

Study Site

Serpentine savannas, defined as barrens with 10-25% tree cover (Flinn et al., 2017), are considered to have an especially high conservation value, and house nearly all of the rare endemic plant and pollinator species characteristic of serpentine barrens (Floyd, 2006, Smith, 2010, Flinn et al., 2017). Located in the midst of suburban Baltimore, Soldiers Delight Natural Environmental Area includes the largest remaining serpentine savannah ecosystem in the eastern United States (Floyd, 2006, Tyndall & Hull, 1999).

The soil at Soldiers Delight is a sandy loam with a low concentration of calcium and a high concentration of magnesium and nickel (Tyndall, 2012). Dominant grassland species include true prairie grasses, such as *Schizachyrium scoparium* Michx. (little bluestem; Poaceae) and *Sorghastrum nutans* Nash. (indian grass; Poaceae) (Tyndall & Hull, 1999, Tyndall, 1994). There are also over 39 rare, threatened, or endangered plant species at Soldiers Delight, including the *Gentianopsis crinita* Froel. (fringed gentian flower; Gentianaceae), *Symphyotrichum depauperatum* Nesom. (serpentine aster, Asteraceae), *Cerastium arvense L. var. villosum* (serpentine chickweed) and *Agalinis decemloba* Greene (ten lobe false foxglove; Scrophulariaceae) (Tyndall & Hull, 1999, Tyndall, 1994, Tyndall, 2005, Floyd, 2006, Flinn, et al., 2017).

The serpentine savanna ecosystem at Soldiers Delight is actively managed using a combination of cutting trees and controlled burns to limit the encroachment of Virginia Pine and Eastern Red Cedar (Tyndall & Hull, 1999, Tyndall, 1994, Floyd, 2006, Prince, et. al, 2004). However, rare and endangered serpentine flora are also threatened by competition from invasive species and consumption by herbivores (Flinn et al. 2017). White tailed deer density at Soldiers Delight has increased dramatically in the past few decades (Floyd, 2006), and have had an uncharacterized impact on the unique flora and fauna of this rare ecosystem.

Deer Exclusion Plot Construction

Exclusion plots are frequently used to measure the effects of large herbivores on plant and insect communities (Rambo & Faeth, 1999, Pasari et al., 2014, Stephan et al. 2017, Averill et al., 2017). We identified two sites within Soldiers Delight with similar physical, topographic, and edaphic characteristics (39.4030190, -76.8240320, and 39.4025640, -76.8214420), shown in Fig. 1. At each site, we constructed five 5m x 5m deer exclosure plots, each paired with an adjacent control plot. Due to shallow soil depth at the study site, exclosures were constructed using 5-gallon buckets of cement anchoring 2 m tall wooden posts, and 2 m tall polypropylene fence with 2" mesh openings, to permit movement of pollinators and birds. Holes (4 x 6 inches) were cut in the bottom of the fence in order to allow small mammals to access plots, and 1 cm thick wire was wrapped around the bottom of each structure to prevent fawns from accessing the plots.

Plant Sampling

In ecosystems with low diversity, measuring changes in species abundance is considered to be more informative than measuring biodiversity alone, thus both were determined in this work (Mendenhall et al., 2011, Murphy & Romanuk, 2014). To quantify the impact of deer browsing on the diversity and abundance of flowering plant species, monthly plant inventories were conducted. Within each experimental plot, two 1 x 1 m quadrats were established, and percent cover of plant species was recorded for each sampling event. Additionally, for each plot, the number of flowering plants, the number of flowers on each plant, and the height of each plant was recorded. Sampling occurred every 2-3 weeks in order to capture peak bloom for all species present.

Some pollinator species, including bumblebees, preferentially visit patches with high floral densities (Westphal et al., 2003, Hegland & Boeke, 2006). For each flowering species encountered during the growing season, 30 inflorescences were photographed next to a ruler, and average floral areas for each species were calculated using the program ImageJ (Schneider et. al, 2012). The area of compound flowers *Solidago rugosa* Mill (Asteraceae) and *Packera anonyma* Weber & Love (Asteraceae) was measured using the program GIMP (The GIMP Team, 2020), by dividing the number of pixels of all flowers on the plant by the number of pixels in a 1 cm square. This number was divided by the number of influorescences in an image to determine area per influorescence. The area for *Polygala verticillata* L. (Polygalaceae) was calculated for the entire flower rather than individual influorescences due to their tiny size (about 8 sq mm). The average floral area per species was multiplied by the number of inflorescences of each species present in each plot to obtain a standardized measure of species blossom density (Hegland & Boeke, 2006). Floral areas for each species in each plot were added together to calculate total floral density per plot.

Statistical analyses

To quantify patterns in community composition data over the growing season, we used a nonmetric multidimensional scaling (NMDS) ordination to divide sampling points throughout the year into distinct season groups based on their plant community compositions (NMDS; McCune & Grace, 2002). The input data were total counts for flowering plant species at each sampling date. The NMDS was performed using the package 'vegan' in R, using the Bray-Curtis dissimilarity index as a distance measure (Oksanen et. al, 2019). Based on their distinct groupings, sampling dates were divided 5 significantly different seasons: spring, early summer, mid-summer, late summer, and fall. Community composition differences between these seasons were compared using a permanova.

Counts of flowering plant number per plot, total flower number per plot, and flower number per plant for each species were compared between control and exclusion treatments for each unique combination of date, plot, and treatment using a negative binomial regression with repeated measures. All negative binomial regressions were run using the package "MASS" in R (Venable & Ripley, 2002).

Total blossom area, mean herbaceous plant cover, and mean plant height per species for each unique combination of date, plot, and treatment were analyzed using general linear mixed effects model with repeated measures (GLMMs). In all models, "plot" and "quadrat" were treated as random factors. The GLMMs were validated visually (Zuur et al., 2010) leading to Box-Cox transformations of some response variables. All GLMMs were run using the package "lme4" in R (Bates et al., 2015), and best models were determined based on AIC values. Linear regressions were then used to assess how blossom density area related to herbaceous plant percent cover for each plot and treatment.

To determine which flower species were particularly driving differences in flower counts between exclusion and control plots, average values for each species in control and exclusion plots were calculated for five plant functional responses: total plant number per plot, total flower number per plot, average blossom area per plot, average vegetative area per plot, and average height per plant. To determine the magnitude of difference between exclusion and control plant responses, the ratio of the response between exclusion and control for each species and each functional response was calculated. Responses were weighted equally by adding all ratio values for each vegetation response and dividing each individual ratio value by the sum. An NMDS was performed comparing ratios of plant functional responses between exclusion and control plots across species, to determine which species showed the greatest differences in plant response between control and exclusion treatments. NMDS analysis was performed using the package ‘vegan’ in R, using the Euclidean distance as a dissimilarity index as a distance measure (Oksanen et. al, 2019).

To assess community response to exclusion treatments, community weighted means (Garnier et al., 2004, Violle et al., 2007) were calculated for four plant functional responses: total flower number per plot, average blossom area per plot, average vegetative area per plot, and average height per plant. An NMDS was performed comparing plant functional trait responses between exclusion and control plots using the package ‘vegan’ in R, using the Euclidean distance as a dissimilarity index as a distance measure (Oksanen et. al, 2019). Number of flowers per plant was found to be significantly correlated to average blossom area, and was not used as a functional response variable for this analysis. The functional trait responses between control and exclusion treatments were compared using a permanova.

Bray-Curtis dissimilarity indices between control and exclusion treatments at each date were calculated for 6 response variables: average vegetative plant area per plot, average blossom density area per plot, total number of flowering plants, total number of flowers, flower number per plant, and average plant height. To assess how community-wide dissimilarity varied over time between exclosure and control treatments, dissimilarity values were plotted over time. For each response variable, a linear or quadratic regression was run to assess how dissimilarity changed over the growing season. AIC values were used to determine whether linear or quadratic equations best represented the variance of the data.

Results

The total number of flowering plants on the landscape fluctuated over the course of the growing season, peaking during the late summer (Figure 2). Based on NMDS and permanova analysis, community composition was found to change over time, producing 5 significantly different season groupings: spring, early summer, mid-summer, late summer, and fall ($F_{4,9}$ = 18.535 p = 0.005, dimensions = 2, stress = 0.04).

NMDS was used to assess how plant community functional responses differed between control and exclusion treatments. Both the magnitude and variability of all plant functional traits were higher in the exclusion treatment compared to the control treatment (dimensions = 2, stress = 0.02; Figure 3). Permanova results showed that plant functional responses were significantly greater in exclusion treatments than control treatments ($F_{1,8} = 4.81$, p = 0.02), and pairwise analysis showed that all plant response variables differed significantly between the two treatments (Table S2).

Flowering plant count in deer exclusion treatments was consistently higher than in control plots ($X^2_{1,94} = 12.79$, $p<0.001$). Deer presence had a significant negative impact on all measured plant response variables: species and floral counts, vegetative and floral area per plot, number of flowers per plant, and average plant height per species (Table 1). Based on NMDS analysis, flower species that were particularly preferred by deer included *Liatris spicata* L. (Asteraceae), *Oenothera biennis* L. (Onagraceae), *Sabatia angularis* L. (Gentianaceae), *Solidago nemoralis,* and *Symphyotrichum depauperatum* (dimensions = 2, stress < 0.001; Figure 4). Individual species scores for this ordination are shown in Table S3.

The linear relationship between vegetative and floral area was positive and highly significant (p value < 0.001, $R^2 = 0.92$). Bray-Curtis dissimilarity between control and exclusion treatments showed a slight but nonsignificant linear increase over time for flower count and plant area (p value = 0.26, p value = 0.15; Figure 5). Community dissimilarity for plant height increased linearly over the growing season (p value = 0.04, $R^2 = 0.42$). Plant count community dissimilarity followed a quadratic curve over time, peaking in the middle of summer, and then decreasing in the fall (p value = 0.02, $R^2 = 0.58$).

Discussion

Deer presence had a significant effect on plants growing in the eastern serpentine savannah. In studying the effects of deer presence on plant and flower count, vegetative and floral area, flowers per plant, and plant height for herbaceous plants, we found that throughout the growing season (Figure 2) and across the plant community (Figure 3), deer presence consistently reduced values for all plant response traits measured (Table 1). These results are consistent with numerous studies that have found deer presence dramatically reduces the abundance of flowering plants in prairie (Anderson, 2001) and temperate forest ecosystems (Ruhren & Handel, 2003, Sakata et al., 2015). Across ecosystem types, herbaceous plants are impacted by deer more than other plant taxa due to higher nitrogen content and an inability to outgrow a deer's "zone of accessibility," approximately 2 m from the ground (Alverson et al. 1988, Ruhren & Handel, 2003).

Although deer presence caused lower response values for the plant community as a whole, not every plant species experienced this herbivory pressure in the same way. Species most impacted by deer herbivory included *S. depauperatum, O. biennis, S. nemoralis, S. angularis, and L. spicata*. As shown in Figure 4, deer presence had the greatest negative impact on the height of *S. depauperatum* plants, while plant area was most affected for *S. rugosa*, and flower count was most affected for *O. biennis.* The only species that did not follow the community trend of decline due to deer presence was *Polygonum tenue* Michx. (Polygonaceae), which was only present in the control and exclusion treatments for Plot 1. In the control plot it grew taller and more abundant. The unexpected results from this species may indicate that *P. tenue* is unpalatable for deer, or reflect overall scarcity, rather than actual response to deer herbivory.

Comparison of Bray-Curtis dissimilarity for community-wide plant responses between control and exclusion treatments over the growing season showed a small, non-significant linear increase over time for the following response variables: plant area, floral area, flower number, and flowers per plant (Figure 5). Community dissimilarity for plant height showed a significant positive linear trend over time, indicating that as the growing season proceeds, plant height between control and exclusion treatments increasingly diverges. Late blooming perennials persist on the landscape for the duration of the growing season, and may be browsed continuously, increasing the impact on relative plant height over time. Community dissimilarity of plant count over time was the only response variable that had a significant quadratic relationship. Dissimilarity between treatments increased between the spring and late summer, peaking in late August and early September, before dropping back down during the fall season. This decline in dissimilarity likely reflects local weather patterns rather than deer impact. A seasonal drought in the mid-Atlantic during the fall of 2019 caused a decrease in overall herbaceous plant abundance across both treatments, causing the plant numbers to become relatively more similar.

No differences in overall herbaceous plant diversity were observed between control and exclusion treatments. Because this study was only conducted for one year, these results are not unexpected. However, a continuation of this study might find different results. Low floral diversity in some Midwest prairie ecosystems has been attributed to decades of intense deer browsing (Anderson, 2001). Serpentine barrens are ecosystems characterized by low diversity, and are also particularly vulnerable to herbivory pressures (Strauss & Boyd, 2011, Flinn et al., 2017, Safford & Malleck, 2011). Because most flowering herbaceous plants did not evolve under a strong vertebrate herbivory pressure, they are not resilient to browsing (Anderson, 2001). As a result, species that are preferentially consumed by deer at high densities may be driven to extinction, changing community composition and reducing biodiversity (Anderson, 2001, Latham & McGeehin, 2012). *S. depauperatum*, one of the species that we found was most impacted by deer, is critically endangered and near-endemic to eastern serpentine barrens (Rajakaruna, 2009, Latham & Mcgeehin, 2012, Flinn et al., 2017). Already imperiled due to a combination of habitat loss, tree encroachment, and species invasion, serpentine grasslands could lose biodiversity and be permanently degraded by continued intense browsing pressure (Latham & McGeehin, 2012, Flinn et al. 2017).

Florivory by deer not only has direct impacts on the abundance of herbaceous plants, but also indirect impacts that cascade throughout ecosystems. Many insect species in temperate grassland ecosystems are dietary specialists that have developed associations with one or a few native plant species over millions of years (Latham & McGeehin, 2012). These same insects are vital links in the foodweb for a variety of secondary consumers (Latham & McGeehin, 2012), and the loss of their host plants threatens the stability and biodiversity of the entire community. Additionally, numerous studies of deer impacts on flowering plants have observed higher density of insect pollinators in areas where deer are excluded, compared to areas of deer presence (Anderson, 2001, Ruhren & Handel, 2003). Low floral abundance leads to a reduction in pollinator visitation, causing further population decline over time (Anderson, 2001, Ruhren & Handel, 2003). While our research indicated that deer detrimentally affect eastern serpentine plant species, the impact of deer browsing on native pollinators and plant reproductive success across grasslands across the mid-Atlantic region is unknown.

The findings of this study have clear and important management implications. First, in serpentine areas where deer browsing is intense, management programs should be implemented to monitor flowering plant species that are preferentially browsed by deer (Anderson, 2001, Latham & McGeehin, 2012). Using season-specific indicator species will ensure that browsing impact is monitored throughout the growing season (Anderson, 2001). Ultimately, the local deer population must be reduced in order to sustain or restore rare eastern serpentine flora (Ruhren & Handel, 2003). Latham & McGeehin (2012) explain that any attempts to reintroduce extirpated species without drastically limiting the deer population will "be a lesson in futility". We suggest that the number of permits for recreational deer hunting on serpentine barrens be increased, and to determine whether this method is effective, that the vegetation response to hunting be monitored over time (Latham and McGeehin, 2012). If recreational hunting is not sufficient to limit the detrimental impacts of deer browsing to serpentine vegetation, sharpshooter culling may be necessary to reduce deer populations to a sustainable level (Latham and McGeehin, 2012).

Globally, temperate grasslands are considered to be one of the most critically imperiled ecosystems, having declined severely in total area and ecosystem functionality in the past century (Hoekstra et al., 2005, Latham & McGeehin, 2012). In the United States alone, more than 50% of temperate grassland habitat has been lost to anthropogenic land-use change

(Hoekstra et al., 2005). Serpentine grasslands in the eastern United States are of particular conservation concern, due to their high fragmentation, history of severe disturbance, and abundance of rare and endemic species (Latham & McGeehin, 2012, Flinn et al., 2017). As natural habitats become increasingly fragmented by anthropogenic land use changes, it is particularly important to study ecosystem interactions in naturally fragmented ecosystems (Wolf & Thorp, 2011). As such, serpentine barrens represent more than current zones of high conservation value (Flinn et al., 2017); they are excellent model systems for predicting and understanding ecosystem interactions of the future.

Figures and Tables

Figure 1: location of field sites in Owings Mills Maryland

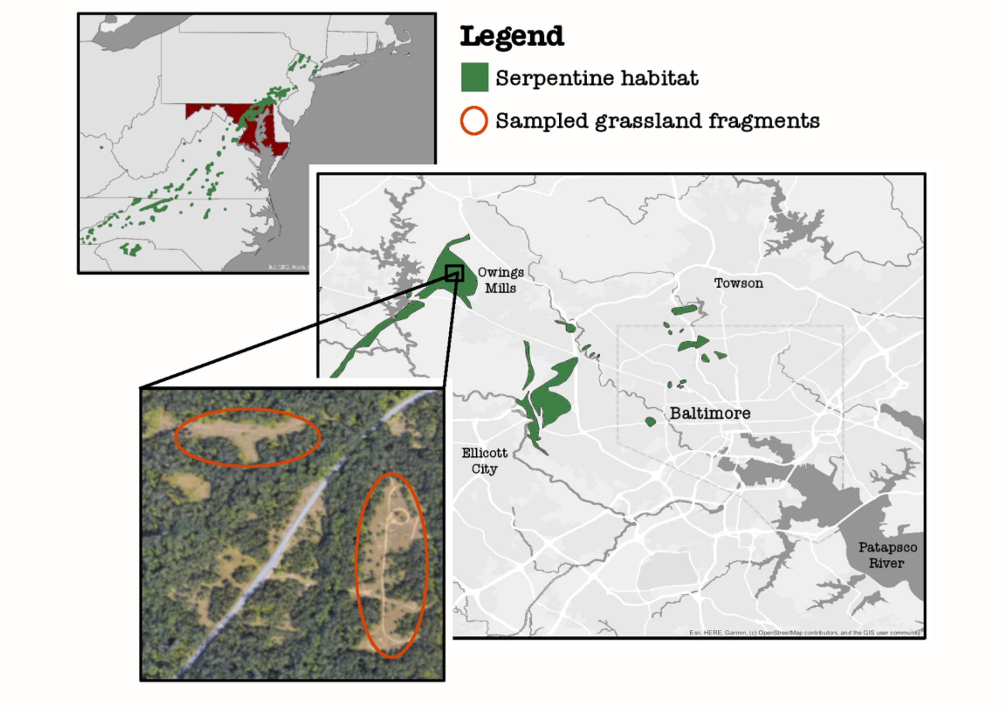


Figure 2: Total flowering plant count between deer exclusion (E) and control plots (C) over the course of the growing season

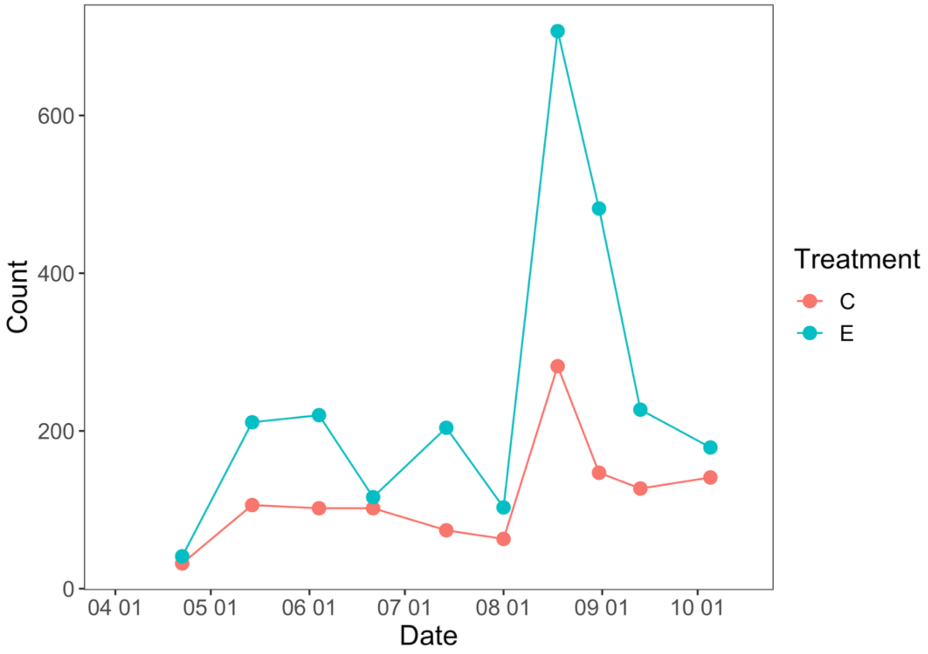


Figure 3: NMDS plot showing net vegetation response based on community weighted means of plant response variables to deer presence and absence. Points represent net average community response values for each control and exclusion plot, and arrow length reflects the relative contribution of each plant response variable to the magnitude of community response. The larger area for the exclusion treatment polygon reflects greater overall variability in plant response values compared to the control treatment.

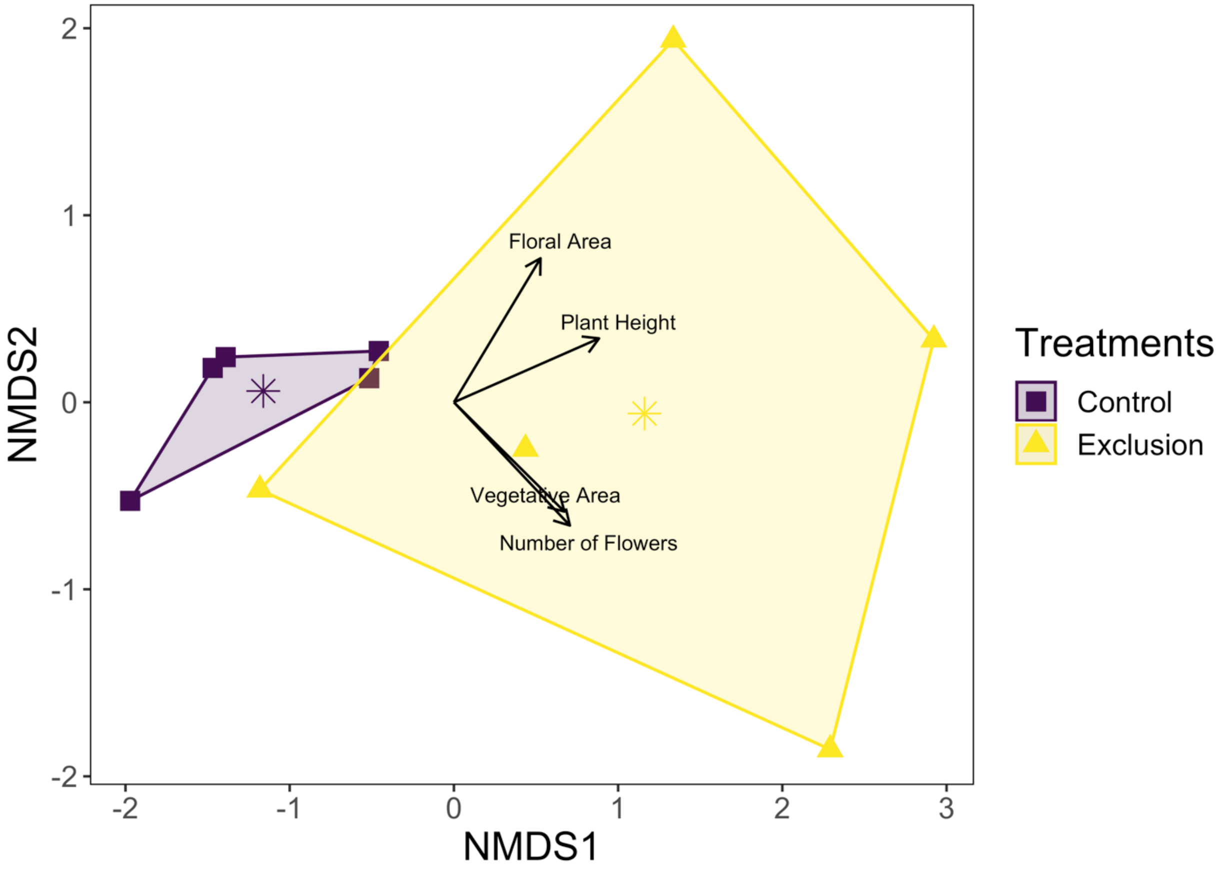


Table 1: Effect of deer presence on vegetation over time using analysis of deviance (Type II test). P values for all explanatory variables were highly significant and equal to <0.001, except for Treatment in the Average Height per Treatment GLMM, which had a p value of 0.014.

Response Variable	Model Type	Best Model	Explanatory Variable	X^2	df
Number of Plants	Negative Binomial	$n \sim Treatment + Season$	Treatment	12.79	1
			Season	31.16	4
Number of Flowers	Negative Binomial	$n \sim Treatment + Season$	Treatment	17.20	1
			Season	48.70	4
Flowers per Plant per Species	Negative Binomial	$n \sim Treatment + Species$	Treatment	103.78	1
			Species	11.63	12
Average Floral Area (cm^2)	GLMM	$n^{0.2} \sim Treatment + Season$	Treatment	13.00	1
			Season	35.33	4
Average Vegetative Area (%)	GLMM	$n^{0.3} \sim Treatment + Season$	Treatment	37.05	1
			Season	106.66	4
Average Height per Species (cm)	GLMM	$n^{0.1} \sim Treatment + Season + Species$	Treatment	6.024	1
			Season	44.92	4
			Species	426.03	12

Figure 4: NMDS plot showing that the species *Liatris spicata* (LISP), *Oenothera biennis* (OEBI), *Sabatia angularis* (SAAN), *Solidago nemoralis* (SONE), and *Symphyotrichum depauperatum* (SYDE) were most affected by deer presence, using an integrated combination of all plant response variables. Points represent average plant response values across species. Length of arrows corresponds to magnitude of the ratio in species response between disturbed and control plots, with longer length indicating greater differences between control and exclusion. Asterisks indicate p values < 0.05.

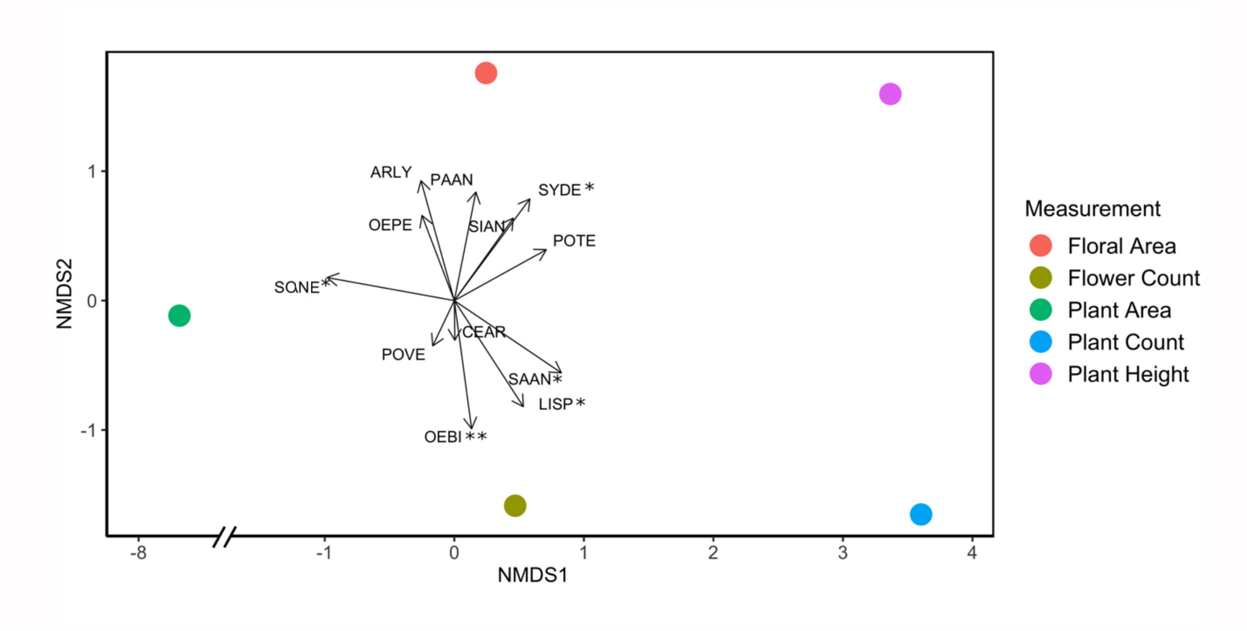
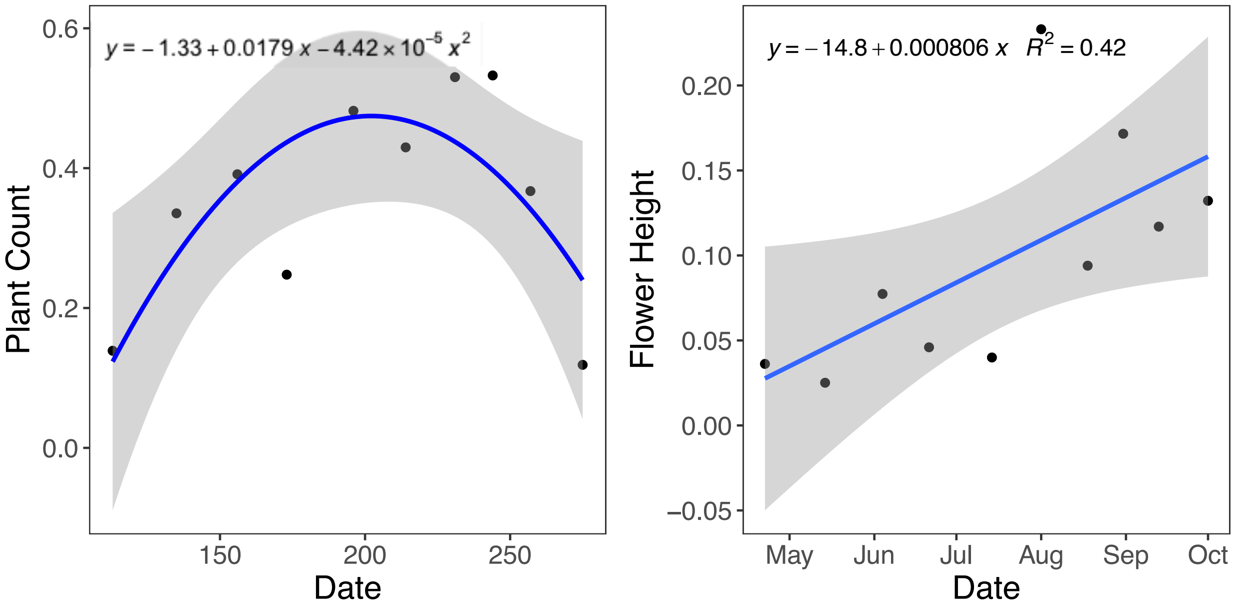


Figure 5: Bray-Curtis dissimilarity of plant response traits over the growing season. Shaded grey areas are 95% confidence intervals.



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Supplementary Materials

Table S1: Means and standard deviations for all plant response variables measured for each flowering plant species

Measurement	Treatment	ARLY	CEAR	LISP	OEBI	OEPE	PAAN	POTE	POVE	SAAN	SIAN	SONE	SYDE
Plant Count	Control	58	70	482	34	83	55	5	118	4	44	5	216
Plant Count	Exclusion	74	147	1278	136	108	65	5	222	11	63	9	371
Flower Count	Control	187	258	2054	67	108	382	24	226	9	118	36	1150
Flower Count	Exclusion	345	919	7433	337	139	842	11	310	24	288	184	2556
Floral Area (cm^2)	Control	1.8 ± 2	3.8 ± 4	3.7 ± 3	7.0 ± 7	2.9 ± 2	4.2 ± 3	1.5 ± 1	3.8 ± 2	9.8 ± 8	3.8 ± 2	0.8 ± 1	5.2 ± 6
Floral Area (cm^2)	Exclusion	2.6 ± 4	6.5 ± 15	5.0 ± 4	8.8 ± 12	2.8 ± 1	7.8 ± 8	0.7 ± 0.3	2.7 ± 1	9.5 ± 6	6.6 ± 8	2.4 ± 2	6.8 ± 8
Plant Height (cm^2)	Control	24.3 ± 10	13.7 ± 5	32.0 ± 10	23.4 ± 5	13.1 ± 4	35.9 ± 9	23.6 ± 7	9.7 ± 4	24.2 ± 7	20.9 ± 5	31.8 ± 11	34.9 ± 11
Plant Height (cm^2)	Exclusion	21.7 ± 10	15.1 ± 6	39.1 ± 14	27.1 ± 7	14.5 ± 4	40.7 ± 11	11.8 ± 2	8.1 ± 3	30.1 ± 10	19.8 ± 5	44.9 ± 12	43.3 ± 16
Flowers per Plant	Control	3.2 ± 3	3.7 ± 4	4.3 ± 3	2.0 ± 2	1.3 ± 1	6.9 ± 5	4.8 ± 3	1.9 ± 1	2.3 ± 2	2.7 ± 2	7.2 ± 5	5.3 ± 6
Flowers per Plant	Exclusion	4.7 ± 7	6.3 ± 14	5.8 ± 5	2.5 ± 3	1.3 ± 1	13.0 ± 13	2.2 ± 1	1.4 ± 1	2.2 ± 1	4.5 ± 5	20.4 ± 18	6.9 ± 8
Plant Area (cm^2)	Control	4.6 ± 2	8.5 ± 8	25.8 ± 56	3.1 ± 2.6	3.1 ± 2.6	21.2 ± 15	11.3 ± 12	13.5 ± 16	0.0	11.9 ± 23	1.5 ± 1	40.8 ± 33
Plant Area (cm^2)	Exclusion	12.7 ± 13	25.3 ± 17	45.8 ± 52	19.9 ± 26	19.9 ± 26	30.4 ± 26	4.8 ± 4	38.4 ± 39	10 ± 0	7.2 ± 13	18.6 ± 6.5	61.6 ± 42

Table S2: NMDS results from Figure 4 comparing the trait responses for each species to deer exclusion treatment. Significant p values indicate that all plant response variables were significantly different between control and exclusion treatments.

Response Variable	NMDS1	NMDS2	r2	Pr(>r)	
Plant Height	0.93312	0.35956	0.898	0.001	***
Number of Flowers	0.7304	-0.68301	0.9333	0.001	***
Floral Area	0.56559	0.82469	0.871	0.006	**
Vegetative Area	0.75451	-0.65629	0.7944	0.014	*

Table S3: NMDS results from Figure 2 comparing the trait responses for each species to deer exclusion treatment. Significant p values indicate species that experienced the strongest and most negative responses to the deer presence

Species	Code	NMDS1	NMDS2	r2	Pr(>r)	
Arabis lyrata	ARLY	-0.27	0.96	0.93	0.13	
Cerastium arvense	CEAR	0.01	-1.00	0.09	0.92	
Liatris spicata	LISP	0.54	-0.84	0.96	0.03	*
Oenothera biennis	OEBI	0.13	-0.99	1.00	0.01	**
Oenothera perennis	OEPE	-0.35	0.93	0.50	0.48	
Packera anonyma	PAAN	0.19	0.98	0.73	0.32	
Polyganum tenue	POTE	0.87	0.48	0.66	0.42	
Polygala verticillata	POVE	-0.43	-0.90	0.15	0.81	
Sabatia angularis	SAAN	0.83	-0.56	0.99	0.02	*
Sisyrinchium angustifolium	SIAN	0.58	0.81	0.61	0.38	
Solidago nemoralis	SONE	-0.98	0.18	1.00	0.02	*
Symphyotrichum depauperatum	SYDE	0.60	0.80	0.96	0.02	*

Chapter 2: Disturbance disrupts pollinator network stability in a low diversity grassland

Abstract

Plants are the foundation of terrestrial foodwebs, so disturbances that degrade mutualist networks may threaten ecosystem functionality and ecosystem services. While mutualist networks are generally robust the loss of weakly interacting species, disturbances that impact strongly interacting keystone generalist species can decrease pollinator network stability. This study assessed how metrics of mutualist network stability were impacted when keystone pollinator groups were negatively affected by deer browsing disturbance. Deer exclusion plots were used to compare plant and pollinator response between disturbed and undisturbed habitat throughout a growing season. Pollinators were sampled with pan traps and visual surveys were used to document plant-pollinator interactions. Highly abundant flower species *Liatris spicata* was found to be a keystone plant species, while the pollinator group of Dipteran flies were found to be keystone pollinators. Although flies were equally as abundant between control and exclusion plots, the diversity and intensity of their interactions (species strength) was significantly decreased in exclusion plots. It was found that while community stability metrics of interaction strength asymmetry (ISA) and connectance stayed constant between disturbed and undisturbed habitats, nestedness, which reflects network redundancy, decreased in disturbed habitats. As a result of decreased species strength of a keystone generalist pollinator group, community nestedness and overall network stability also decreased.

Introduction

Globally, habitat loss and disturbance have led to dramatic declines in the diversity and abundance of both plants and their pollinators (Biesmeijer, et al., 2006, Ricketts et al., 2008, Winfree et al., 2009). Losses of biodiversity in mutualist communities may disrupt plant-pollinator associations and alter network dynamics (Kaiser-Bunbury et al., 2010, Aslan, et al., 2013). Because plants are the foundation of terrestrial foodwebs, disturbances that degrade mutualist networks may threaten overall ecosystem functionality and ecosystem services (Harris & Johnson, 2004, Memmott et al., 2004, Potts et al., 2010). Determining factors that contribute to mutualist network resiliency or vulnerability to different types of disturbance will improve overall understanding of plant-pollinator interactions and may inform conservation and restoration efforts (Vazquez & Simberloff, 2002, Harris & Johnson, 2004, Winfree et al. 2009, Koski et al., 2015, Carman & Jenkins, 2016, Kremen et al., 2018).

Across ecosystems, most mutualist networks share certain important characteristics (Vasquez et al., 2009, Bascompte, & Jordano, 2009). A core group of highly interacting keystone generalist species are expected to have disproportionately important ecological roles as mutualists compared to more specialized species (Gilbert, 1980, Koski et al., 2015). Within pollinator networks, abundance frequently predicts which species are keystone mutualists (Koski et al., 2015). Abundant, common plants and pollinators are expected to have many interaction partners, while rare endemic species are expected to have fewer interaction partners (Vasquez et al., 2009, Koski et al., 2015). Mutualist networks generally have a high degree of redundancy, or nestedness, whereby specialist pollinators are reliant on keystone generalist plants, and specialist plants are reliant on keystone generalist pollinators (Vasquez & Aizen, 2004, Bascompte & Jordano, 2009, Vasquez et al., 2009, Potts et al., 2010, Koski et al., 2015). This nested structure implies asymmetrical interaction strength between generalist and specialist species, and also indicates the importance of a core group of generalist species to overall network structure and function (Bascompte & Jordano, 2009, Vasquez et al., 2009). Mutualist networks also exhibit low overall connectance- the proportion of possible interspecific interactions that actually occur (Bascompte & Jordano, 2009, Vasquez et al., 2009). Because most plant-pollinator interactions are relatively weak, a few strong interactions provide the structural basis for most plant-pollinator networks (Bascompte & Jordano, 2009, Vasquez et al., 2009).

Past studies have examined the impacts of habitat loss, fire, grazing, and mechanical disturbance on pollinator networks (Winfree et al., 2009). Disturbance may impact mutualist networks by reducing both the diversity and abundance of plants and pollinators (Winfree et al., 2009). Species that are habitat or dietary specialists often have little flexibility to cope with environmental changes, and are particularly vulnerable to disturbance (Harris & Johnson, 2004, Biesmeijer, et al., 2006, Ricketts et al., 2008, Winfree et al., 2009). As a result, pollinator networks in disturbed communities are predicted to be simpler, have fewer specialist species, and be more dominated by generalists (Janzen, 1974, Vasquez & Simberloff, 2003, Harris & Johnson, 2004, Carmen & Jenkins, 2016). Because of their nested structures, most mutualist networks are resilient to the losses of weakly interacting species, but very vulnerable to the loss of strongly interacting generalist species (Vasquez & Aizen, 2004, Bascompte & Jordano, 2009, Vasquez et al., 2009). Losses of essential keystone species in interaction networks may lead to extinction cascades, whereby the loss of one or a few highly connected species triggers additional extinctions throughout a network system (Landi, 2018). In low diversity systems where keystone species are especially important, their losses may result in complete disassembly of mutualist webs (Rodriguez-Cabal et al., 2013). Identifying keystone mutualists and determining whether their abundance or interactions are altered due to disturbance is therefore necessary to quantify the impact of disturbance to ecosystems (Koski et al., 2015).

Although individual keystone species may have strong impacts on network structure and function, flowers and pollinators are often present on the landscape for only a short period of time (Caradona et al, 2017). Mutualist networks based on pollinator functional groups rather than species identities can show patterns and trends throughout the growing season, even as plant and pollinator species composition vary over time (Fontaine et al., 2006, Koski et al., 2015). To identify keystone mutualist functional groups, three network parameters are commonly used: **strength**, the number of interaction partners a species has and the frequency of those interactions, **node specialization index (NSI)**, the number of interaction partners that a species or group shares with other species, and **degree of specialization (d^0)**, which indicates whether flower species are visited by opportunists or common pollinator species (Bascompte et al., 2006, Dormann, 2011, Koski et al., 2015). High strength, low NSI, and low d^0 indicate keystone mutualists that are especially important for maintaining connectivity within mutualist networks (Koski et al., 2015).

Ecosystem resilience, or resistance to disturbance is often predicted by network complexity and biodiversity (Bascompte & Jordano, 2007, Carman & Jenkins, 2016). Resilience may also be conferred by high levels of connectance, by strong symmetrical pairwise interactions between plants and pollinators, and by a high degree of nestedness (Vasquez & Aizen, 2004, Okuyama & Holland, 2008, Bascompte & Jordano, 2009, Vasquez et al., 2009, Passmore et al., 2012, Potts et al., 2010, Koski et al., 2015). Although mutualist networks are generally robust to disturbance and species loss, if keystone mutualists are strongly affected by disturbance, network stability can be degraded (Bascompte & Jordano, 2009, Rodriguez-Cabal et al., 2013, Landi, 2018). By comparing metrics of **nestedness**, **connectance**, and **interaction strength asymmetry (ISA)** between disturbed and undisturbed areas of the same habitat, ecosystem stability and resilience to disturbance can be estimated (Kaiser-Bunbury et al., 2010, Passmore, 2012).

This study assessed mutualist network response to overbrowsing by *Odocoileus virginianus* Zimmerman, 1780 (white tailed deer, Cervidae) in a low diversity serpentine grassland system over the course of one growing season. Unlike grazing or habitat fragmentation, browsing is a selective type of disturbance that disproportionately impacts certain plant species over others (Rooney, 2001, Rooney & Waller, 2003, Latham et al., 2005, Rawinski, 2008, Averill et al., 2017). Through selective browsing, especially of plant reproductive structures, *O. virginianus* can reduce the density and diversity of flowering plant communities, disrupt plant pollinator associations, (Vazquez & Simberloff, 2003, Geddes et al., 2006, Wang et al., 2008) and damage important mutualist networks (Miller et al., 1992, Wang et al., 2008, Rodriguez-Cabal et al., 2013, Sakata & Yamasaki, 2015). In low diversity ecosystems with limited possibilities for ecological interactions, targeted disturbances like browsing may have especially strong impacts on mutualist networks (Gilbert, 1980, Koski et al., 2015).

Specific objectives for this research included: 1. Determining keystone plants and pollinator groups and assessing whether their abundances or interactions were affected by deer disturbance, and 2. Quantifying how network stability and resilience responded to disturbances to keystone mutualists over the growing season. Common, abundant flower species and pollinator functional groups were predicted to be important keystone species with many interaction partners. While overall abundance of plant and pollinators was predicted to be lower in disturbed habitat, network structure and stability were not predicted to change unless a keystone pollinator group was impacted.

Methods

Study Site

Covering only 3,400 acres in total, serpentine grasslands in the eastern United States are home to both globally rare endemic plant species and regionally endangered plant and pollinator species (Flinn et al., 2017, Rajakaruna, 2009). Serpentine ecosystems are heavily fragmented, low-diversity ecosystems that harbor rare and endemic plant species with unique adaptations to combat harsh edaphic conditions (Latham & McGeehin, 2012, Flinn et al., 2017). Herbaceous plants growing on serpentine soils typically have smaller flowers and reduced pollen and nectar sources, thus more limited floral resources for pollinators, compared to plants in surrounding habitats (O'dell & Rajakaruna, 2011, Wolf & Thorpp, 2011). Threatened by a combination of species invasions, land-use change, and an unsustainably large deer population, eastern ecosystems are considered to be one of the highest conservation priorities in the United States (Prince, et. al, 2004, Floyd, 2006, Latham & McGeehin, 2012).

Located in suburban Baltimore, Soldiers Delight Natural Environmental Area includes the largest remaining eastern serpentine grassland ecosystem in the United States (Floyd, 2006, Tyndall & Hull, 1999). Dominant grass species at this site include *Schizachyrium scoparium* Michx. (little bluestem, Poaceae) and *Sorghastrum nutans* Nash. (indian grass, Poaceae) (Tyndall & Hull, 1999, Tyndall, 1994). Over 39 rare, threatened, or endangered plant species can be found at Soldiers Delight, including the *Gentianopsis crinita* Froel. (fringed gentian flower, Gentianaceae), *Symphyotrichum depauperatum* Nesom. (serpentine aster, Asteraceae), *Cerastium arvense L. var. villosum* Hollick & Briton (serpentine chickweed, Carophyllaceae) and *Agalinis decemloba* Greene (ten lobe false foxglove, Scrophulariaceae) (Tyndall & Hull, 1999, Tyndall, 1994, Tyndall, 2005, Floyd, 2006, Flinn, et al., 2017). Numerous rare pollinator species are also found at Soldiers Delight, including *Hesperia leonardus* Harris 1862 (Leonard's skipper, Hesperiidae), *Hesperia metea* Scudder 1863 *(*cobweb skipper; Hesperiidae*)* and *Satyrium edwardsii* Grote & Robinson (Edwards hairstreak; 1867). The population of white tailed deer at Soldiers Delight has dramatically increased in the past few decades (Floyd, 2006). The increased browsing pressure has had a significant, but undescribed impact on the rare flowering plants at Soldiers Delight and, presumably, their pollinators.

Deer Exclusion Plot Construction

Exclusion plots are often used to assess the effects of large herbivores on plant and insect communities (Rambo & Faeth, 1999, Pasari et al., 2014, Stephan et al. 2017, Averill et al., 2017). Two sites at Soldiers Delight with similar physical, topographic, and edaphic characteristics were identified (39.4030190, -76.8240320, and 39.4025640, -76.8214420), shown in Fig. 1. Across the two sites, we constructed five 5m x 5m deer exclusion plots, each paired with an adjacent control plot. Due to shallow, rocky soil at the study site, exclusion plots were anchored using 5-gallon buckets of cement with 2 m tall wooden posts. 2 m tall polypropylene fence with 2" mesh openings was stretched between posts, and 4'' by 6'' holes were cut in the bottom of the fence in order to allow small mammals to move in and out of the plots. 1 cm thick wires were then wrapped around the base of each structure to prevent fawns from entering the plots.

Plant Sampling

To quantify the impact of deer browsing on flowering plant abundance, monthly plant inventories were conducted. For each sampling event, the number of flowering plants and the total number of open flowers was recorded for each plot (CaraDonna et al., 2017). To capture peak bloom for all species present, sampling was conducted every 2-3 weeks throughout the growing season of 2019.

Certain pollinator species, particularly bumblebees, will preferentially visit patches of vegetation with high floral areas (Westphal et al., 2003, Hegland et al., 2006). For each flowering species observed, we photographed 30 inflorescences next to a ruler, and determined average floral areas for each species using the program ImageJ (Schneider et. al, 2012). Because ImageJ has issues determining the area of objects with central gaps, the area for compound flowers *Solidago rugosa* Mill (Asteraceae) and *Packera anonyma* Weber & Love (Asteraceae) was measured in the program GIMP (The GIMP Team, 2020). For these species, the total number of pixels of flowers for each plant were divided by the number of pixels in a 1 cm square. This number was then divided by the number of inflorescences of the plant to determine average area per inflorescence. Due to tiny inflorescence size of *Polygala verticillata* L. (Polygalaceae; about 8 sq mm), floral area for this species was calculated for entire flowers rather than individual inflorescences. Average floral area for each species was multiplied by the number of inflorescences of each species present in each plot, to obtain a standardized measure of species floral area (Hegland, 2006). Species floral areas from each plot were additionally added together, to calculate total floral area per plot.

Pollinator Sampling

Pan traps are a common and effective method for collecting aerial pollinators such as wasps, bees, and flies (Campbell & Hanula, 2007, Tuell & Isaacs, 2009, Rubene et al. 2015, Moreira et al., 2016). Brightly colored plastic bowls in yellow, white, and blue colors were covered in 1x1" mesh squares to prevent the unintentional killing of endangered butterfly species. These three colors are frequently used in combination for pollinator capture studies in the eastern United States flies (Campbell & Hanula, 2007, Rubene et al. 2015). At each sampling event, one trap of each color was positioned at ground level, and one trap of each color was positioned at vegetation height (1 m tall), on a wooden stand (Tuell & Isaacs, 2009, Moreira et al., 2016). A random number generator was used to determine which corners of a plot the raised and ground-level traps would be placed, and also the color order of the traps on the wooden stands (Moreira et al., 2016). Each trap was filled ¾ of the way full with water and a few drops of dish soap as a surfactant. Traps were installed in at approximately 10:00 am on one morning, and collected at approximately 6:00 pm the following evening, collecting insects for a period of about 32 hours. The insects from each trap were stored in 70% ethanol and then identified to the highest taxonomic level possible.

Visual insect surveys were also conducted to determine plant-pollinator associations and frequency of flower visitation between control and exclusion treatments (Westphal et al., 2003). Surveys were conducted for twenty-minute periods, whereby one researcher would observe pollinator activity in the control plot and another researcher would simultaneously observe the exclusion plot. Each researcher would record the identity of each pollinator that entered a plot, the amount of time that it spent in the plot, the number of inflorescences it visited, and the species of each visited inflorescence. Surveys were conducted under weather conditions favorable for pollinators: temperature > 18 ºC, sunshine, and low wind (<6 m/s) (Lazaro et al., 2016). During each sampling period, each plot was observed two to three times, depending on weather conditions.

Statistical analyses

To quantify changes in plant community composition over the growing season, nonmetric multidimensional scaling was used (NMDS; McCune & Grace, 2002). The input data were total counts for flowering plant species at each sampling date. The NMDS was performed using the package 'vegan' in R, using the Bray-Curtis dissimilarity index as a distance measure (Oksanen et. al, 2019). Based on NMDS and permanova analysis, 5 significantly different plant community groups were present over time: spring, early summer, mid-summer, late summer, and fall.

Based on captures from pan traps and insects observed during visual surveys, seven pollinator functional groups were created: ants, butterflies, skippers, bees, beetles, wasps, and flies. Each of these pollinator functional groups was found to be present during multiple seasons, and interacted with multiple flower species.

To examine pollinator community response to deer disturbance, pollinator response variables were compared between control and exclusion plots over time. Total count of pollinators visiting plots, count of pollinators caught in pan traps, count of pollinators that visited flowers per plot, and average number of flowers visited were compared between control and exclusion treatments for each unique combination of season, pollinator functional group, plot, and treatment using a negative binomial regression with repeated measures. A negative binomial regression with repeated measures was also used to compare floral abundance between control and exclusion plots for each plant species. All negative binomial regressions were run using the package "MASS" in R (Venables & Ripley, 2002). Average time spent visiting flowers for each unique combination of season, plot, insect type, and treatment were analyzed using linear mixed effects model with repeated measures (LMMs). In the LMM, "plot" and "quadrat" were treated as a random factors. The model was then validated visually (Zuur et al., 2010) leading to Box-

Cox transformations of some response variables. LMMs were run using the package "lme4" in R (Bates et al., 2015), and best models were determined based on AIC values.

Shannon's diversity index was calculated for pollinating taxa caught in pan traps, and linear mixed effects models were used to assess how overall diversity varied between treatments over time, and also how diversity varied between treatments in pollinator functional groups. Linear regressions were then used to assess how flower visitation related to flower number, floral area, and time spent in plots by pollinators for each plot and treatment.

Plant–flower visitor networks were constructed using the bipartite package in R (Dormann et al. 2008). To compare network response to disturbance, nestedness, connectance, and interaction strength asymmetry (ISA) were calculated for control and exclusion treatments at each season and plot. Connectance was calculated as the fraction of realized links relative to total possible links (Dunne et al. 2002). Nestedness was calculated by quantifying deviations between a theoretical perfectly nested matrix and the matrix of interest (Rodríguez-Gironés & Santamaría, 2006). Nestedness values ranged from 0-100, with zero representing perfect nestedness. ISA is equal to the difference between relative dependencies of mutualists divided by the maximum mutualist dependency value (Bascompte, et al., 2006). Species network metrics of strength, NSI, and d^0 were also calculated for pollinator functional groups at each unique combination of plot and treatment to determine which plant and pollinator species were keystone species. Strength was calculated as the sum of dependences of all organisms relying on a given mutualist value (Bascompte, et al., 2006). NSI was calculated as the as the mean geodesic distance between node positions (Dalsgaard, et al., 2008). We calculated d^0 as the coefficient of variation of interactions of a mutualist, normalized to values between 0 and 1 (Julliard et al., 2006, Poisot et al., 2012). All species and community metrics were calculated using the bipartite package in R (Dormann et al. 2008). LMMs with repeated measures were then used to compare species and community network metrics between treatments for pollinator groups.

Results

Ten flowering plant species and seven pollinator groups were found to interact in the mutualist network at Soldiers Delight (Figure 1, Table S1). No pollinators were observed to visit plant species *Polygonum tenue* or *Viola spp*. Total count of pollinators visiting plots was equal between exclusion and control plots for all insect functional groups except for butterflies and day-flying moths, which were more common in control plots ($F_{6,408}$=31.7, p<0.001; Table 1, Table S2). Counts of pollinators that visited flowers per plot and counts of pollinators caught in pan traps were equal between control and exclusion plots for all insect groups ($F_{1,261}$=2.6, p=0.10; $F_{1,357}$=0.73, p=0.40). However, average time spent visiting flowers by individual pollinators, and average number of flowers visited by each pollinator were significantly higher in exclusion plots compared to control plots ($F_{1,360}$=39.2, p<0.001; $F_{1,337}$=39.4, p<0.001).

Floral abundance, floral area, and time spent in plots by pollinators were all positively correlated with floral visitation ($F_{1,23}$=67.9, p<0.001, R^2 = 0.92; $F_{1,23}$=43.5, p<0.001, R^2 = 0.65; $F_{1,23}$=11.6, p=0.002, R^2 = 0.34). Shannon's diversity did not vary between treatments for different pollinator functional groups and did not vary between treatments over time ($F_{5,40}$=3.6, p=0.87 $F_{4,12}$=1.0, p=0.91). Floral abundance was higher overall in exclusion treatments compared to control treatments, but there were no significant differences in floral abundance between treatments for individual flower species ($X^2_{10,167}$=8.2, p=0.61; Table S2).

Community metrics of nestedness, connectance, and ISA were compared between control and exclusion treatments. While connectance and ISA did not vary significantly between control and exclusion plots ($F_{1,8}$=0.21, p=0.66, $F_{1,28}$=1.5, p=0.25), nestedness was significantly lower in control plots compared to exclusion plots ($F_{1,8}$=5.8, p=0.04; Figure 2).

With 1760 total flowers counted over the growing season, flower species *Liatris spicata* L. (Asteraceae) was very abundant during mid and late summer. Due to its high species strength ($F_{9,56}$=19.2, p<0.001) and low d^0 ($F_{9,56}$=5.1, p<0.001), *L. spicata* was determined to be a keystone species (Figure 3).The second most abundant species, *Symphyotrichum depauperatum* also had a very low d^0 ($F_{9,56}$=5.1, p<0.001). Values for NSI were similar across all plant species except specialist *Sabatia angularis* L. (Gentianaceae), which was mostly found to be pollinated by bees in the genus *Ceratina* ($F_{9,56}$=2.3, p=0.03).

Skipper butterflies (Hesperiidae) were the most abundant pollinator group (Table S4), and were dominant pollinators for *L. spicata*, and also an important pollinator for *S. depauperatum.* Despite their high abundance, the overall species strength for skippers was relatively low, and the d^0 was relatively high (Figure 4). Although less abundant, flies were a dominant keystone pollinator group with high species strength ($X^2_{6,55}$=46.3, p<0.001) and low d^0 ($X_{6,62}$=48.2, p<0.001). While values for ISA were similar between treatments for most taxa, the species strength of flies was significantly lower in control plots as compared to exclusion plots ($X_{6,62}$=13.6, p<0.03) NSI was low and constant across all pollinator taxa ($X_{6,62}$=10.6, p=0.1)

Discussion

This study assessed how metrics of mutualist network stability were impacted when keystone pollinator groups were negatively affected by disturbance. While ISA and connectance were constant across disturbed and undisturbed treatments, browsing had the overall effect of decreasing pollinator network nestedness. Nestedness, which reflects network redundancy, is an important factor that maintains stability and resilience in pollinator networks (Vasquez & Aizen, 2004, Okuyama & Holland, 2008, Bascompte & Jordano, 2009, Vasquez et al., 2009, Passmore et al., 2012). A decrease in nestedness following disturbance likely reflects that keystone mutualists were negatively impacted (Bascompte & Jordano, 2009, Rodriguez-Cabal et al., 2013, Landi, 2018).

The hypothesis that abundance would predict whether or not taxa were keystone pollinators was partially supported. *L. spicata* was both the most abundant flowering plant species, and the most connected keystone plant species at Soldiers Delight. The correlation between abundance and connectivity can be explained by the concept of interaction neutrality (Vasquez et al., 2009). Interaction neutrality describes a scenario whereby all individual mutualists have the same probability of interacting with other individuals, regardless of their taxonomic identity (Vasquez, 2005, Vasquez et al., 2007, Vasquez et al., 2009). As a result, abundant species will interact more frequently and with a greater diversity of partners than rarer species (Vasquez, 2005, Vasquez et al., 2007, Vasquez et al., 2009). When the abundance of mutualists in networks are unevenly skewed, the distribution of interactions between mutualists will also be skewed (Vasquez et al., 2009). Although skipper butterflies were the most abundant pollinator group across all sites, they had low species d^0. These results suggest that skippers are relatively more specialized than other pollinator groups.

Although they were not the most abundant pollinator, flies were found to be the most important keystone pollinator, with high species strength and low species specificity (low d^0). From this functional group, 85% of observed pollinating flies were syrphid (Syrphidae), 4% were bee flies (Bombylidae), and 11% were unidentified non-Syrphid Dipterans. Many studies have emphasized the roles of flies, particularly syrphid flies, as highly connected and abundant generalist pollinators in mutualist networks (Branquart & Hemptinne, 2000, Orford et al., 2015, Klecka, et al., 2018). While flies were not observed to be abundant relative to other pollinator groups in this study, the small size and active flight pattern of syrphid flies likely made them difficult to detect (Weems, 1958). We assume that flies were probably more abundant as pollinators than were observed.

While the interactions of most pollinator groups were not impacted by disturbance, flies were found to be less connected and have lower species strength in control plots compared to exclusion plots. The impact of disturbance on flies and not other pollinator groups may be related to their relatively small body size. Small-bodied pollinators have generally been shown to forage over shorter distances than larger pollinators (Greenleaf et al., 2007, Carman & Jenkins, 2016). Large-bodied pollinator groups that forage across wide spatial areas may be less affected by local disturbances, and therefore equally as abundant and interactive with flowers between disturbed and undisturbed areas (Memmott et al., 2004, Fortuna & Bascompte, 2006, Carman & Jenkins, 2016). However, small pollinators like syrphid flies may be more sensitive to disturbance (Carman & Jenkins, 2016).

A reduction in species strength for flies may explain lower network nestedness in control plots compared to exclusion plots. As a keystone pollinator group, flies have a disproportionate impact on the structure and function of the overall mutualist network. Even though flies were equally as abundant between control and exclusion plots, the diversity of their interactions were significantly decreased. As a result of limited interaction diversity of a keystone generalist pollinator group, community nestedness may have decreased due to deer browsing (Bascompte & Jordano, 2009, Rodriguez-Cabal et al., 2013, Landi, 2018).

This study was novel in that it assessed how pollinator networks responded to browsing - a distinct and targeted form of disturbance. Other studies of mutualist network response to disturbance have focused on grazing, or habitat fragmentation, or fire, which are all forms of disturbance that impact ecosystems more homogeneously (Winfree et al., 2009). In this study, although overall flower abundance was lower in browsed areas compared to control areas, abundance and interaction diversity of individual flowering plant species was not significantly different between treatments. In ecosystems with plant species particularly targeted by browsers, however, browsing may have stronger impacts on community stability (Vazquez & Simberloff, 2003, Geddes et al., 2006, Wang et al., 2008). Rodriguez-Cabal et al. (2013) found that exotic ungulate browsing resulted in a trophic cascade that disassembled an entire interaction web.

Despite perturbations that limit the diversity and abundance of mutualists and their interactions, most pollinator networks have been shown to be dynamic, resilient systems (Kaiser-Bunbury et al., 2010, Burkle et al., 2013, Carradona et al., 2017). Carradona et al. (2017) describes "interaction re-wiring”, an attribute of mutualist networks whereby mutualist interactions are regularly reassembled over time due to seasonal and interannual changes. If temporal flexibility in interactions is an intrinsic component of mutualist networks, interaction re-wiring can be expected to buffer network stability and robustness in the face of species loss Kaiser-Bunbury et al., 2010, Burkle et al., 2013, Carradona et al., 2017. Future studies of pollinator networks should evaluate factors that make networks resilient or vulnerable to targeted disturbances such as acute browsing.

Figures and Tables

Figure 1: Cumulative plant-pollinator interactions in control (A) and exclusion plots (B), including dominant plant species *Liatris spicata* (LISP), and then excluding it (C,D).

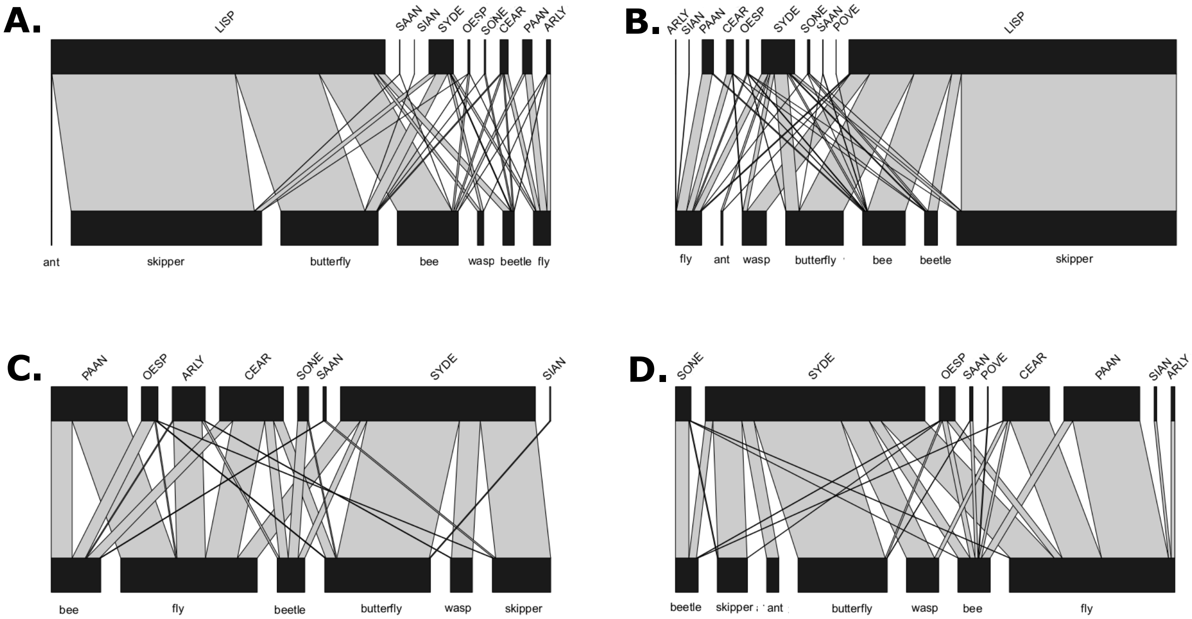


Table 1: Effect of deer presence on pollinators over time using analysis of deviance (Type II test). P values for all explanatory variables were highly significant and equal to <0.001.

Response Variable	**Model Type**	**Best Model**	**Explanatory Variable**	**X^2**	**df**
Count of Pollinators Visiting Plots	Negative Binomial	*n ~ Season + Type + Type*Season + Type*Treatment*	Season	76.7	4
			Type	117.1	6
			Type*Season	98.8	22
			Type*Treatment	31.7	6
Count of Pollinators Visiting Flowers	Negative Binomial	*n ~ Season + Type + Type*Season*	Season	38.7	4
			Type	101.9	6
			Type*Season	69.8	19
Count of Flowers Pollinated per Species	Negative Binomial	*n ~ Treatment + Species*	Treatment	39.4	1
			Season	119.9	4
			Type	54.1	6
			Type*Season	63.9	19
Average Time Spent Visiting Flowers	LMM	$n^{0.2}$ *~ Treatment + Season + Type*	Treatment	16.3	1
			Season	6.7	4
			Type	30.7	6
Count of Pollinators Collected in Bowl Traps	Negative Binomial	*n ~ Type + Type*Season*	Type	57.6	9
			Type*Season	90.6	26

Figure 2: Metrics of community mutualist network resilience between control and exclusion treatments. Nestedness scores are based on a scale of 0-100 with 0 being perfectly nested. A lower nestedness score for exclusion treatments indicates that the community is more nested. Interaction strength asymmetry and connectance are equal for mutualist networks across treatments.

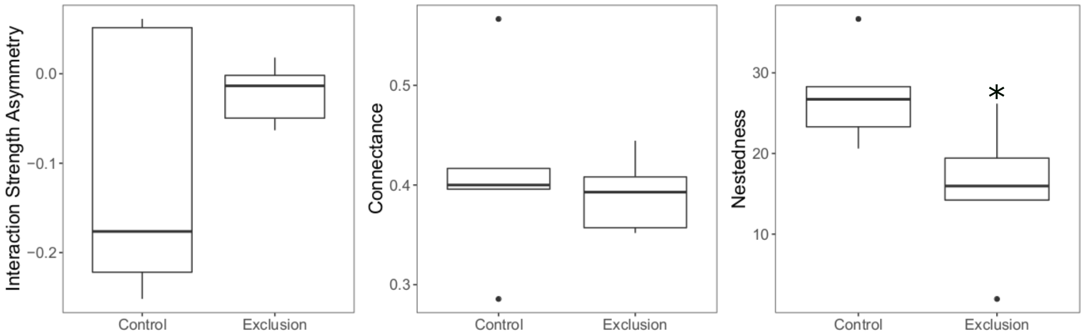


Figure 3: Species network metrics for plant species. Asterisks indicate significantly different species values. Keystone plants are expected to have high species strength, low species specificity, and a low node specialization index.

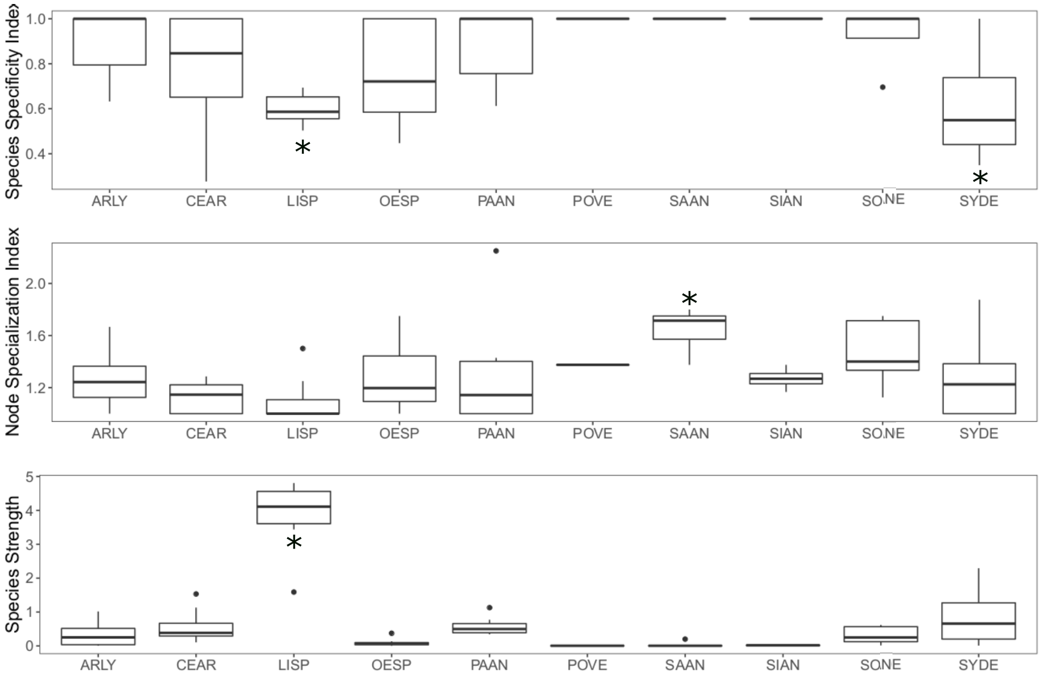
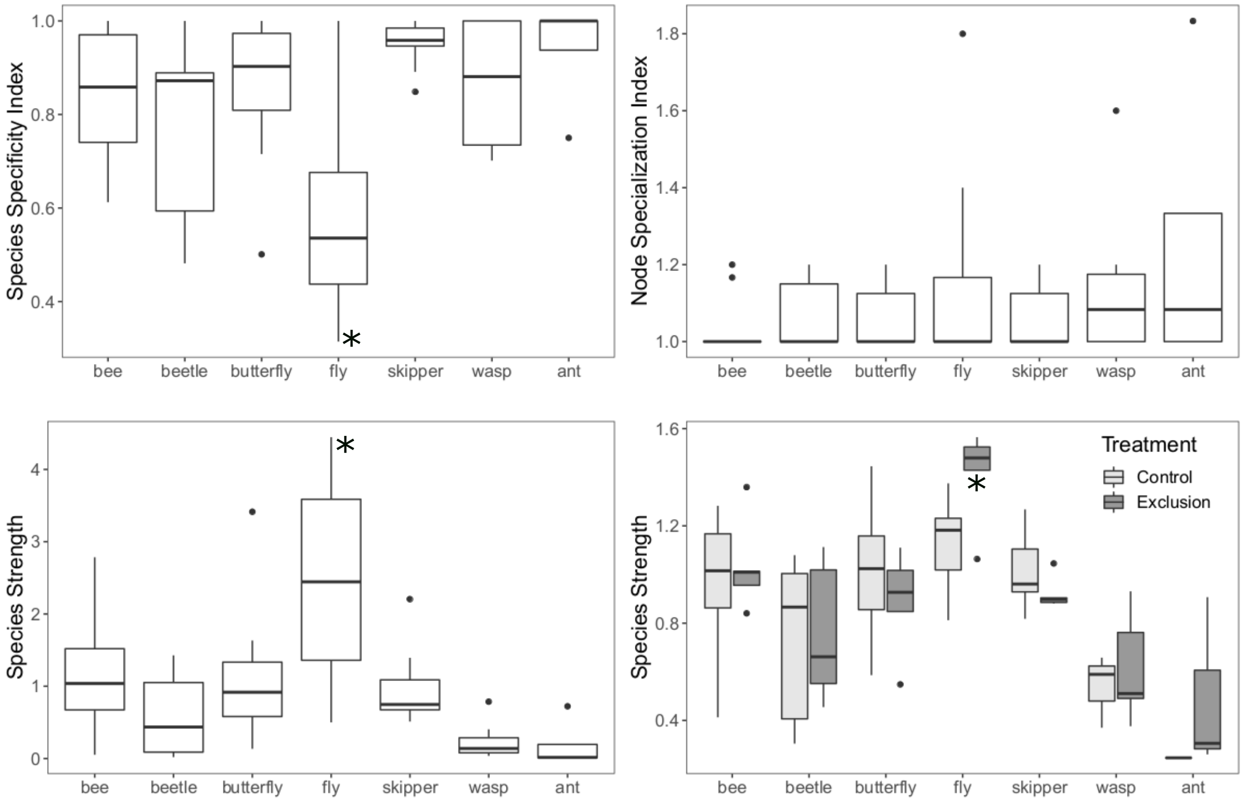


Figure 4: Species network metrics for pollinator species. Asterisks indicate significantly different species values. Keystone pollinator groups are expected to have high species strength, low species specificity, and a low node specialization index. Species strength of flies is significantly higher in exclusion plots compared to control plots.



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Supplementary Materials

Table S1: Total flower counts for all flowering plant species in experimental plots, and the number of flowers visited by pollinators in control and exclusion plots for each species.

Plant Species	Species Code	Total Flower Count	Flowers visited in controls	Flowers visited in exclusions
Arabis lyrata	ARLY	132	30	9
Cerastium arvense	CEAR	217	58	112
Liatris spicata	LISP	1760	2413	5185
Oenothera spp	OESP	361	15	38
Packera anonyma	PAAN	120	74	181
Polygala verticillata	POVE	340	0	2
Polyganum tenue	POTE	10	0	0
Sabatia angularis	SAAN	15	3	8
Sisyrinchium angustifolium	SIAN	107	1	6
Solidago nemoralis	SONE	14	11	37
Symphyotrichum depauperatum	SYDE	587	177	524
Viola spp	VISP	1	0	0

Table S2: Total counts for all pollinator groups observed in experimental plots during visual surveys, and means and standard deviations for all response variables measured for each pollinator group.

Measurement	Treatment	Ant	Bee	Beetle	Butterfly	Fly	Skipper	Wasp
Total count that entered plots	Control	4	136	49	267	97	373	43
Total count that entered plots	Exclusion	18	170	77	99	156	301	56
Count that visited flowers	Control	1	66	33	111	60	225	14
Count that visited flowers	Exclusion	14	99	76	71	117	257	31
Mean time spent in plots (s)	Control	195.8 ± 390	49.1 ± 101	194.6 ± 346	50.1 ± 124	60.5 ± 154	102.3 ± 175	27.4 ± 92
Mean time spent in plots (s)	Exclusion	539.6 ± 415	88.8 ± 159	847.8 ± 437	151.8 ± 245	218.1 ± 332	198.1 ± 233	109.6 ± 207
Total flowers visited	Control	5	440	82	703	129	1378	45
Total flowers visited	Exclusion	32	676	205	913	414	3478	384

Vita

Allyson Richins was born on August 27, 1993 in Riverside CA. She was raised in Las Cruces, New Mexico and received her Bachelors of Science in 2015 in Biology with a concentration in Ecology from the University of New Mexico. During her undergraduate degree, she completed a senior honors thesis on aridland grasshopper foraging strategies using stable isotopes under the mentorship of Drs. Seth Newsome and Dave Lightfoot. Upon graduation, Allyson traveled around Central and South America, working for NGOs and conservation research projects and working with a variety of wildlife, including grey whales, sea turtles, and pumas. She then spent a year working for the University of Wisconsin as a forest entomology technician, and then a tropical plants field technician in Hawaii. Allyson joined the lab of Catherine Hulshof at Virginia Commonwealth University in August 2018, where she has enjoyed working on various field and data-related projects in addition to her thesis.