Title

The Effects of Anthropogenic Global Changes on Prairie Plant-Pollinator Mutualisms

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Publication Date

2024

The Effects of Anthropogenic Global Changes on Prairie Plant-Pollinator Mutualisms

By

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DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTORATE OF PHILOSOPHY

in

Ecology

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

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2024

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Abstract

Plant-pollinator mutualisms are essential to biodiversity and food security, yet pollinators and the plants that depend on them are declining due to anthropogenic stressors. Understanding how multiple interacting anthropogenic stressors affect the structure and dynamics of plant-pollinator mutualisms across spatial scales remains an emerging research area. In particular, evidence remains equivocal as to whether plant invasions have positive or negative effects on plant-pollinator mutualisms. Invasive plants, highly attractive to pollinators, can paradoxically support native pollinator diversity, while in other cases invasive plants can decrease pollinator and create novel communities via invasional meltdowns.

California grasslands provide an ideal opportunity to explore these questions and apply the findings to ecological restoration. California grasslands are a hotspot for plant and insect diversity, but are vulnerable to anthropogenic changes, such as climate change, plant invasions, and nutrient deposition. My research occurred in California grasslands in the Coast Range. The area contains a mosaic of heterogenous soils and plant communities: nutrient deficient serpentine soils provide a refugium for a diverse community of native plants, while nutrient-rich non-serpentine soils contain invasive-dominated plant communities. The invasion of exotic annual grasses in what were historically forb-dominated meadows provides a system in which I can examine whether invasive grasses have indirect effects on pollinators via their effects on the forb community. This patchy landscape provides an opportunity to explore questions related to the spillover of pollinators across plant community boundaries and consequent impacts on plant fitness. Moreover, California grasslands are vulnerable to a suite of interacting anthropogenic stressors in addition to plant invasions, particularly climate change, defaunation, and nutrient enrichment. The system has high interannual variability in precipitation and temperature and is sensitive to increased extremes in precipitation under climate change. My doctoral work examines how aridity, plant invasions, and nutrient enrichment alter the structure and diversity of plant communities and their pollinators across space and time. My research combines approaches from a diverse fields including spatial ecology, network theory, community ecology, botany, entomology, restoration ecology, and data science.

My first chapter integrates an invasive grass removal experiment with dynamical consumer resource models to examine whether removing a serpentine-tolerant invasive grass species has downstream effects on pollinators and plant-pollinator interaction networks. While much work has explored the effects of invasive forbs on pollinators, a gap remains as to whether plant-to-plant competition between grasses and forbs for non-pollinator resources (e.g. soil, light) can have consequences for the diversity, structure, and abundance of plant-pollinator interactions. We found that grass removal increased pollinator abundance and diversity, increasing the functional redundancy of the network and enhancing the role of a core generalist wildflower as a hub for pollinators. By comparing these empirical findings to our dynamical model, we demonstrated that consumer-resource theory could benefit from including pollinator spatial ecology and nesting biology to account for increases in diversity that are likely due to pollinator dispersal and increases in bare ground patches for ground-nesting bees.

My second chapter examines whether invasive wildflowers can have cross-ecosystem effects on the pollination, fitness, and plant-pollinator network structure of native wildflowers via the spillover of shared pollinators across ecological boundaries. Patchy mosaics of serpentine and non-serpentine soils provide an opportunity to test these questions. We conducted an

observation study of meadows containing serpentine-non-serpentine ecosystem boundaries across a landscape level invasion gradient for two invasive wildflowers that share pollinators with two serpentine wildflowers that are closely related. The relative abundance of invasive and native plants at the boundary influenced whether invasive species had competitive or facilitative boundary effects on their serpentine neighbors. As the ratio of invasive to native wildflowers at the boundary increased, pollinator abundance and diversity to the native plant, native plant seed set, and the role of the native plant as a core hub in the network decreased. This suggests as the landscape context becomes more invaded, the invasive species has a competitive cross-ecosystem effect on the native species via shared pollinators.

For my third chapter, I am collaborating with the global Nutrient Network to ask whether nutrient deposition and large herbivore exclusion have interactive effects on plant communities and their pollinators across biogeographic gradients. I analyzed the global, multiyear data set on plant cover to examine how grasslands forbs have responded to joint effects of fertilization and large mammalian herbivore exclusion. I found that large herbivores can rescue forbs from the negative effects of increased grass competition under nutrient enrichment. This chapter tests whether predictions about the effects of interacting global changes on grassland forbs result in generalizable patterns at a global scale. Losses of forb diversity under nutrient enrichment may have downstream consequences on pollinators.

Acknowledgements

For thousands of years, the lands where this research took place has been the home of Patwin, Wappo, Pomo, Miwok , Shóqowa and Mono peoples.

Thank you to my advisors and committee members Susan Harrison, Fernanda Valdovinos, and Neal Williams for supporting my career journey. Thank you to Cathy Koehler, Paul Aigner, and Ben Amann at the UC McLaughlin Reserve for providing information on the natural history and field sites. Likewise, thank you to John Bailey and Greg Solberg at the Hopland UC Extension Center and Michelle Flavell and Andrea Northup-Warner at the Sierra Foothills UC Extension Center for maintaining field facilities. Thank you to my coauthors and collaborators in the Nutrient Network: Lauren L. Sullivan, Erika Hersch-Green, Eric W. Seabloom , Elizabeth T. Borer, Pedro Tognetti, Peter B. Adler, Lori Biederman, Miguel N. Bugalho, Maria Caldiera, Juan Cancela , Luísa Carvalhiero, Jane Catford, Chris R. Dickman, Aleksandra J. Dolezal, Ian Donohue Anne Ebeling, Nico Eisenhauer, Kenneth J. Elgersma, Anu Eskelinen, Catalina Estrada, Magda Garbowski, Pamela Graff, Daniel S. Gruner, Nicole Hagenah, Sylvia Haider, Stanley W. Harpole, Yann Hautier, Anke Jentsch, Nicolina Johanson, Sally E. Koerner, Luciola S. Lannes, Andrew MacDougall, Holly Martinson, John Morgan, Harry Olde Venterink, Devyn Orr, Brooke Osborne, Pablo Peri, Sally A. Power, Xavier Raynaud, Anita C. Risch, Mani Shrestha, Nicholas G. Smith, Carly Stevens, G. F. (Ciska) Veen, Risto Virtanen, Glenda M. Wardle, Amelia A. Wolf, and Alyssa L. Young. Thank you to Peter Wilfahrt and Ingrid Slette for Nutrient Network data curation and to Anita Krause for providing advice on fieldwork. Thank you to additional research collaborators: Marina LaForgia,, Em Jochim, Gillian Bergmann, Leif Richardson and Alan Hastings for providing insights into their

respective areas of expertise. Thank you to Thomas Zavortink, Socrates Letana and the Bohart Museum of Entomology for providing identification of insect specimens. Thank you for Rachel Vannette for providing knowledge on microbial ecology and to my QE committee Xiaoli Dong, Louie Yang, Neal Williams, and Jen Funk for guiding the initial development of the research. Thank you to my AOE advisor Rick Karban and to GGE staff members Joanna Lewis and Angie Nguyen for providing support on degree requirements. Thank you to Paul Havemann and the UC Reserve system for providing drone imagery and to Alison Colwell and the Davis Herbarium for providing reference specimens and plant presses. Thank you to Bita Rostami, Alexis Grana, Isabel Mendoza, Nat Walts, Rebekah Shane, Kyle Bianchi, Izzie Wolter, KT Lynch, Seth Tantuico, Ngoc Thu Tat, Monica Rodriguez, Ryan Li, Anastasia Karp, Ali Appelgate, and Zach Schneider for assistance in the field and/or lab. Thank you to the many mentors who have previously supported me throughout my career journey so far: Rodolfo Dirzo, Deborah Gordon, and Lee Anderegg. Thank you to my students and co-instructors in ESP 100, BIS2B, ECL200A, and ECL200B. Thank you to my colleagues in Northern California Botanists, the California Invasive Plant Council, Hands on the Land, the UC Davis Pesticide Oversight Committee, and the GGE Executive Committee for working with me on community engagement and service. Thank you to my friends and family for being so supportive throughout my PhD journey, especially my parents.

I would further like to thank the various organizations that have funded this research: the Irene Brown Memorial Fund, the Davis Botanical Society, the UC Davis Hill Fellowship, the UC Davis Jastro & Shields Fellowship, the Nature Conservancy, the California Native Grasslands Association, and the Northern California Chapter of the Phi Beta Kappa Honor Society.

This dissertation is dedicated to my grandparents: Ruth Nelson, Sam Nelson, Madelene G. Vargo, and Stephen Vargo who always encouraged me to “stay in school”.

Chapter 1: Invasive Grass Removal Restores Plant-Pollinator Mutualisms

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Keywords: Invasive Species; Plant-Pollinator Networks; Restoration

Abstract

Plant-pollinator mutualisms contribute to biodiversity and ecosystem function, and are the frequent targets of restoration efforts. Whether restoration efforts can aid in the recovery of plant-pollinator dynamics remains an emerging question. While attractive, animal-pollinated invasive plant species can affect plant-pollinator mutualisms via competition for pollinators with native species, a knowledge gap exists as to whether invasive species can affect plant-pollinator networks via their effects on plant-to-plant competition for non-pollinator resources. We investigated this knowledge gap by combining a consumer-resource model of plant-pollinator network dynamics with the experimental removal of a noxious, wind-pollinated, invasive grass (barbed goatgrass, *Aegilops triuncialis*). Invasive grass removal resulted in the recovery of native plant and pollinator abundance, increasing pollinator visitation to native wildflowers and enhancing the role of an abundant generalist native wildflower as a floral resource for pollinators. Our empirical data further found increases in pollinator diversity and ground-nesting bee abundance, and changes in network structure, suggesting that network ecology may benefit from including pollinator dispersal ecology and nesting requirements into network models.

Significance Statement

The effects of ecological restoration on the dynamics of plant-pollinator communities have yet to be fully elucidated. A knowledge gap exists as to whether invasive species can affect plant-pollinator networks via their effects on competition for plant recruitment. Invasive, wind-pollinated grasses provide an opportunity to address this gap. Our integrated data-theory approach that combined a mathematical model with grass removal experiments found that removing an invasive grass species restored the native plant community, increasing pollinator diversity and abundance. Our network analyses showed that invasive grass removal augmented the role of an abundant native wildflower as a critical generalist hub for pollinators in the network, illustrating a mechanism by which plant-to-plant competition for recruitment can have downstream effects on plant-pollinator interactions.

Introduction

Within restoration ecology, the paradigm is shifting from restoring target species to restoring the stability and function of plant-animal interactions (Genes and Dirzo 2022). Plant-pollinator mutualisms contribute to biodiversity and ecosystem function (Ollerton et al. 2011), and are the frequent targets of restoration efforts (Kaiser-Bunbury et al. 2017, Noreika et al. 2019, Sexton and Emery 2020), as they are declining under anthropogenic global changes, particularly invasive species (Kremen et al. 2004, Potts et al. 2010, Vanbergen and Initiative 2013, Valdovinos 2019). Removing animal-pollinated invasive plants as a restoration practice can restore plant-pollinator interaction diversity, functional redundancy and fruit set (Kaiser-Bunbury et al. 2009, 2017). It is unclear, however, how the removal of invasive species will affect the population dynamics of plants and pollinators, partly because empirical research is often conducted for shorter time scales than what is needed for evaluating population declines or recoveries. Combining models with empirical research can help evaluate the long-term effects of invasive species removal on the populations of plants and pollinators in plant-pollinator networks.

Traditional population dynamics models of mutualisms have typically used Lotka-Volterra type modeling (Gause and Witt 1935, Vandermeer and Boucher 1978, Travis and Post 1979), which defines mutualistic interactions as net, direct positive effects between species (reviewed in (Hale and Valdovinos 2021)). These models use a positive term in each species' growth equation based on the population size of its mutualistic partner. While this approach has improved our understanding of various mutualism types and their impact on long-term stability including that of networks (Bascompte et al. 2006, Bastolla et al. 2009); reviewed in (Valdovinos

2019), it offers limited insight into more complex dynamics including indirect effects among species of the same guild (e.g., plant effects on other plant species that share pollinators) (Dritz et al. 2023). More recent consumer-resource models of plant-pollinator networks decompose mutualisms into the biological processes that produce them, such as the consumption of floral resources by pollinators and the exchange of pollination services provided to the plant (Holland and DeAngelis 2010, Valdovinos et al. 2013). Using this type of modeling can disentangle the extent to which different processes across the plant's life cycle influence community dynamics. For example, these models can separate the dynamics of competition among plant species for seed recruitment (e.g., soil resources) from the dynamics arising from their competition for shared pollinators (Valdovinos et al. 2013, 2016, 2018, Valdovinos 2019).

Restoration via the removal of an invasive, wind-pollinated grass allows us to test for the effects of plant competition for soil resources on pollinator community dynamics without potentially confounding direct effects of competition for shared pollinators. Previous studies have focused on manipulating the abundances of animal-pollinated invasive plant species with flowers that are attractive to pollinators (Lopezaraiza–Mikel et al. 2007, Bartomeus et al. 2008, Kaiser-Bunbury et al. 2017, Russo et al. 2019) and thus are unable to disentangle the effects of competition among plant species for shared pollinators from their competition for seed recruitment. Whether plant competition for seed recruitment between grasses and wildflowers can affect the dynamics and stability of plant-pollinator mutualisms remains an open question. Invasive grasses can decrease both the diversity and abundance of native forbs available to pollinators (Arathi and Hardin 2021) and can decrease the availability of bare ground patches that provide essential habitat for ground-nesting bees (Harmon-Threatt 2020). Together these suggest that removal of invasive grasses may confer benefits to pollinators. Alternatively, invasive grass removal at within-meadow scales may not affect pollinators if pollinator foraging responds more to changes in floral resources at the landscape scale (Hackett et al. 2024).

Abundant wildflower species can act as core network hubs that support a diversity of pollinators (Sih and Baltus 1987, Russo et al. 2019, Sandacz et al. 2023) and can stabilize plant-pollinator network structure if these abundant plants are retained during perturbations (Bastolla et al. 2009, Valdovinos et al. 2016). By attracting both dietary generalist and specialist pollinators, these core wildflowers contribute strongly to the underlying structure of the plant-pollinator network (Bastolla et al. 2009). These common, core wildflower species could thus be especially reduced by plant invasions: theory and past empirical findings suggest that plant invasions disproportionately reduce the abundances of common native plants rather than rare ones (Powell et al 2013). Populations of these core, abundant plant species essential to network structural stability may thus be disproportionality decreased by grass invasions. Invasive grass removal may have stronger effects on the recruitment and pollination of abundant wildflower species than rare ones (Powell et al. 2013, Case et al. 2016). Alternatively, invasive plant species can have less of an impact on abundant native plants in cases where abundant native plants show biotic resistance (Zavaleta and Hulvey 2007), in which case network structure may show some resistance to invasion effects through the retention of abundant core wildflower. Moreover, the recovery of an abundant wildflower species following grass removal may not affect pollinators if pollinator responses are more driven by changes in floral diversity (Martins and Antonini 2016).

Whether the effects of grass invasions on abundant native wildflowers extend to their interactions with pollinators and their contributions to plant-pollinator network structure remains equivocal.

To test whether invasive grasses can affect plant-pollinator networks via their effects on abundant native wildflowers, we examine the effects of removing an invasive grass species on the restoration of plant-pollinator mutualisms using an integrated data-theory approach. Our approach combines a dynamical consumer-resource model and grass removal experiments from a California serpentine grassland. Our study species *Aegilops triuncialis* (barbed goatgrass), is an invasive annual wind-pollinated grass that is of low value to pollinators (Lyons et al. 2010, Meimberg et al. 2010, Pajkovic et al. 2014). Barbed goatgrass forms dense, monotypic stands, displacing a diverse community of native wildflower species, including an abundant native wildflower at our site, *Lasthenia californica* (goldfields) (Aigner and Woerly 2011, Case et al. 2016). Goldfields offers a floral resource for to wider diversity of flower-visiting insects and relies on insect pollination for reproduction (Ornduff 1966, Hendrickson et al. 2018). Its small, composite flower shape provides accessible floral rewards to a diversity of pollinator types, particularly short-tongued bees and flies (Thorp and Leong 1998). Our research builds on prior work in our system that demonstrated that removing barbed Goatgrass resulted in the recovery of the native wildflower community, including that of goldfields (Aigner and Woerly 2011, Case et al. 2016, Gornish et al. 2018). Our integrated data-theory approach investigates whether invasive goatgrass removal can affect pollinators and their interactions with wildflowers. Pairing our consumer-resource model with goatgrass removal experiments, we tested the following predictions: (1) invasive grass removal will increase wildflower abundance, (2) consequently increasing pollinator abundance and visitation and (3) enhancing the role of goldfields as a core generalist hub for pollinators in the plant-pollinator network.

Materials and Methods

Population Dynamics

We used an integrated approach that combined dynamical consumer-resource models of plant-pollinator mutualisms from (Valdovinos et al. 2013) to complement our grass removal experimental data (Table S3). The model from (Valdovinos et al. 2013) defines the population dynamics (over time of *t*) of each plant (p_i, Eqn. 1) and pollinator (a_j, Eqn. 2) species in the network, as well as the dynamics of floral rewards for each plant species (R_i, Eqn. 3), and the (per capita) foraging effort (α_{ij}, Eqn. 4) that each pollinator assigns to each plant species as follows:

$$\frac{dp_i}{dt} = \gamma_i \sum_{j \in A_i} e_{ij}\sigma_{ij}V_{ij} - \mu_i^P p_i \qquad (Eq.\ 1)$$

$$\frac{da_j}{dt} = \sum_{i \in P_i} c_{ij}V_{ij}b_{ij}\frac{R_i}{pi} - \mu_j^A a_j \qquad (Eq. 2)$$

$$\frac{dR_i}{dt} = \beta_i p_i - \phi_i R_i - \sum_{j \in A_i} V_{ij}b_{ij}\frac{R_i}{pi} \qquad (Eq. 3)$$

$$\frac{d\alpha_{ij}}{dt} = G_j\alpha_{ij}\left(c_{ij}\tau_{ij}b_{ij}R_i - \sum_{k\in P_j}\alpha_{kj}c_{kj}\tau_{kj}b_{kj}\,R_k\right) \qquad (Eq.\ 4)$$

This model assumes that plants and pollinators are obligate mutualists. The population growth of each plant species (Eqn. 1) is governed by both the quantity (V_{ij}) and quality (σ_{ij}) of visits received from each of its pollinator mutualists, the expected quantity of seeds produced from a pollination event (e_{ij}), the fraction of those seeds produced that successfully recruit to adult plants (γ_i), and density-independent plant mortality loss (μ_i^P). Increased seed production does not necessarily increase seeds that recruit to adults ($\gamma_i s_i$ in Eq. 1, where $s_i = \sum_{j\in A_i} e_{ij}\sigma_{ij}V_{ij}$), the proportion of seeds that recruit successfully to adults is determined by function γ_i, the seed establishment fraction. Visit quantity (Eq. 5) and quality (Eq. 6) are defined as:

$$V_{ij} = a_j p_i \tau_j \alpha_{ij} \qquad (Eq. 5)$$

$$\sigma_{ij} = \frac{V_{ij}}{\sum_{k\in P_j} V_{kj}} \qquad (Eq. 6)$$

where τ_j is the visitation efficiency of pollinator species *j*. Visit quality (σ_{ij}) is the quantity of conspecific pollen from plant species *i* pollinator species *j* carries relative to the total quantity of pollen pollinator species *j* carries from all plant species visited.

The fraction of seeds that recruit to adults, the seed establishment fraction, (γ_i) is limited by competition among plants for soil resources (Tilman et al. 1997) (Eq. 7):

$$\gamma_i = g_i\left(1 - \sum_{l \neq i \in P_j} u_l P_l - w_i P_i\right) \qquad (Eq. 7)$$

where g_i is the maximum fraction of seeds that recruit to adult plants, and w_i and u_l are intraspecific and interspecific competition coefficients for seed recruitment, respectively, for non-pollinator resources such as soil resources.

The population growth of pollinator species *j* (Eqn. 2) is governed by the efficiency with which pollinators convert floral rewards to energy (c_{ij}), density independent mortality loss (μ_j^A) where b_{ij} is the extraction efficiency of floral rewards for pollinator species *j* in each visit. Floral reward consumption scales linearly with floral award abundance (R_i). The model assumes a Type I functional response where floral reward dynamics are constrained by a saturating rate (ϕ_i), which consequently saturates pollinator consumption of floral rewards.

The growth rate of plant species *i*'s floral rewards (Eqn. 3) is governed by the rate at which plant species *i* produces floral rewards (β_i), the reward saturation rate (ϕ_i), and total consumption from all pollinator species that visit plant species *i.*

The foraging effort pollinator *j* allocates to plant species *i* (Eq. 4) is governed by the rate of adaptive foraging (G_j), pollinator resource conversion efficiency (c_{ij}), resource consumption of plant *i* per unit effort, and average floral resource consumption across all plant species pollinator *j* visits. Foraging effort (α_{ij}) across all plant species that are visited by pollinator *j* sums to 1, with values of foraging effort ranging between 0 and 1. The model assumes a closed system in which pollinators do not enter nor leave the meadow and further assumes the effect is a population-level response, while our empirical data (described below) may capture changes in pollinator abundances arising from the redistribution of pollinators in response to changing floral resources.

Simulation Design

To integrate the Valdovinos et al. 2013 model with our grass removal experiment, we used the combined control and restored network from the experimental data to generate simulated network data in MATLAB version 24.1.0.2537033 ("MATLAB" 2024). We made the following modifications to include goatgrass as a wind-pollinated plant: (1) we assumed goatgrass seed production and population dynamic equations were independent of pollinator abundances in the plant-pollinator network and (2) We assumed that the seed production of goatgrass was equal to that of the average of all animal-pollinated wildflower species in the network. To calculate w_i, the coefficient of intraspecific plant competition for non-pollinator resources such as soil resources, we took the inverse of the mean cover for each plant species. For an unresolved plant species, we took the inverse of the mean of all cover values for all wildflowers in the network.

We set the variance of the plant parameters to 0.1 and ran the simulation 500 times for both the control and goatgrass removed scenarios. We removed goatgrass from the simulation by running the model once with goatgrass and then removing it. We assumed pollinators were adaptively foraging and that background mortality of plants and pollinators was low (Table S3). We extracted plant abundances, pollinator abundances, pollinator visitation, and per-capita pollinator visitation from the simulations as outputs.

Study System

The study took place in a serpentine grassland at the University of California, Donald & Sylvia McLaughlin Natural Reserve in California's inner North Coast Range in Morgan Valley (Lake County, CA 38.861°N, 122.408°W) with vegetation typical of this ecosystem (Harrison et al. 2020). Prior to the experimental treatments, the site was heavily invaded by the wind-pollinated *Aegilops triuncialis* (barbed goatgrass) but had few additional non-native species (Aigner and Woerly 2011, Case et al. 2016, Gornish et al. 2018). *A. triuncialis* is an invasive annual grass that can tolerate serpentine soils (Lyons et al. 2010, Meimberg et al. 2010) and displaces the native wildflower community (Aigner and Woerly 2011), altering patterns of nutrient cycling (Drenovsky and Batten 2007). One of the most abundant wildflowers at the site was *Lasthenia californica* (goldfields), an annual, spring-blooming forb that is self-incompatible at the stigma and insect-pollinated (Ornduff 1966, Hendrickson et al. 2018).

Study Design

For our grass removal experiment, we used a randomized block experimental design that is a subset of a larger *A. triuncialis* removal experiment detailed in Aigner & Woerly 2011 (Aigner and Woerly 2011) (Figure S4). Removal treatments were applied during the spring of 2008-2010 in a serpentine meadow. Pollinator observations occurred in the spring and summer of 2011-2013. We focused on the within-meadow scale (~650m2) responses of pollinator foraging within a single meadow to invasive grass removal, as these finer scale, within-meadow responses have been previously shown to be relevant for plant competition, coexistence and population dynamics in annual plant-pollinator systems (Johnson et al. 2022).

Aigner and Woerly (2011) established a grid of 2m x 2m plots separated by 2-m buffers and defined 10 blocks based on initial *A. triuncialis* density (number of grass culms per meter squared). They randomly assigned nine *A. triuncialis* removal treatments and a control (*A. triuncialis* left in place) within each (Figure S4). Each treatment had ten plots. For our pollinator study, we focused on the control treatment and the four treatments that Aigner and Woerly (2011) found most effective at reducing *A. triuncialis*: hand pulling, mowing, application of the grass-specific herbicide clethodim in early May, and application of the grass-specific herbicide fluazifop in early May (50 plots total) (Figure 1). Blocks accounted for little variation in plant response (Aigner and Woerly 2011), so we did not consider them for the pollinator analysis.

All plant cover data were obtained in 2011 and 2012 based on visual estimates using a 1 m x 1 m frame placed in the center of each plot. We estimated vegetative cover using square pieces of cardboard representing 0.1%, 1%, 5%, 10%, and 25% cover as references (see Aigner & Woerly 2011). Cover was estimated by two trained observers per plot in 2011 and one trained observer per plot in 2012. Within each treatment, we divided plots evenly between observers.

Floral Visitor Observations

Trained observers visited each plot and recorded plant-floral visitor interactions and floral visitor abundance for a given time period. We used survey methods that were feasible in terms of time and budget for land managers to replicate. All observations were done during morning and afternoon on sunny days when pollinators were active. Each plot was visited for one-minute observation periods in 2011 and for two-minute observation periods in 2012 & 2013. We recorded all flower visits by flying insects during the observation period, recording the taxon of the floral visitor and the flower. Pollinators were collected as voucher specimens outside the observation period. We identified pollinators to the level of morphospecies, with identification of voucher specimens confirmed to species by Dr. Robbin Thorp. We classified bees as groundnesters vs cavity-nesters based on information obtained from existing literature (LeBuhn 2013). We further classified floral visitors into the following functional groups based on existing literature: butterflies/moths, syrphids, bombyliids, non-bee mimic flies, short-tongued bees, medium to long-tongued bees, and kleptoparasitic bees. We conducted 17 observation sessions over a three-year period. In 2011, observation dates were April 16, 22, 27, and June 30. In 2012, observation dates were April 21, 22, May 2, and September 7, 8, and 11. In 2013, observation dates were April 12, May 22, 24, 31, and August 14, 15, and 28. A floral visit was recorded if an insect made contact with the reproductive parts of a flower. If a plot had no flowering plants present, pollinator visitation data were not collected.

Statistical Analysis

Statistical analyses were performed in R using the 'lme' and 'bipartite' packages (Dormann et al. 2009, R Core Team 2022, Bates et al. 2015). For all linear and generalized linear models, we scaled the variable year using the scale() function in R that centers a numeric matrix for each of the years, and we calculated plant and pollinator community variables at the plot level for each year. For all models, we checked data were normally distributed using the qqnorm function in R; we then plotted the residuals to check for heteroscedasticity. To determine whether goatgrass removal would affect wildflower cover, we used linear models to examine if goatgrass cover, total grass cover for all annual grass species present, goldfields cover, and total cover for all native forb species visited by pollinators varied by treatment, year, and their interaction (e.g. Cover ~ Treatment*Year). For the wildflower cover analysis, we used all native wildflowers species whose flowers were visited by pollinators (Table S2). We found no significant differences in pollinator community response between treatments using removal method for goatgrass, so we pooled all goatgrass removal treatments together in our analyses.

We used a linear model to further examine if Shannon diversity for pollinators varied by goatgrass removal, year, and their interaction. After testing for overdispersion, we used a generalized linear model with a Poisson distribution to test whether pollinator morphospecies richness varied by treatment, year, and their interaction. To examine whether removing goatgrass improved habitat for ground-nesting bees, we used a generalized linear model with a negative binomial distribution to test whether ground-nesting bee abundance varied by treatment, year, and their interaction. We further tested whether the proportion of ground nesting bees (ground nesting bee abundance/total pollinator abundance) varied by treatment, year and their interaction using a generalized linear model with a gaussian distribution. We used a generalized linear model with a negative binomial distribution to test whether total pollinator abundance across all morphospecies varied by treatment and year, and their interaction.

One potential limitation is that pollinators are highly mobile animals and could move between plots. To address this limitation, we checked for spatial autocorrelation. We recorded latitude and longitude at the center of each of our study plots. We calculated Moran's I to test for spatial autocorrelation in pollinator richness, pollinator abundance, and pollinator Shannon diversity and constructed semivariograms for each of these variables using the 'nlme' and 'gstat' R packages (Pebesma 2004, Pinheiro et al. 2017). Pollinator richness and Shannon diversity were not spatially autocorrelated. Pollinator abundance was spatially autocorrelated in an isotrophic manner. To account for spatial autocorrelation in pollinator abundance, we ran a linear model of the effect of treatment on pollinator abundance with and without an exponential correlation structure that accounted for spatial autocorrelation using the ‘nlme’ package in R. We chose an exponential correlation structure as this structure had the lowest AIC, although the difference in AIC between the different spatial correlation structures (Exponential, Gaussian, Linear, Rational Quadratic, Spherical) varied by less than two units of AIC, suggesting they were equally parsimonious. All spatial correlation structures were more parsimonious than the model that didn’t account for spatial autocorrelation (difference in AIC=22.8 > 2 AIC units).

To test whether goldfields would strongly influence network structure, we further assessed the individual contribution of each plant species to nestedness using the nestednesscontribution() function in the R ‘bipartite’ package (Dormann et al. 2009). This function estimates the degree to which the interactions of each plant species increase or decrease community nestedness by comparing to a random null model that is designed to control for the effect of differences in degree. Networks included plant-pollinator interaction data from both spring and summer across three years. We compared observed network pollinator niche overlap as measured by the Morisita-Horn Index (Horn 1966) using the ‘swap web’ null model to test for whether plant-pollinator network structure differed between restoration treatments using the ‘bipartite’ package in R (Dormann et al. 2009). The ‘swap web’ null model generates marginal totals identical to those observed and the same connectance as observed. To account for differences in network size, we compared networks to null models via z-scores.

Results

Simulated Plant-Pollinator Dynamics

When goatgrass was removed from our network model, wildflower abundances increased, resulting in increased pollinator abundance and visitation, which is consistent with our first and second predictions, respectively. Total wildflower abundance increased when goatgrass was removed compared to when it was present (Figure 1A), because the removal of goatgrass released wildflowers from competition with this grass species for resources limiting seed recruitment (determined by function γ_i of the plant equation, see Eq. 7 in Methods). Pollinator abundance and pollinator visitation to wildflowers increased when goatgrass was removed (Figure 1B, Figure 1D) because the increase in wildflower abundance increased the floral rewards available to pollinators (Eq. 3, see Methods) which increased their reward consumption and, therefore, their abundance (Eq. 3) and visits to plants (Eq. 5, see Methods). Increased pollinator visitation under goatgrass removal increased seed production ($s_i = \sum_{j \in A_i} e_{ij} \sigma_{ij} V_{ij}$ in Eq. 1), but this increased seed production (s_i) does not necessarily increase seeds that recruit to adults ($\gamma_i s_i$ in Eq. 1), which is determined by function γ_i. Indeed, through simulation results, we confirmed that the increase in plant abundance shown in Fig. 2A is primarily due to the reduced competition for seed recruitment following the removal of goatgrass, rather than the resulting increase in pollinator visits.

Ground nesting bees, however, showed a negligible increase in abundance when *A. triuncialis* was removed (Figure 1E) due to their variation in dietary specialization. Several of the ground-nesting bee species present in our data are specialists that have fewer floral rewards available to them in the model (Eq. 3), which results in restricted reward consumption (Eq. 2, see Methods), limiting their abundances (Eq 2), while other ground-nesting bees were generalists in diet that have a greater variety of food sources available to them in the model.

Consistent with our third prediction, the abundance of and pollinator visitation to an abundant generalist native wildflower, goldfields, increased when goatgrass was removed

(Figure 1C). Removing goatgrass released goldfields from grass competition for resources limiting seed recruitment to adult plants (γ_i, Eq. 7, see Methods), increasing goldfields abundance (Eq. 1) and consequently increasing the availability of its floral rewards to pollinators (Eq. 3) and pollinator visitation to goldfields (Eq. 5). As a generalist wildflower, goldfields supports a functionally diverse community of both generalist and specialist pollinators; thus, increases in goldfields' rewards can increase pollinator abundance for species across all pollinator functional groups through increased floral reward consumption (Eq. 3). In our model, generalist plant species visited by both generalist and specialist pollinators, while specialist pollinators visited generalist wildflowers. Because goldfields is a generalist wildflower that is visited by a diversity of pollinator species, in our model, goldfields may receive more visits (Eq. 5) including from specialist pollinators that deposit conspecific pollen (Eq. 6) than specialist wildflowers which are typically visited by only generalist pollinators. Because specialist pollinator visit a single species of plant, they carry a single type of pollen and thus provide higher quality visits.

Data from Removal Experiments

To complement our model results, we observed a total of 814 individual pollinators across three years of sampling. We documented a total of 21 plant species and 50 pollinator morphospecies across removal and control treatments and years, comprising 134 unique pairwise interactions. In total, we observed 13 bombyliid flies, 97 syrphid flies, 321 other flies, 10 kleptoparasitic bees, 22 butterfly and moths, 144 long-tongued bees, 163 short-tongued bees, 2 wasps, and 42 other individual insects across all treatments and years.

Plant Community Response to Removal Experiments

We found evidence to support our first prediction: consistent with our model, removing the invasive goatgrass increased native wildflower abundance compared to the control (Table 1). Removal treatments had lower goatgrass cover than the controls (t=-8.503, p=2.43 x10^{-13}; Figure 2A; restored 4.5% +/- 5.43, control 28.11% +/- 22.82). Likewise, the removal treatments had lower total annual grass cover than the controls (t= -7.210, p=1.28 x10^{-10}; Figure 2B). Goatgrass cover and total annual grass cover did not significantly differ by year or by an interaction of year and treatment. Removal treatments had higher cover of the generalist wildflower species goldfields than controls (t=3.034, P = 0.003; Figure 2C; restored 2.32% +/- 2.74, control 0.44%+/- 0.98). Goldfields cover did not differ by year, and there was no treatment-by-year interaction. Removal treatments had higher cover of wildflowers visited by pollinators in the network than the controls (t=5.050, p=2.10 x10^{-6}; Figure 2D; restored 27.59% +/- 12.90, control 11.78% +/- 12.34). Total cover of pollinator-visited wildflowers did not differ by year, and there was no treatment-by-year interaction.

Pollinator Community Response to Removal Experiments

We found evidence to support our second prediction: pollinator communities in the goatgrass removal treatments had increased richness, increased abundance, and higher Shannon diversity than the controls where goatgrass was left in place (Table 1). The removal treatments had higher pollinator morphospecies richness (z=3.881, p=1.04 x10^{-4}; Figure 3A), higher total pollinator abundance (z=5.009, p=5.47 x10^{-7}; Figure 3B), and higher pollinator Shannon diversity (t=4.434, p=1.96 x10^{-5}; Figure 3C) than the controls. Pollinator morphospecies richness, Shannon diversity index, and total abundance did not significantly differ by year or an interaction of year and treatment. Pollinator richness (Moran's I, p= 0.17) and Shannon diversity (Moran's I, p=0.817) were not spatially autocorrelated. Although pollinator abundance was spatially autocorrelated (Moran's I, p= 0.00025), pollinator abundance was still higher in restored plots than controls when accounting for spatial autocorrelation (t=3.55, p=0.0005).

The removal treatments had a higher abundance of ground-nesting bee morphospecies than the control treatments (z=3.882, 1.04 x10^{-4}; Figure 3D). The removal treatments had a non-significantly higher proportion of ground-nesting bees relative to all pollinators observed (t=1.737, p= 0.085).

Plant-Pollinator Network Response to Removal Experiments

In support of our third prediction, the most abundant forb species, goldfields, had the highest individual contribution to nestedness. For both the restored and control network, goldfields contributed the most to nestedness among plant species. For the restored network, goldfields had an individual contribution to nestedness significantly higher than null expectations (z=5.443, p=5.23x10^{-8}), while the control network, where goatgrass was left in place, had an individual contribution to nestedness that was non-significantly higher than null expectations (z=1.404, p>0.05). Goldfields hosted all observed pollinator functional groups: butterflies/moths, syrphids, bombyliids, non-bee mimic flies, short-tongued bees, medium to long-tongued bees, and kleptoparasitic bees. The restored network had higher niche overlap in plant species visited among pollinators, as measured by the Morisita-Horn Index, than expected by the null model (z= 6.371, p=1.88 x10^{-10}), while the control network did not differ from null expectations (z=-1.121, p=0.262) (Figure S2, for further information on network responses see Supplemental Results).

Discussion

Our integrated data-theory approach demonstrated that invasive grass removal can affect the pollinator community via the recovery of native wildflowers, illustrating that grass-wildflower competition for resources other than pollinators can have downstream effects on pollinators. Consistent with our predictions, removing goatgrass increased wildflower abundance, pollinator abundance, and pollinator visitation, enhancing the role of a common native generalist wildflower goldfields as a core network hub for pollinators. While our consumer-resource model showed negligible increases in ground-nesting bees following goatgrass removal and could not test for effects goatgrass removal on pollinator diversity and network structure, our empirical data showed that goatgrass removal increased ground-nesting bee abundance, pollinator diversity, and network pollinator niche overlap. Our consumer-resource model does not account for two processes that could explain these empirical findings: the effects of nesting habitat availability on pollinator population dynamics and the movement of pollinators in and out of the meadow.

Our model results show that invasive grass removal released wildflowers from competition with grasses for resources limiting seed recruitment. This is consistent with empirical work showing that grass removal can open up niche space for native wildflowers, resulting in increased availability of soil and light resources to wildflowers and increased seed recruitment (Aigner and Woerly 2011, Case et al. 2016, Arathi and Hardin 2021). Enhanced wildflower recruitment to adulthood following goatgrass removal increased floral rewards abundance. Pollinators prefer to forage in areas with a higher quality and quantity of floral rewards (Sih and Baltus 1987, Fuccillo Battle et al. 2021), so increased floral reward abundance under goatgrass removal can increase the food resources available to pollinators. The modeled increase of floral reward consumption resulting from goatgrass removal increased pollinator abundance, consistent with empirical findings of the effects of invasive wildflower removal (Kaiser-Bunbury et al. 2009, 2017) and other restoration practices (Tarrant et al. 2013, Winsa et al. 2017, Noreika et al. 2019, Sexton and Emery 2020, Gao et al. 2021, Deprá et al. 2022) on pollinators. Although increased wildflower recruitment under goatgrass removal directly affected the final plant abundances in our model, pollinator visitation determined if wildflowers persisted (see also Valdovinos et al. 2013). If pollinator consumption of floral resources increases under grass removal, animal-pollinated wildflowers may receive a higher quality and quantity of pollinator visits, which can increase their fecundity and fitness as shown by both theoretical and empirical findings (Valdovinos et al. 2016, Kaiser-Bunbury et al. 2017, Valdovinos and Marsland 2021), contributing to the longer term recovery of wildflower communities following restoration.

Removing the invasive grass enhanced the role of an abundant, generalist wildflower as a core hub for pollinators in both our model and experimental data, consistent with the idea that invasive species may strongly affect common native species (Powell et al. 2013). These findings contrast with evidence that common native plant species may show biotic resistance to invasive species (Zavaleta and Hulvey 2007). This result suggests that ways in which invasive species suppress abundant, generalist native plants (Powell et al. 2013, Case et al. 2016) can extend to effects on pollinators. As such, goldfields appears to play a core role in increasing pollinator diversity in restored plots relative to controls. Similar important influence of dominant flowering species on the structure and stability of plant-pollinator communities has been shown in other studies (Kaiser-Bunbury et al. 2009, Malena et al. 2021). Indeed, invasive flowering plants themselves may have a similar effect (Bartomeus et al. 2008, Russo et al. 2019). The loss of core generalist plant species can destabilize plant-pollinator communities (Sandacz et al. 2023). Alternatively, other studies have found that floral diversity is critical for restoring pollinators (Tarrant et al. 2013, Martins and Antonini 2016).

Consistent with empirical work on invasive wildflower removal (Tarrant et al. 2013, Winsa et al. 2017, Noreika et al. 2019, Sexton and Emery 2020, Gao et al. 2021, Deprá et al. 2022), our removal experiment showed that invasive grass removal increased pollinator diversity and pollinator niche overlap. Similarly, shrub removal restored pollinator composition in semi-natural grasslands (Winsa et al. 2017, Noreika et al. 2019). Our finding of increased pollinator niche overlap may suggest that grass removal can enhance the functional redundancy of pollinators (Kaiser-Bunbury et al. 2009). Increased functional redundancy of pollinators is associated with increased stability, as redundancy of species within pollinator niches may make the network less sensitive to specialist pollinator species losses under future perturbations (Memmott et al. 2004, Williams 2011), consistent with positive theoretical relationships between mutualistic diversity and stability (Okuyama and Holland 2008). In contrast, our simulated results did not capture these changes in network structure and pollinator diversity arising from grass removal because our model assumed a closed system in which pollinators did not enter or leave the meadow (Valdovinos et al. 2013, 2016).

Pollinating insects are often highly mobile (Magrach et al. 2017, Mola and Williams 2019), that use resources across patchy, heterogenous landscapes (Hackett et al. 2024). Pollinator dispersal across habitats can affect the structure and diversity of plant-pollinator interactions (Henriksen et al. 2024), influencing plant fitness (Artz and Waddington 2006, Magrach et al.

2017). By treating meadows as open systems, future consumer-resource models of plant-pollinator networks could incorporate pollinator movement by adding a metacommunity component in which pollinators can move between habitat and plant patches (Amarasekare 2004, Wang 2019) or by adding a source-sink model that captures how pollinators move across the landscape to track pulses of floral rewards (Bogdziewicz et al. 2018). Alternatively, these models could take a more spatially explicit approach by adding a term to plant population dynamical equations that accounts for the effect of plant spatial configuration on pollinator foraging behavior when calculating pollinator visitation and its consequent effects on seed production (Underwood et al. 2020, Bruninga-Socolar et al. 2022). As central place foragers, bees generally forage within a radius of their nest, so consumer-resource models could place pollinator consumption of floral rewards within fixed radii based on pollinator foraging range (Alignier et al. 2023).

Invasive grass removal may further increase bee abundance through increasing ground-nesting bee habitat quality. Removing goatgrass, which often occurs in tall, dense patches, may open up more patches of bare ground for pollinators to nest. The restored plots had a non-significantly higher abundance of ground-nesting bees caught in emergence traps compared to the control (Paul Aigner, *unpublished data*). This finding is supported by previous empirical work demonstrating that restoration practices increase ground-nesting bees through increasing the availability of bare ground (Buckles and Harmon-Threatt 2019, Brokaw et al. 2023).

Overall, our data-theory integrated approach shows that changes in grass-wildflower competition can alter the outcome of plant-pollinator consumer-resource interactions, affecting the community dynamics of plant-pollinator mutualisms. Our empirical findings illustrate the importance of incorporating pollinator spatial ecology and nesting biology into consumer-resource models to improve our understandings of how mutualistic communities recover from anthropogenic perturbations following restoration.

Acknowledgements The authors would like to thank Dr. Xiaoli Dong, Dr. Jennifer Funk, Dr. Neal Williams, Dr. Louie Yang, Dr. Emily Meinecke, Dr. Pamela Reynolds, Dr. Ash Zemenick, Dr. Christian John, Wesley Brooks, Dr. Jean-Gabriel Young, the Valdovinos Lab, and members of the UC Davis Ecological Networks seminar class for providing feedback on the data analysis. The authors would like to thank Dr. Neal Williams, Dr. Susan Harrison and Jasen Liu for providing detailed feedback on the manuscript. Cathy Koehler, Leslie Scott, Courtney Gomola, C.S.J., Paola Arenas and Shirley Wu assisted with collecting cover data. Paola Arenas and Lauren Quinones helped collect pollinator data. Dr. Robbin Thorp provided input on specimen identification in 2013. This work was performed at the University of California Natural Reserve System McLaughlin Reserve DOI: (10.21973/N3W08D).

Funding The authors declare that no funds, grants, or other support were received during preparation of this manuscript.

Author Contributions: Study Design & Conceptualization; Paul Aigner, Fernanda Valdovinos Data curation; Paul Aigner, Rebecca Nelson, Sabine Dritz; Formal analysis; Rebecca Nelson, Sabine Dritz, Paul Aigner, Fernanda Valdvinos; Methodology; Paul Aigner, Rebecca Nelson, Sabine Dritz, Fernanda Valdovinos; Visualization; Rebecca Nelson, Sabine Dritz, Fernanda Valdovinos, Paul Aigner; Writing - original draft; Rebecca Nelson; Writing - review & editing. Rebecca Nelson, Sabine Dritz, Fernanda Valdovinos, Paul Aigner.

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Figures and Tables

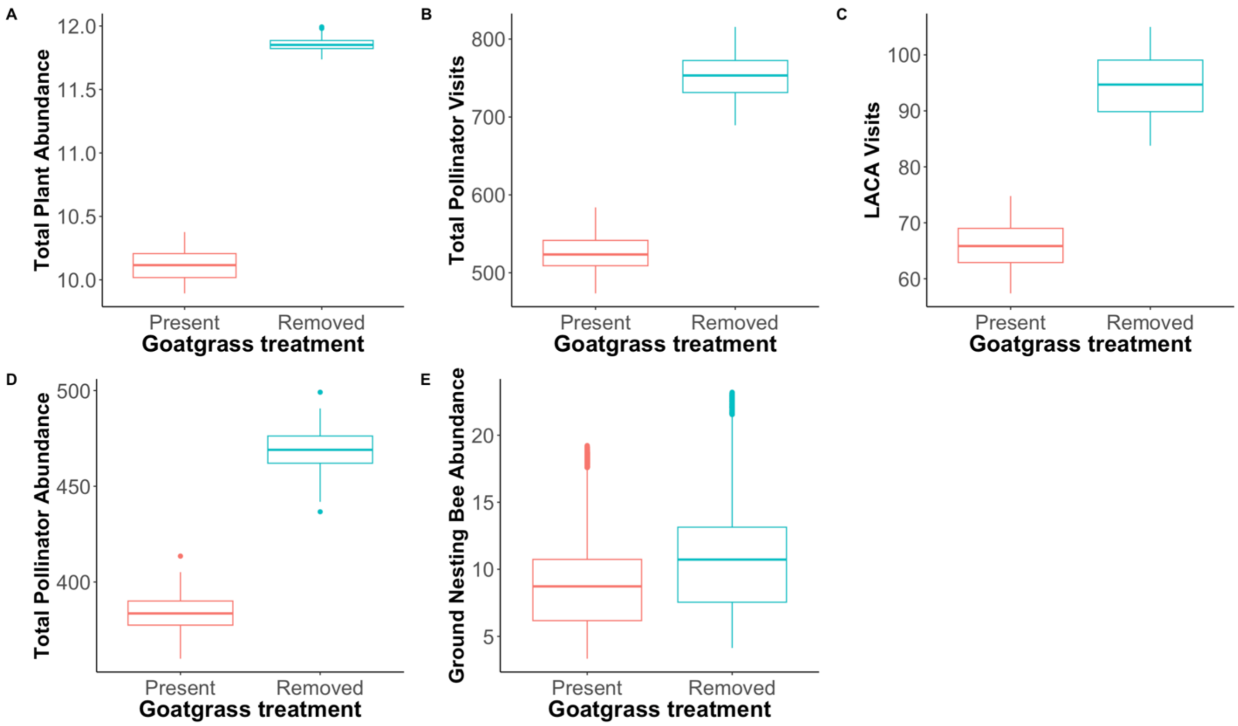

Figure 1 Simulation results for (a) total native plant abundance, (b) total pollinator visits to plants, (c) total pollinator visits to *Lasthenia californica* (LACA), and (d) total pollinator abundance, and (e) total ground nesting bee abundance under low plant and low pollinator mortality with adaptive foraging. Red shows scenario for which goatgrass is present, while blue shows scenario for which goatgrass was removed.

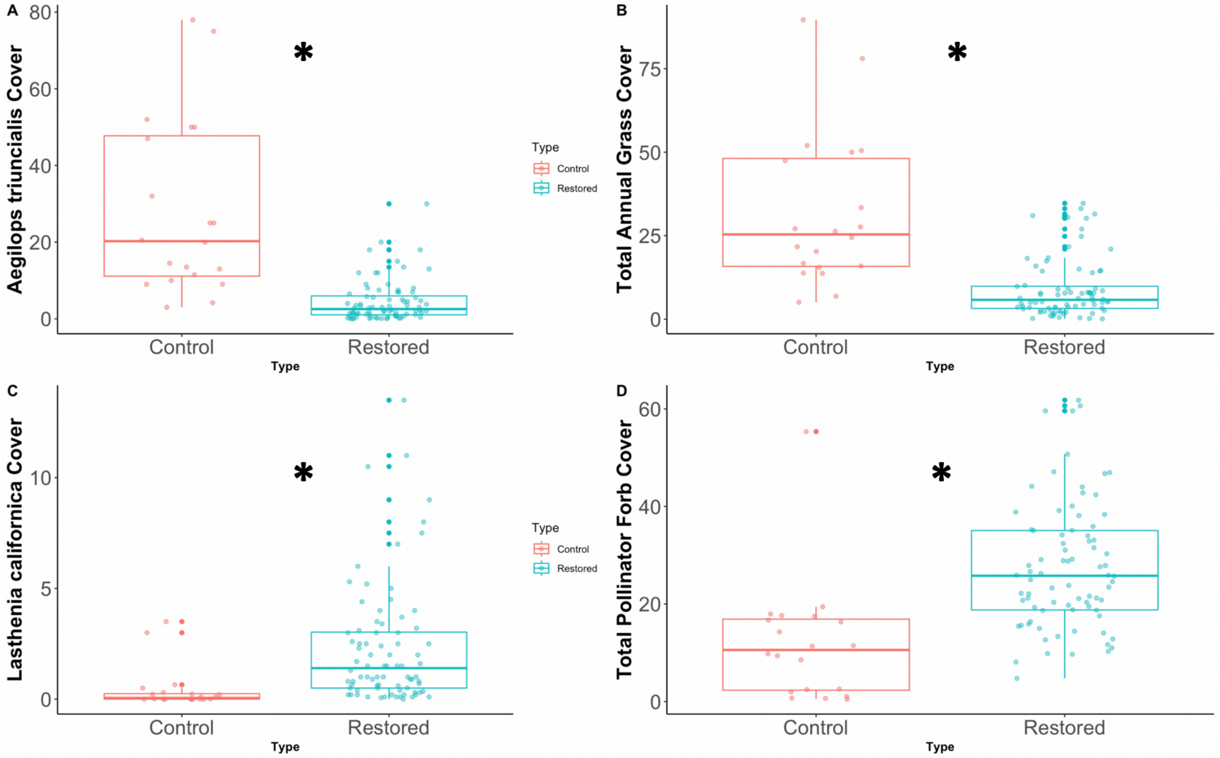

Figure 2. Plant Community metrics by treatment type for (a) *Aegilops triuncalis* cover (b) total annual grass cover (c) *Lasthenia californica* cover and (d) total native forb cover for species visited by pollinators. Control refers to the control treatment and restored refers to *A. triuncialis* removal treatments. Asterisks denote significant differences between the restored and control treatments for an alpha level of 0.05.

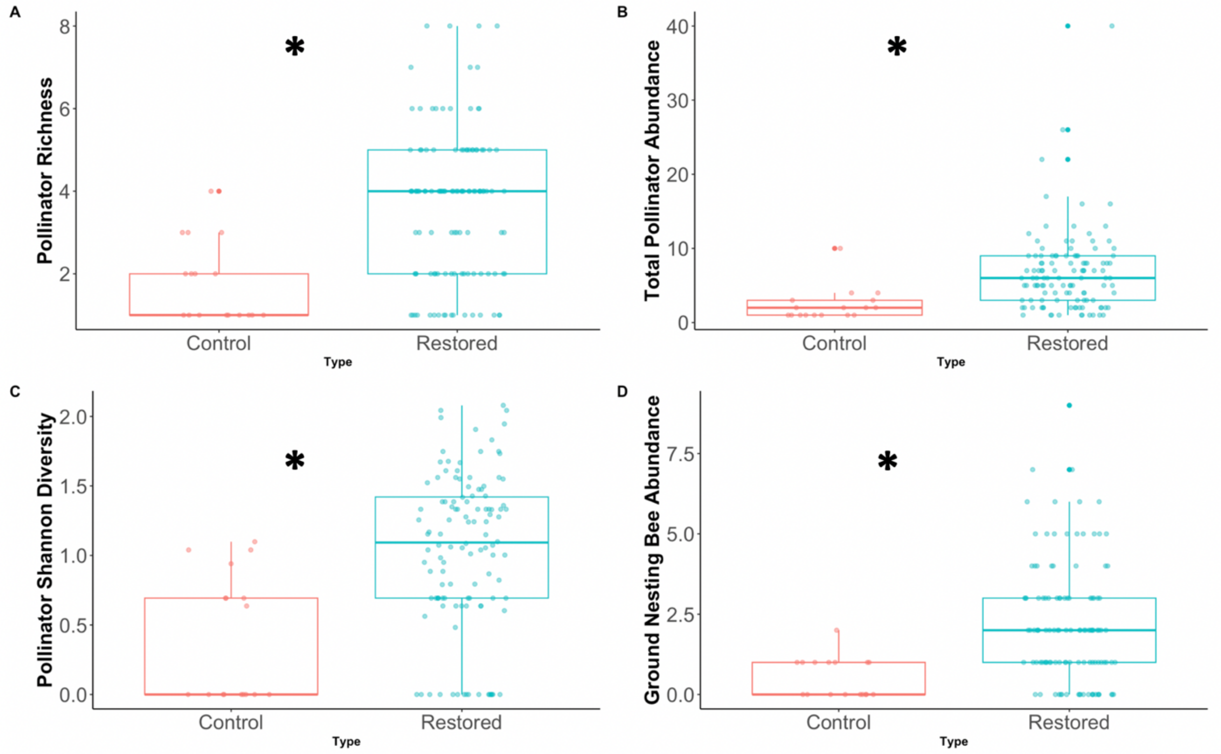

Figure 3. Pollinator Community Metrics by Treatment Type for (a) pollinator morphospecies richness, (b) total pollinator abundance, (c) pollinator Shannon diversity, and (d) total abundance of ground-nesting bee species. Control refers to the control treatment and restored refers to the *A. triuncialis* removal treatments. Asterisks denote significance for an alpha level of 0.05

Table 1 Summary of main statistical results. Bolded p-values are significant at an alpha level of 0.05.

Explanatory variable	Response Variable	Test statistic	p-value
Treatment (Control vs Restored)	*A. triuncialis* cover	t=-8.503	**2.43×10^{-13}**
	Total annual grass cover	t=-7.210	**1.28×10^{-10}**
	L. californica cover	t=3.034	**0.003**
	Pollinator-visited forb cover	t=5.050	**2.10×10^{-6}**
	Pollinator Richness	z=3.881	**1.04×10^{-4}**
	Total pollinator abundance	z=5.009	**5.47×10^{-7}**
	Pollinator Shannon Diversity	t=4.434	**1.96×10^{-5}**
	Ground-nesting Bee Abundance	z=3.882	**1.04×10^{-4}**
	Proportion of Ground-nesting bees relative to all pollinators	t=1.737	0.085
	Pollinator Community Composition	F=4.274	**0.001**

Chapter 1 Supplemental Appendix

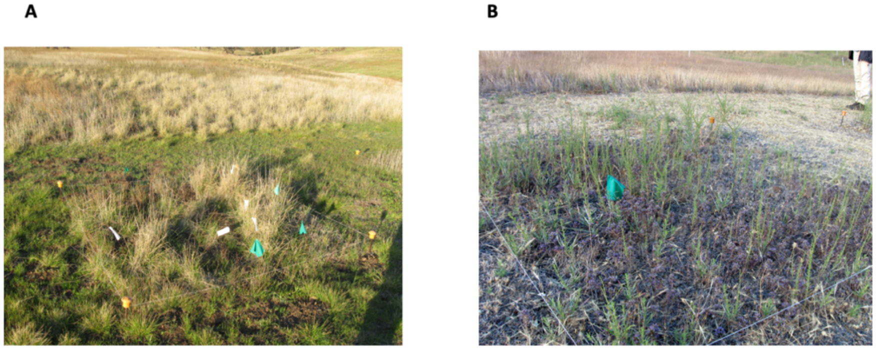

Figure S1. Examples of plots from (A) the control treatment and (B) the restoration treatments. The control plot in (A) is dominated by *A. triuncialis*, while the restored plot in (B) is dominated by a mixture of native forb species.

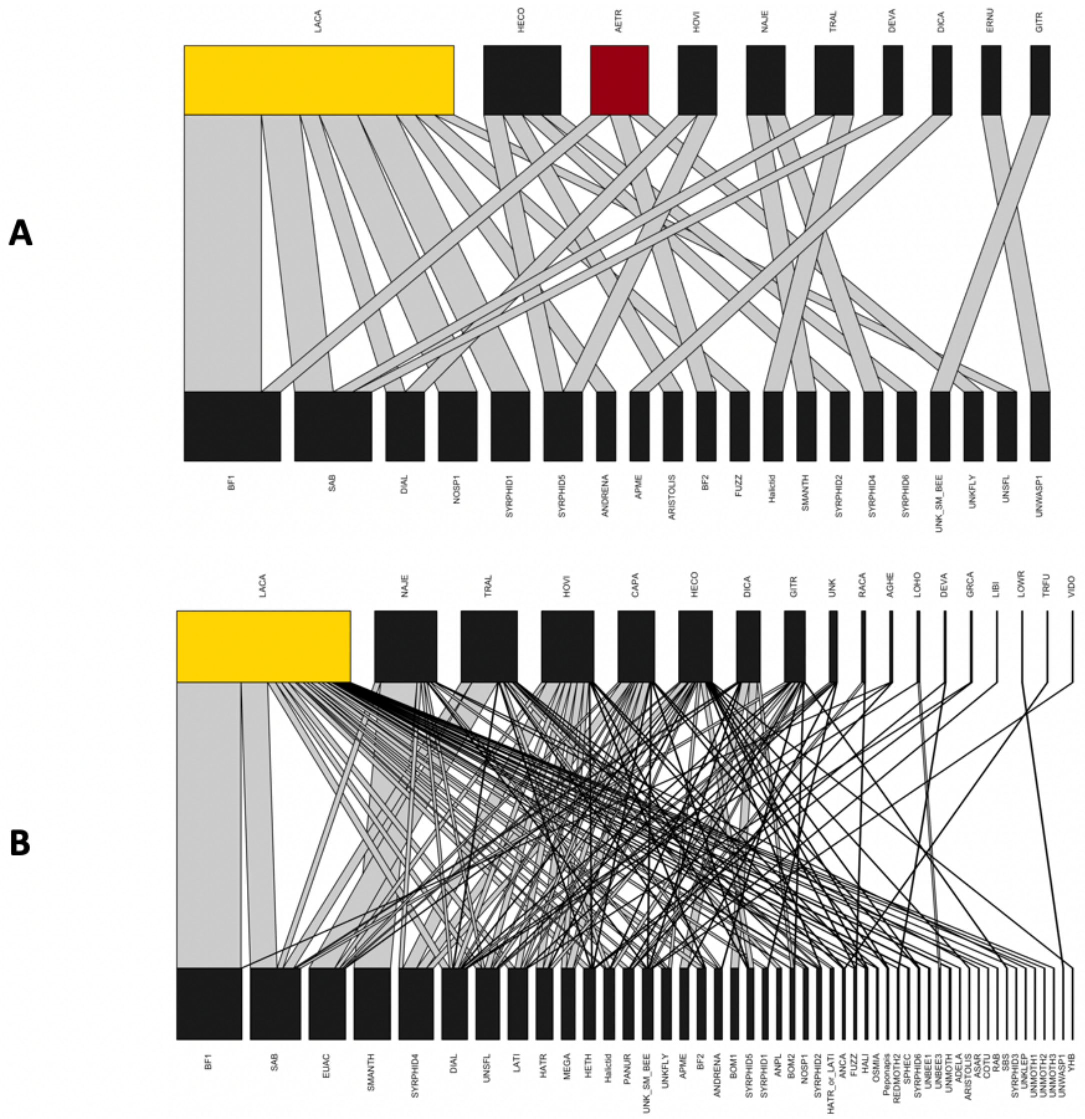

Figure S2. Plant-Pollinator Networks by *Aegilops triuncialis* Removal Treatment. Bipartite plant-pollinator networks across all three years of data collection for (A) the control treatment (B) the restored treatments. Each rectangle in the top row represents a plant species, abbreviated by a four-letter species code. *Lasthenia californica* (labeled LACA) is shaded in yellow. *Aegilops triuncialis* (labeled AETR) is shaded in dark red, although we did not consider pollinators perching on this grass to be actual visits. Each rectangle in the bottom represents a pollinator morphospecies with its abbreviated species code. Gray lines between rectangles represent interactions between plants and pollinators. The thickness of lines as well as the size of each rectangle is proportional to the frequency of a given interaction, plant, or pollinator respectively in the network data. See Supplementary Table 2 for full list of native forb abbreviations. UNK refers to an unidentified forb. See Supplementary Table 1 for a full list of pollinator morphospecies and their abbreviations

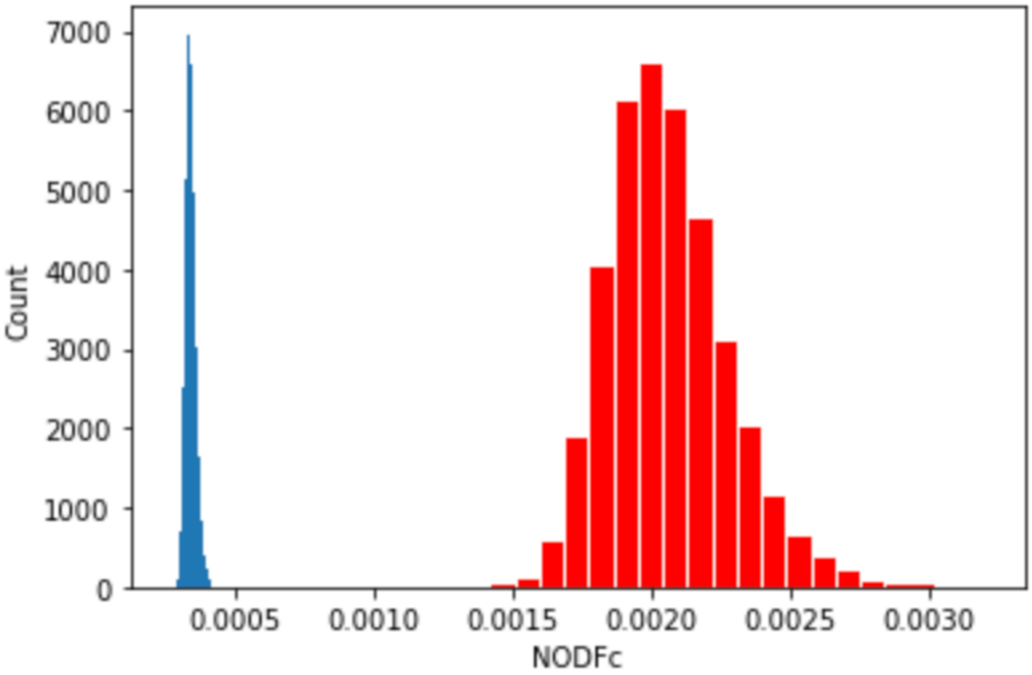
7000
6000
5000
4000
3000
2000
1000
0
Count
0.0005
0.0010
0.0015
0.0020
0.0025
0.0030
NODFc

Figure S3. NODFc (relative nestedness) posterior probability distribution from Bayesian inference for restored and control networks. The restored network is depicted in blue, and the control network is depicted in red. The maximum posterior NODFc is .00334 for the control and 0.000479 for the restored network. The mean posterior NODFc is 0.00205 for the control network and 0.000338 for the restored network

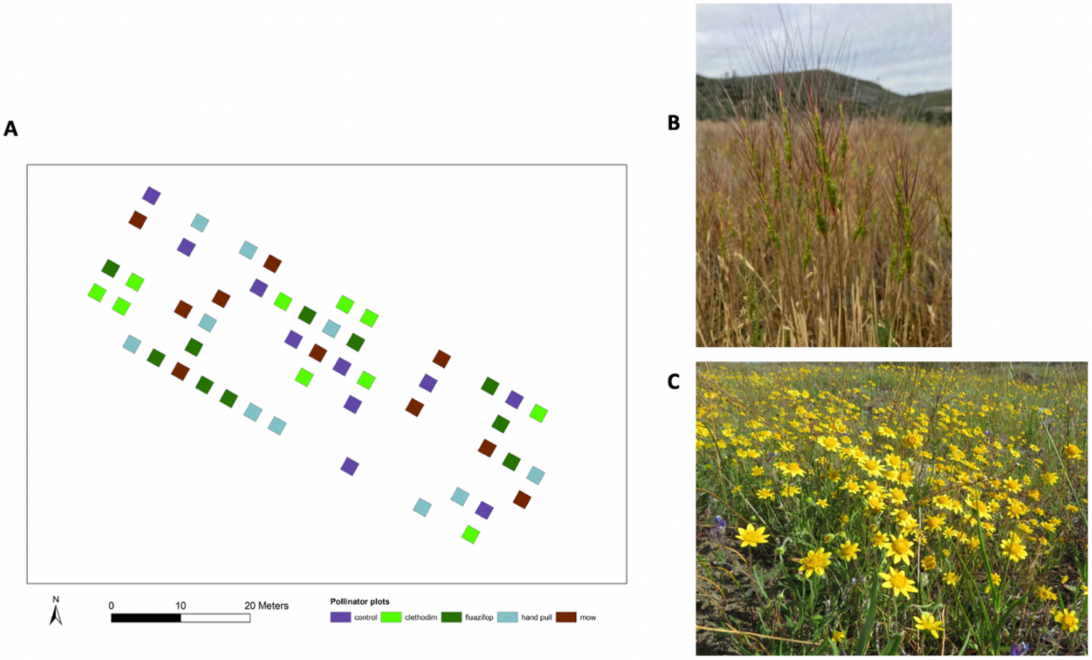

Figure S4. Study design. (A) Map of study site and experimental design. Each square represents a study plot from Aigner and Woerly 2011 that we surveyed for pollinators. (B) A serpentine plant community invaded by *Aegilops triuncialis* (barbed goatgrass). Image credit: Paul Aigner. (C) A typical native serpentine meadow plant community. The main forb shown in the picture is *Lasthenia californica* (goldfields). Image credit: Rebecca Nelson

Supplementary Table S1. Species abbreviations from network diagram accompanied by a description of each morphospecies and functional group.

ARTH	**Morphospecies Description**	**Functional Group**
ADELA	*Adela trigrapha*	Lepidopterans
ANCA	*Anthophora californica*	Long-tongued bees
ANDRENA	*Andrena spp.*	Short-tongued bees
ANPL	*Andrena plana*	Short-tongued bees
ANSP1	Andrena species #1; *Andrena anguistitarsata*	Short-tongued bees
ANLE	*Andrena lewisorsum*	Short-tongued bees
APME	*Apis mellifera*	Long-tongued bees
ASAR	*Ashmeadiella aridula astraguli*	Short-tongued bees

ARISTOLIS	*Eristalis sp.*	Syrphids
BF1	Tiny black dipteran	Other Flies
BF2	Large black dipteran	Other Flies
BOM1	*Bombylius* sp. 1	Bombyliiads
BOM2	*Bombylius* sp. 2	Bombyliiads
COTU	*Coenonympha tullia*	Lepidopterans
DIAL	*Dialictus spp.*	Short-tongued bees
EUAC	*Eucera actuosa*	Long-tongued bees
FUZZ	Fuzzy dipteran	Other Flies
HALI	*Halictus ligatus*	Short-tongued bees
Halictid	unknown halictid	Short-tongued bees
HATR	*Halictus tripartitus*	Short-tongued bees
HATR_or_LATI	*Halictus tripartus* or *Lassiglossum titusi*	Short-tongued bees

HETH	*Heliothades diminutive*	Lepidopterans
LATI	*Lasioglossum titusi*	Short-tongued bees
NOSP1	*Nomada spp.* 1	Kleptoparasitic bees
MEGA	*Megachile sp.*; MEGA recorded on Sept 7,8,11 of 2012 is *Megachile onobriychitdius*	Long-tongued bees
OSMIA	*Osmia spp.*	Long-tongued bees
PANUR	*Panurginus spp.*	Short-tongued bees
REDMOTH2	Red moth with delta-shaped wings	Leps
SAB	Small bee with striped abdomen, likely *Andrena plana, Andrena sp. 1,* or *Halictus tripartitus,* or *Halictus ligatus*	Short-tongued bees
SBS	Small black syrphid	Syrphids
SMANTH	*Anthophora torticornis*	Long-tongued bees
SPHEC	*Sphecodes sp.*	Kleptoparasitic bees

SYRPHID1	Medium syrphid fly, rounded yellow "notches" on each side of abdomen	Syrphids
SYRPHID2	Medium syrphid fly with color stripes on abdomen	Syrphids
SYRPHID3	Large black syrphid fly	Syrphids
SYRPHID4	Orange syrphid fly	Syrphids
SYRPHID5	Small black syrphid fly with striped abdomen with red tip	Syrphids
SYRPHID6	Small to medium 6-10 mm syrphid with Anthidium-patterned abdomen	Syrphids
UNBEE1	*Andrena subchalybea*	Short-tongued bees
UNBEE3	*Andrena microchlora*	Short-tongued bees
UNKFLY	Unidentified dipteran	Other Flies
UNMOTH1	Gray/brown moth, darker thorax than head, wings cross in back	Lepidopterans
UNMOTH2	Moth almost like HETH but white spots surrounded by much darker circles of brown	Lepidopterans

UNMOTH3	Moth similar to HETH but white mottling on wings was in linear horizontal pattern, not round, white, less circular than UNMOTH2.	Lepidopterans
UNSFL	Unidentified syrphid fly	Syrphids
UNWASP1	Large yellow & red, red wings, vertical and horizontal stripes	Wasps
YHB	Small (6mm) yellow hairless bee with incomplete abdominal bands like *Anthidium*, *Xeromelecta*, *Anthidiellum*, etc	Short-tongued bees
UNK_SM_BEE	*Lassioglossum incompletum*	Short-tongued bees
RAB	*Nomada sp*. or *Sphecodes sp.*	Kleptoparasitic bees
UNMOTH	Unknown moth	Lepidopterans
UNKLEP	Unknown lepidopteran	Lepidopterans
Peponapis	*Peponapis*-like bee	NA

Supplementary Table S2. Native forb species observed being visited by pollinators. Taxonomic names follow Baldwin et al. (2012). Abbreviations used in the network diagram are noted in the second column. *Navarretia pubescens* does not appear in network diagram, as plant was visited by an unidentified bee, whose morphospecies could not be determined.

Species Name	Abbreviation	Family	Naming Authority
Acmispon wrangelianus	LOWR	Fabaceae	(Fisch. & C.A. Mey.) D.D. Sokoloff
Agoseris heterophylla	AGHE	Asteraceae	(Nutt.) Greene
Calycadenia pauciflora	CAPA	Asteraceae	A. Gray
Delphinium variegatum	DEVA	Ranunculaceae	Torr. & A. Gray
Dichelostemma capitatum	DICA	Themidaceae	(Benth.) Alph. Wood
Eriogonum nudum	ERGU	Polygonaceae	Benth.
Gilia tricolor	GITR	Polemoniaceae	Benth.
Grindelia camporum	GRCA	Asteraceae	Greene
Hemizonia congesta	HECO	Asteraceae	D.C.
Holocarpha virgate	HOVI	Asteraceae	A. Gray
Lasthenia californica	LACA	Asteraceae	Lindl.
Leptosiphon bicolor	LIBI	Polemoniaceae	Nutt.
Lomatium hooveri	LOHO	Apiaceae	(Mathias & Constance) Constance & Ertter

Microseris douglasii	MIDO	Asteraceae	(D.C.) Sch. Bip.
Navarretia jepsonii	NAJE	Polemoniaceae	Jeps.
Navarretia pubescens	NAPU	Polemoniaceae	Benth. (Hook & Arn.)
Ranunculus californicus	RACA	Ranunculaceae	Benth.
Trifolium albopurpureum	TRAL	Fabaceae	Torr. & A. Gray
Trifolium fucatum	TRFU	Fabaceae	Lindl.
Viola douglasii	VIDO	Violaceae	Steud.

Supplementary Table S3. Model state variables, functions, and parameters. Values were drawn from a uniform random distribution with specified means and variance of 10% for plants' parameters and variance 0% for pollinators' parameters respectively. Parameter values were obtained from (Valdovinos et al. 2013, 2018) with the exception of w_i which was informed by empirical plant coverage data.

Definition	**Symbol**	**Dimension**
State Variables		
Plant *i* population density	p_i	$individuals\ area^{-1}$

Animal *j* population density	a_j	$individuals$ $area^{-1}$
Total floral resource density for plant population *i*	R_i	$mass$ $area^{-1}$
Foraging effort of animal *j* on plant *i*	α_{ij}	none
Functions		
Visitation rate of animal *j* to plant *i* (visit quantity)	$V_{ij} = \alpha_{ij}\tau_j p_i a_j$	$individuals$ $area^{-1}time^{-1}$
Quality of visits (per capita) of animal *j* to plant *i* (per	$\sigma_{ij} = \frac{V_{ij}}{\sum_{k\in P_j} V_{kj}}$	none

capita) (visit quality)		
Fraction of seeds *i* that recruit to adults (Seed Establishment Fraction)	$\gamma_i = g_i\left(1 - \sum_{l\neq i\in P_j} u_l P_l - w_i P_i\right)$	none
Parameters		
Visitation efficiency	τ_j	$visits\ area^{-1}time^{-1}individuals^{-1}individuals^{-1}$
Expected number of seeds produced by pollination event (seed production)	e_i	$individuals\ visits^{-1}$
Per capita plant mortality rate	μ_i^P	$time^{-1}$

Conversion efficiency of floral resources to pollinator births	c_j	$individuals\ mass^{-1}$
Per capita pollinator mortality rate	μ_j^A	$time^{-1}$
Pollinator extraction efficiency of floral resource per visit	b_{ij}	$individuals\ visits^{-1}$
Maximum fraction of total seeds that recruit to adult plants	g_i	none
Interspecific plant competition	$\boldsymbol{u_i}$	$area\ individuals^{-1}$

coefficient for non-mutualistic resources		
Intraspecific plant competition coefficient for non-mutualistic resources	$\boldsymbol{w_i}$	$area\ individuals^{-1}$
Production rate of floral resources	β_i	$mass\ individuals^{-1} individuals^{-1}$
Self-limitation of floral rewards production	ϕ_i	$time^{-1}$
Pollinator adaptive foraging rate	G_j	none

Chapter 2: Invasive plants affect native plant pollination through pollinator-mediated cross-boundary effects

Rebecca A. Nelson, Neal Williams, Fernanda S. Valdovinos, and Susan Harrison

Abstract

One way that invasive plants may affect native plants, either positively or negatively, is by changing the foraging behavior and/or abundance of shared pollinators. Although this question has been primarily explored within single ecosystems, pollinators are highly mobile foragers that may readily cross ecological boundaries. Here, we examine whether focal native species can be affected by invasive species with which they share pollinators, even though the native and invasive species grow on different soils and are therefore separated by an ecological boundary. We asked this question in a mosaic of 22 native-dominated meadows on serpentine soil, embedded in a matrix of non-serpentine meadows. The non-serpentine meadows contain dense stands of two insect-pollinated invasive species: *Vicia villosa,* flowering in spring; *Centaurea solsitialis*, flowering in summer. The serpentine meadows contain two relatively uncommon native species that co-flower and share pollinators with these invasives: *Trifolium fucatum* and *Helianthus exilis*, respectively. In a three-year study, we determined that the abundance ratio of each exotic species to its co-flowering native species, within radii of 250-1000 meters of focal patches of the native plant, was associated with decreased visitation rates, seed set, and contribution to plant-pollinator network structure for the native species. We conclude that invasive plants can have indirect cross-boundary effects on native plants in neighboring ecosystems, via competition for shared pollinators.

Keywords: Cross-Ecosystem Effects, Spillover, Invasive Species, Plant-Pollinator Mutualism

Introduction

Cross-boundary effects occur when organisms or matter from one ecosystem enter a neighboring ecosystem (Polis et al. 1997, Scherer-Lorenzen et al. 2022). Spillover, a type of cross-boundary effect, is the process by which organisms move across ecosystem boundaries (Tscharntke et al. 2005). As highly mobile organisms, pollinator forage across ecosystem boundaries and rely on spatially heterogenous landscapes for floral resources (Hackett et al. 2024). Such spillover of pollinators has been especially well studied in croplands, which provide trackable systems to explore mechanisms and also represent landscapes in which potential increased crop pollination services have tangible financial benefits (Tscharntke et al. 2005, Winfree et al. 2007, Ekroos et al. 2008). Proximity of crop fields to wildland habitats, for example, can enhance pollinator visitation to crops, pollinator diversity on crops and crop pollination through spillover of wildland pollinators into croplands (Kremen et al. 2002, 2004, Ekroos et al. 2008, Williams 2011, Garibaldi et al. 2013). Pollinator spillover between crop-wildland boundaries has further demonstrated that land use changes associated with agricultural intensification can affect the strength of spillover (Kremen et al. 2002, Ekroos et al. 2008, Magrach et al. 2017). For example, the spillover of honeybees from orange groves into wildlands negatively decreased the fitness of an abundant native plant species through honeybees displacing other more effective pollinators (Magrach et al. 2017).

Pollinator spillover, however, across wildland-wildland ecosystem boundaries has yet to be fully elucidated. Whether findings from crop-wildland boundaries are applicable to wildland-

wildland boundaries, which tend to be more diverse and spatially complex, remains an open question (Ponisio et al. 2016, Hackett et al. 2024). In a naturally-fragmented glade ecosystem, bee movement among habitat patches was rare despite landscape heterogeneity (Harmon-Threatt and Anderson 2023), raising questions about the relative importance of pollinator spillover as a process shaping the diversity and structure of mutualisms more broadly. In natural habitat mosaics in Florida, proximity to a swamp-prairie boundary increased prairie pollinator diversity and abundance as adjacent swamplands provided pollinator nesting habitat (Artz and Waddington 2006). Theory further suggests that the spatial dispersal of pollinators across habitat patches is essential to maintenance of plant-pollinator mutualisms (Amarasekare 2004). Within a single ecosystem, plant species can have indirect negative or positive effects on each other’s reproduction via competition or facilitation for shared pollinators (Brown et al. 2002, Braun and Lortie 2019). Whether plant species within a wildland ecosystem can have cross-boundary effects on the pollination and fitness of plant species within a neighboring wildland ecosystem remains an untested question despite the high mobility of pollinators as foragers across patchy landscapes (Greenleaf et al. 2007, Kennedy et al. 2013).

Despite the global prevalence of cross-boundary effects of invasive species on food webs and nutrient cycling (Peller and Altermatt 2024), a gap remains as to whether abundant invasive plant species can have indirect, cross-ecosystem effects on rare native plant pollination and fitness in natural ecosystems via spillover competition for shared pollinators. Invasive plant species can disrupt plant-pollinator mutualisms by negatively affecting native plant reproduction via indirect effects from shared pollinators (Morales and Aizen 2002, Traveset and Richardson 2006, Parra-Tabla et al. 2021). Theory predicts that invasive plant species can shape the dynamics and fitness outcomes of plant-pollinator mutualisms via these pollinator-mediated, indirect effects (Valdovinos et al. 2009, Dritz et al. 2023). Evidence remains equivocal, however, as to whether abundant invasive plants have competitive or facilitative indirect effects on uncommon native plants via shared pollinators (Brown et al. 2002, Molina-Montenegro et al. 2008). Invasive plant species that are abundant and highly attractive to pollinators can outcompete less common native plants for shared pollinators, decreasing pollinator visitation to native plants and native plant fitness (Brown et al. 2002, Parra-Tabla et al. 2021). Alternatively, invasive plant species can have facilitative effects on native plant species by acting as magnet species that attract more pollinators to the area, increasing pollinator visitation to native plants and native plant fitness (Rodriguez 2006, Braun and Lortie 2019, Etter et al. 2022). Pollinator sharing between invasive and native plants can further decrease native plant fitness through increased heterospecific pollen transfer (Brown et al. 2002, Bjerknes et al. 2007). These contrasting competitive and facilitative outcomes of indirect effects exist in part, because the effects of competition for shared pollinators and competition for belowground resources needs to be robustly partitioned (Underwood et al. 2020). These past findings come from within single ecosystems, raising questions about whether invasive plants can have indirect cross-ecosystem effects on native plant reproduction via pollinator spillover between habitats. If invasive plants indirectly affect native plants via spillover competition for shared pollinators, then ecosystems previously thought to be spatial refugia from the impacts of invasive species are not necessarily safe from their negative effects (Harrison et al. 2000). Addressing this gap has implications for the management of refugia ecosystems that contain rare native plants (Case et al. 2016)

Serpentine grasslands are considered spatial refugia for native plants as their low-nutrient serpentine soils, high in heavy metals, prevent most invasive plant species from establishing (Harrison et al. 2006, Harrison and Rajakaruna 2019). Patchy mosaics of native-dominated grasslands on serpentine soil embedded in a matrix of highly invaded grasslands on non-serpentine, sandstone-derived soils provide an opportunity to address this question (Harrison 1999). The non-serpentine soils contain dense stands of two common, insect-pollinated invasive species: *Vicia villosa,* flowering in spring; *Centaurea solsitialis*, flowering in summer (Barthell et al. 2001, Harmon-Threatt and Kremen 2015). The serpentine soils contain two relatively uncommon native species that co-flower and share pollinators with these abundant invasive species: *Trifolium fucatum* and *Helianthus exilis*, respectively (Wolf et al. 1999, Baldwin and Goldman 2012). Within this system, floral diversity is higher in spring than summer (Harrison et al. 2006), further providing the opportunity to examine whether seasonal differences in spillover effects support negative relationships between community diversity and invasibility (Levine and D'Antonio 1999). Pollinators can track shifts in the relative abundance of floral rewards when foraging (Sih and Baltus 1987, Valdovinos et al. 2013, 2016), suggesting that relative plant abundances on each side of the ecosystem boundary may modulate spillover effects. Pollinators can make these foraging decisions across scales ranging from within a meadow (250m) to moving between meadows across the landscape (500m for small, solitary bees, 1000 m for large social bees) (Moore et al. 2011, Mola and Williams 2018, Alignier et al. 2023).

In a three-year observational study, we examined whether the abundance ratio of each invasive species to its co-flowering native species, within radii of 250-1000 meters of focal patches of the native plant species, was associated with decreased pollinator visitation rates, seed set, and contribution to plant-pollinator network structure for the native species. We hypothesize that as the ratio of invasive flowers to native flowers at the boundary increases, (1) pollinator visitation to native plants will decrease, (2) consequently native plant seed set will decrease, (3) the role of the invasive species as a dominant hub in the network will increase, while that of the native species will decrease, and (4) that season of flowering, spring vs summer, will affect the relative strength of boundary effects: the more diverse, spring-blooming plant-pollinator network will be more robust to spillover competition than the less diverse, smaller network of summer-blooming plants.

Methods

Study System

This study took place at the University of California McLaughlin Reserve in the North Inner Coast Range of California (Lake County, CA 38.861°N, 122.408°W), where a mosaic of native wildflower dominated serpentine grasslands and invaded non-serpentine grasslands occur within a patchy landscape (Harrison 1999). The area experiences a Mediterranean climate with hot, dry summers and cool, wet winters (Harrison et al. 2000).

We focused on two pairs of taxonomically related and co-flowering plant species; within each pair, one species was an abundant exotic species growing on non-serpentine (sandstone-derived) soils and the other was a relatively uncommon native species growing on serpentine soils. The abundant invasive hairy vetch (*Vicia villosa* Roth, Fabaceae) co-flowers with the uncommon native bull clover (*Trifolium fucatum* Lindl., Fabaceae) in spring (April-May) (Baldwin and Goldman 2012, Harmon-Threatt and Kremen 2015). The abundant and invasive yellow star-thistle (*Centaurea solstitialis* L., Asteraceae) co-flowers with the rare serpentine sunflower (*Helianthus exilis* A. Gray, Asteraceae) in summer (July) (Wolf et al. 1999, Barthell et al. 2001, Baldwin and Goldman 2012).

Study Design

To test whether pollinator visitation and associated pollination outcomes for native serpentine plant species varied with the relative abundance of focal invasive non-serpentine plant species and native serpentine plant species on each side of the ecosystem boundary, we conducted a three-year observational study of 22 naturally occurring meadows that contained serpentine-non-serpentine ecosystem boundaries that as a group spanned substantial variation in the abundances of the two invasive species on non-serpentine soils.

Meadow Selection

We define a meadow as an area of contiguous grassland containing a serpentine-non-serpentine ecosystem boundary as defined by a geologic contact between soils, typically about 250 m2 in area (Moore et al. 2011). We defined a contiguous grassland as habitat where the dominant vegetation types were grasses and herbaceous forbs (Sanderson et al. 2009) spanning an area not interrupted by either a non-grassland ecosystem (i.e. chaparral, oak woodland, rock outcroppings), nor by any human-made landscape features (i.e. buildings, trails or roads). We selected focal meadows based on the following criteria: (1) contained an ecological boundary as defined by a geologic contact between serpentine and non-serpentine soils, (2) had the focal native plant species present and flowering, and (3) was located at least 100 m away from any other meadow sampled to minimize pollinator movement between sites.

Across all three years, we surveyed a total of 14 unique meadows in spring and 9 unique meadows in summer that satisfied these selection criteria, resulting in 22 unique total meadows (one meadow was surveyed in both spring and summer). Because the locations of focal native plants varied across years, we only surveyed meadows in a given year that met the selection criteria for that year. Across all three years, we surveyed a total of 22 unique meadow and year combinations in spring and 19 unique meadow and year combinations for summer. Within meadows, we sampled plant-pollinator interactions on focal native plant patches, focal individual native plant flowerheads as well on meadow plant-pollinator networks as along randomized transects to obtain data (described in further detail in the Meadow Plant-Pollinator Networks section of the methods) (Lopezaraiza–Mikel et al. 2007).

We used official McLaughlin Reserve vegetation and soil maps made available to the public on the McLaughlin Reserve website (https://naturalreserves.ucdavis.edu/mclaughlin-reserve/species-lists-maps-and-data) to identify meadows that met our above selection criteria. We further consulted with McLaughlin Reserve Directors Paul Aigner and Catherine Koehler on site selection. Using the CalFlora Observer Pro and Avenza mapping applications on a cell phone, we recorded the latitude and longitude of each meadow at the centroids, or approximate center point of the meadow. The total sampling region across all meadows sampled spanned an area of approximately 7000 acres with meadows ranging from approximately 100 m to 300 m apart from their nearest neighboring meadow.

Focal Patch Selection

Within our selected meadows, we observed plant-pollinator interactions for all focal patches of native plants within a given meadow. We defined a focal plant patch as a contiguous, conspecific cluster of plants that co-flowered. We defined a contiguous, clustered patch as a patch where all individual plants of a given species occurred within at least meter of each other in distance. If an individual focal plant was greater than one meter from its nearest conspecific neighbor, it was considered an isolated individual and not part of the patch. We monitored a total of 50 unique patch and year combinations for spring native clovers and 44 unique patch and year combinations for summer native sunflowers. We used the same criteria to identify all focal invasive plant patches present in a given meadow as well.

We used the polygon mapping feature in the Calflora Observer Pro Application to map each focal invasive and native plant patch to produce a map of geospatial polygons that represent the shape and area of a given patch. We determined the edges of a patch by finding the point at which the nearest conspecific neighboring individual plants were greater than one meter away. To map the patch as a geospatial polygon, we then walked slowly around the edges of the patch, using the CalFlora application to mark the locations of the edges of the patch, generating a polygon in the shape of the patch. We uploaded the geospatial map of plant patch polygons into Google Earth to confirm that the location of the patches was accurate by comparing landscape features in satellite imagery to photographs taken of each meadow in the field. We used ground-truthing to confirm that CalFlora polygons were realistic in their measurements of patch area and extent by measuring the length and width of the patches in the field using a tape measure to estimate the area of patch.

Selection of Spatial Radii

We combined geospatial information on plant patch locations with patch-level floral abundance data from plant abundance surveys described below to determine the mean abundance of floral units of the invasive plant within fixed 250m, 500m and 1000m radii of a given focal patch of native plants. We defined a floral unit as a single flower head or part of a multiple head from which a medium sized bee would have to fly rather than walk to reach another unit of the same plant species (Lopezaraiza–Mikel et al. 2007). We uploaded our plant geospatial data from CalFlora into Google Earth and then used the shape feature to draw 250m, 500m, 1000m radii in relation to the centroid of each focal native plant patch.

We selected radii at 250m, 500m and 1000m to capture three different biological scales of pollinator foraging: 250 m is the typical area of a meadow (Moore et al. 2011) and the scale at which pollinators forage within a given meadow (Alignier et al. 2023); 500 m is the distance at which small, solitary bees move between different meadows when foraging (Greenleaf et al. 2007, Alignier et al. 2023); and 1000 m is the distance at which larger bees (e.g. bumblebees and honeybees) move between different meadows when foraging (Greenleaf et al. 2007, Mola and Williams 2018, Alignier et al. 2023). The most common pollinators of the focal plant species were bees, so we chose to base these spatial radii on relevant scales of bee foraging (Wolf et al. 1999, Barthell et al. 2001, Harmon-Threatt and Kremen 2015).

Patch Level Floral Abundance Surveys

To pair geospatial information and pollinator visitation data for focal plant patches with estimates of floral abundance made independently of the plant-pollinator interaction data, we estimated the abundance of inflorescences as floral units within each focal patch using a log-binned method (Mola and Williams 2018). We defined a floral unit as a single flower head or part of a multiple head from which a medium sized bee would have to fly rather than walk to reach another unit of the same species (Lopezaraiza–Mikel et al. 2007).

Selection of Individual Flowerheads

Within focal patches, we randomly selected and tagged individual focal native plant flowerheads to collect data on pollinator visitation and seed set. We used to the following criteria to select individual native plant flowerheads (1) each native plant flowerhead had to be located within a focal patch and a focal meadow, (2) each flowerhead had to be freshly opened with no signs of senescence (i.e. browning, petals fading or closing), (3) the flowerhead had no signs of florivory at the start of observation, and (4) the flowerhead was on an individual plant that has not been already tagged and selected. We monitored a total of 850 individual clover flowerheads in 2024 and 700 individual sunflower flowerheads in 2023 and 2024, tagging 25-50 individual flowerheads per patch across 22 meadows total.

Pollinator Visitation to Focal Native Plants

We measured the number of pollinator visits to focal native plant patches and individual flowerheads within patches, only during calm, sunny to partly sunny conditions when focal invasive and native flower species were co-blooming. We considered a floral visit to have occurred if a floral visitor contacted the reproductive parts of the flower for at least 1 second. We visually observed and recorded all floral visitors to individuals within the patch and/or individual tagged flowerheads during three-minute observation periods. For each focal patch and/or individual flowerhead observation, we recorded the date, time, meadow identity, patch identity, distance in meters to the nearest focal invasive species, floral visitor identity, plant species identity, and number of flowers visited. We conducted spring pollinator surveys during the following sampling periods: 3/9/2022-4/29/2022, 4/20/23-5/27/23, 4/15/2024-5/11/2024 and conducted summer pollinator surveys during the following sampling periods: 7/3/2022-7/31/2022, 7/5/2023-8/2/2023, and 7/8/2024-7/31/2024, sampling each patch at least six times over the course of the flowering season, making for a total of approximately 1.5 hours of sampling per unique patch and year. We monitored a total of 50 unique patch and year combinations for spring native clovers and 44 unique patch and year combinations for summer native sunflowers, and a total of 700 individual sunflower flowerheads in 2023 and 2024 and 850 individual clover flowerheads in 2024, tagging 25-50 individual flowerheads per patch across 22 meadows total. We tagged our focal clovers and sunflowers with flagging tape and metal plant tags to associate visitation measures with subsequent seed set data.

Floral visitors were identified to morphospecies. We netted voucher specimens of floral visitors which were identified by experts at the Bohart Museum of Entomology (Thomas Zavortink for bees and Socrates Letana for flies) to species, which we then used to sort our floral visitor data into morphospecies (see Supplement). We also photographed floral visitors in the field using a Canon SX60 digital camera. We used short-focus Pentax "butterfly binoculars" to aid in our visual observations of pollinators.

Native Plant Seed Set

We measured seed set for focal native plants. Once individual flowerheads had senesced, we bagged all monitored flowerheads, a total of 700 sunflower flowerheads and 850 clover flowerheads with mesh bags to deter seed predators and collected seedheads. The same focal native plant patches and individual flowerheads used for pollinator observations were also used for seed set measurements. We noted cases where focal flowerheads went missing or were eaten by florivores. We counted the total number of seeds per focal flowerhead. For clovers, we collected additional closed, unbagged seedpods from focal patches in spring 2022. We measured seed set by counting the number of seeds per pod and mass of seeds for spring clovers, and count by counting the total number of seeds per flowerhead for summer sunflowers. We recorded the identity of the patch each seedpod was from, so that data could further be aggregated to patch-level estimate. Seed set data for spring was from 2022 and 2024, and for summer was from 2023 and 2024.

Meadow Plant-Pollinator Networks

We surveyed interaction between plant and pollinator communities in spring and summer in 2022, 2023, and 2024 for our focal meadows, selected with the previously described criteria from which we constructed interaction networks. We adapted survey protocols developed by Lopezaraiza et al. 2007 to survey community-level plant pollinator interactions in focal meadows along randomized 100m transects, surveying any observed plant-pollinator interactions within 1 m of the transect, every five meters under calm, sunny to partly sunny conditions (Lopezaraiza–Mikel et al. 2007). We surveyed each meadow a minimum of six times over the flowering season. Across all three years, we surveyed a total of 22 unique meadow and year combinations in spring and 19 unique meadow and year combinations in summer for a total of approximately 6 observer hours per unique meadow and year .

The path of each transect varied in relation to the natural shape and curvature of the meadow parallel to the meadow’s topography (Lopezaraiza–Mikel et al. 2007). We slowly walked each transect once, recording any observed interactions where a floral visitor contacted the reproductive parts of the flower for at least a second and netted and photographed voucher specimens of floral visitors. At the same time, we recorded the identity of all flowering plants within 1 m of the same transect and recorded abundance in floral units (Lopezaraiza–Mikel et al. 2007). Although the amount of time spent in each meadow was allowed to vary with insect and floral abundance, each floral unit was sampled for the same amount in order to standardize the sampling effort per flower over a standardized unit of area (Lopezaraiza–Mikel et al. 2007).

Data Analysis

A multi-scale approach allowed for multiple units of replication across increasing biological scales: (1) the scale of an individual flowerhead, (2) the scale of a focal patch of native plants, defined as a contiguous, conspecific cluster of plants that co-flowered, (3) the scale of a meadow, an area of contiguous grassland containing a serpentine-non-serpentine boundary as defined by a geologic contact between soils, averaging ~ 250 m2.

At the (1) individual scale and (2) the patch scale, we tested our first and second hypotheses that pollinator visitation and seed set decrease with increasing invasive to native plant ratio at the boundary because these processes occur as interactions between pollinators and individual flowerheads within conspecific patches. At (3) the meadow scale, we tested our third hypothesis that the functional importance of native plants within the plant-pollinator network decreases, while the functional importance of invasive plants in the network increases with increasing invasive to native plant ratio at the boundary. We then compared network effects across seasons to test our fourth hypothesis that the spring network would be more robust to invasive plant effects than the summer network. The meadow scale is appropriate for testing network effects, as the plant-pollinator network was sampled at the scale of an entire meadow.

All data analyses were performed in R version 4.4.1 (R Core Team 2022). We calculated the mean ratio of invasive plant floral abundance for each radii to focal patch native plant patch floral abundance (e.g. Invasive Plant Floral Abundance within 250, 500, or 1000 m radii/Native Plant Floral Abundance within focal patch) across the flowering season and log-transformed these ratios. At both the individual plant and patch scale, we calculated pollinator morphospecies richness, the mean number of pollinator visits, mean number of European honeybee (*Apis mellifera*) visits, mean number of non-honeybee pollinator visits to the native plant species, and seed set for spring and summer.

To test whether pollinator visitation on focal native plants decreased with increasing invasive to native ratio (Hypothesis 1), we ran generalized linear models with negative binomial distributions for pollinator response variables using lme4 R package (Bates et al. 2009). We ran separate models for each spatial radii (250 m, 500 m, 1000 m radii) and season (spring and summer) at both the individual flowerhead and patch scales. To further test if the log-transformed mean ratio of invasive to native floral abundance at the boundary correlated with pollinator community compositions, we ran PERMANOVAs with a Bray Curtis Dissimilarity Index using the Adonis2 function in the vegan R package (Dixon 2003).

To test whether seed set on focal native plants decreased with increasing invasive to native ratio (Hypothesis 2), we ran generalized linear models with Gaussian distributions for seed set response variables (i.e. total number of seeds for both sunflowers and clovers and clover seed mass) using the lme4 R package (Bates et al. 2009). We ran separate models for each spatial scale (250 m, 500 m, 1000 m radii) and season (spring and summer) at both the individual flowerhead and patch scales.

To test whether the network role of focal native plants decreased with increasing invasive to native ratio (Hypothesis 3), we measured individual plant species contribution to nestedness, weighted betweenness centrality, and weighted closeness centrality using the bipartite package in R (Dormann et al. 2009). Nestedness is the degree to which plants interact with both generalist and specialist pollinators (Bascompte et al. 2003). The more strongly a plant species contributes to nestedness, the more that plant species acts as a core hub for pollinators in the network. Betweenness centrality is a measure of how central a species is in the network based on the shortest path between species nodes, while closeness centrality is a measure how central a species is in the network based on the reciprocal sum of the length of the shortest paths between the focal species and all other species nodes in the network (Dormann et al. 2009). To further test whether invasive and native focal plants had indirect effects on each other via perceived apparent competition for shared pollinators within the network, we used the Müller index (Eqn. 1), (Müller et al. 1999, Bergamo et al. 2021, Page and Williams 2023a):

$$d_{ij} = \sum_k \frac{\alpha_{ik}}{\sum_l \alpha_{il}} x \frac{\alpha_{jk}}{\sum_m \alpha_{mk}} \text{ (Eqn. 1)}$$

For all pairwise comparisons of invasive plants against focal native plants, the degree of apparent competition for shared pollinators (d_{ij}) between plant species i and j equals the summed product of the number of interactions between plant i and pollinator k (α_{ik}) relative to the number of interactions of plant species i across all pollinator species (α_{il}) and of the number of interactions between plant j and pollinator k (α_{jk}) relative to the number of interactions of plant species j across all pollinator species (α_{mk}).

For examining the effects of invasive to native plant ratio on plant-pollinator network properties, we used generalized linear models with Gaussian distributions. For network analyses, we used only the ratio of invasive to native plants at the 250 m radii, which is the scale at which we sampled plant-pollinator interaction networks. For meadows containing multiple focal patches of native plants, we used the mean invasive plant to native plant ratio of all the patches. To test whether these network responses were less strong for larger, more diverse spring networks than the smaller, less diverse summer networks (Hypothesis 4), we compared the statistical outcomes of the spring and summer models.

To further test if invasive to native plant ratio varied with pairwise niche overlap in pollinators between focal plant species, we ran generalized linear models for each spatial radii and season, testing for an effect of invasive to native plant ratio on two indices of pollinator similarity between focal invasive-native plant pairs: Jaccard's similarity index and Sorensen's index of similarity (Jaccard 1908, Sorensen 1948).

Results:

In total, we observed 2310 individual pollinator visits to clover serpentine patches and 6126 individual pollinator visits to sunflower serpentine patches. We observed 138 visits to clover individual flowerheads and 1730 visits to sunflower individual flowerheads. We observed a total of 11843 pollinator visits across all spring plant-pollinator networks for 364 unique floral visitor morphospecies and 91 unique plant species, and we observed a total of 11337 pollinator visits across all summer plant-pollinator networks for 244 unique floral visitor morphospecies and 42 unique plant species (see Supplement for more information on network sizes). In spring, the log-transformed mean invasive vetch to native clover floral abundance ratio varied from -1.13 to 7.12 at 250 m, from -0.74 to 7.33 at 500 m, and 0.0025 to 8.95 at 1000 m. In summer, the log-transformed mean invasive thistle to native sunflower floral abundance ratio varied from -3.04 to 7.06 at 250m, from -3.04 to 7.06 at 500m, and from 0.21 to 7.41 at 1000 m.

Table 1. Pollinator Responses by Boundary Context. The statistical outputs of negative binomial models, testing for an effect on log-transformed invasive to native plant ratio on pollinator morphospecies richness, pollinator visitation, honeybee visitation, and non-honeybee visitation at 250 m (within-meadow pollinator foraging), 500 m (solitary bee foraging range), and 1000 m (Apidae social bee foraging range) for focal native plant patches and individual flowerheads for both spring native bull clovers (*T. fucatum*) and summer native serpentine sunflowers (*H. exilis*). We did not observe any honeybee visits to tagged individual clovers.

Season	**Scale**	**Radii**	**Pollinator Richness**	**Pollinator Visitation**	**Honeybee Visitation**	**Non-Honeybee Visitation**
Spring	**Patch**	**250 m**	z=-2.68, **p=0.00739**	z= -7.23, **p<0.0001**	z=-3.65, **p=0.000265**	z=-3.34, **p=0.000845**
		500 m	z=-2.86, **p=0.00428**	z=-5.20, **p<0.0001**	z=-1.83, p= 0.067	z=-2.85, **p=0.00441**
		1000 m	z=-3.31, **p=0.000921**	z=-4.88, **p<0.0001**	z=-2.10, p= 0.0358	z=-3.23, **p= 0.00122**
	Individual	**250 m**	z=-0.36, p=0.718	z=-0.55, p=0.5828	NA	z=-0.55, p=0.5828
		500 m	z=-0.11, p=0.913	z=0.217, p=0.828	NA	z=0.217, p=0.828

		1000 m	z=-0.06, p=0.950	z=0.454, p=0.650	NA	z=0.454, p=0.650
Summer	**Patch**	**250 m**	z=-2.45, **p=0.0142**;	z =-3.22, **p=0.00127**	z=-4.01, **p<0.0001**;	z= -2.52, **p=0.0116**;
		500 m	z=-2.28, **p=0.0224**;	z= -3.18, **p=0.0015**	z= -4.14, **p=<0.0001**;	z -2.45, **p=0.0144**;
		1000 m	z= -2.73, **p=0.00628**	z =-2.86, **p=0.00428**	z=-3.58, **p=0.000345**	z= -2.10, **p=0.0358**
	Individual	**250 m**	z=0.54, p=0.588	z=0.46, p=0.643	z=-4.60, **p<0.0001**	z=3.52, **p=0.000438**
		500 m	z=-0.128, p=0.898	z=-0.287, p=0.774	z=-5.399, **p<0.0001**	z= 2.80, **p=0.00514**
		1000 m	z=4.92, **p<0.0001**	z=5.50, **p<0.0001**	z-2.25, p= **0.0244**	z= 8.79, **p<0.0001**

Pollinator visitation to Native Plants

Consistent with our first hypothesis, pollinator visitation at the patch scale varied with boundary context in both spring and summer. In spring, pollinator morphospecies richness on the native clover decreased with increasing invasive to native plant ratio (Figure 1A, Table 1). The total number of pollinator visits to the native clover decreased with invasive to native plant ratio (Figure 1B, Table 1), as did the total number of honeybee visits to the native clover (Table 1), and the total number of non-honeybee pollinators to the native clover (Table 1). With the exception of honeybee visitation, this negative relationship between pollinator response variables and invasive to native plant ratio was consistent across the 250 m, 500 m and 1000 m scales which correspond to the scale of within-meadow pollinator foraging, solitary bee foraging range, and large social bee foraging range (Table 1). Community composition of pollinators on clover significantly varied with invasive to native plant ratio (250 m: F= 8.322, p=0.001; 500m: F=6.7731, p=0.001; 1000m: F=7.1601, p=0.001).

Likewise, in summer, pollinator morphospecies richness on the native sunflower patches at the patch scale decreased with increasing invasive to native plant ratio (Figure 1D, Table 1). Pollinator visitation to sunflower decreased with invasive to native plant ratio (Figure 2E, Table 1) as did the total number of honeybee visits to sunflower (Table 1) and the total number of non-honeybee pollinator visits to sunflower (Table 1). The negative relationship between pollinator response variables and invasive to native plant ratio was consistent across the 250 m, 500 m and 1000 m scales (Table 1). Pollinator community composition on sunflower varied with invasive to native plant ratio (250m: F=5.5986, p=0.001; 500m: F=6.0165, p=0.001; 1000m: F=5.5383, p=0.001).

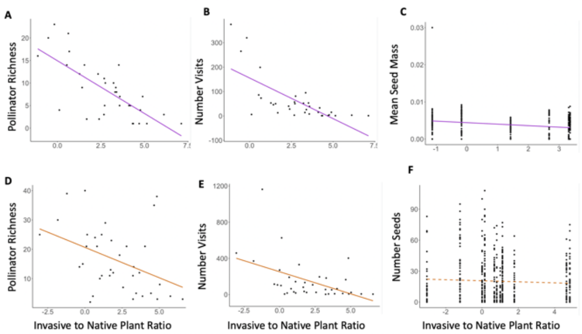

Figure 1. The relationship between invasive plant floral abundance at 250 M to native plant floral abundance for (A) pollinator richness and (B) number of pollinator visits, and (C) mean seed mass per seedpod for bull clover in the spring and for (D) pollinator richness and (E) number of pollinator visits, and (F) total number of seeds per floral unit for serpentine sunflower in summer. The invasive plant for spring is hairy vetch, and the invasive plant for summer is

	yellow star-thistle. Each dot represents the mean values for a given patch over a given season. Solid regression lines denote a statistically significant relationship at an alpha level of 0.05.

The impact of invasive species on visitation to native clovers and sunflowers at the individual flowerhead scale differed depending on the scale at which invader prominence was measured. Contrary to our first hypothesis, in spring, pollinator response variables did not correlate with invasive to native plant ratio (Table 1). Contrary to our first hypothesis, in summer, pollinator morphospecies richness and total number of visits to sunflower flowerheads both increased with the ratio of invasive thistle to native sunflower at the 1000m scale, at which large social bees can disperse across the landscape, (richness: z=4.917, $p<0.0001$, number visits: z=5.502, $p<0.0001$) but not at the 250m or 500m scales (Table 1). The pattern at individual flowerhead scale was further complicated by differences amount pollinator groups. The total number of honeybee visits decreased with invasive to native plant ratio consistent with our first hypothesis (Table 1), while the total number of non-honeybee floral visits increased with invasive to native plant ratio contrary to our first hypothesis (Table 1).

Native Plant Seed Set

In support of our second hypothesis, both clover and sunflower seed set for individual flowers decreased with increasing invasive plant to native plant ratio. In spring, the mean seed mass of clover per seedpod decreased with increasing invasive to native plant ratio at 250 m (2022: t=-4.186, p= 3.74e-05; 2024 t=-3.511, p=0.000452 in 2024), 500 m (2022: t=-3.066, p=0.00237; 2024: t=-2.197, p=0.0281) and 1000 m (2022: t=-3.434, p=0.00068; 2024: t=-1.802 , p=0.0716). Likewise, the mean seed mass per individual clover seedhead decreased with increasing native to invasive plant ratio at 250 m (t= -6.154, p<0.0001), 500 m (t=-3.643, p=0.000292) and 1000 m (t=-2.31, p=0.0212). The total number of clover seeds per pod (250 m t=t2.771, p= 0.00594; 500m & 1000 m ns) decreased with increasing invasive to native plant ratio (Figure 1C). In summer, the total number of sunflowers seeds per flowerhead decreased with increasing invasive to native ratio (1000m: t=-1.984, p= 0.0478; 250 m and 500 m nonsignificant) (Figure 1F).

Meadow Plant-Pollinator Networks

In support of our third hypothesis, the functional importance of focal plant species in the network varied with invasive to native plant ratio at the meadow scale (Figure 2, Figure S1). In spring, as the ratio of invasive vetch to native clover increased, the indirect effect of clovers on vetch via shared pollinators in the network as measured by the Müller index decreased (t=-2.659, p=0.015508) (Figure 2A), but the indirect effect of vetch on clover did not significantly change (Figure 2B). Likewise, as the ratio of invasive to native plant increased, the individual contribution of native bull cover to network nestedness decreased (t=-2.299, p= 0.03303) (Figure 2C) but the individual contribution of vetch to network structure did not significantly change (Figure 2D). In other words, as the as the amount of vetch relative to clover increased, the clover exerted a weaker influence on the network’s nested structure, becoming less of a hub for pollinators. In spring, as the ratio of invasive to native plant at the boundary increased, the betweenness centrality of bull clover in the plant-pollinator decreased (t=-2.679, p=0.014), while that of vetch did not significantly change (t=0.135, p=0.89388). Closeness centrality for neither clover (t=0.067, p=0.94733) nor vetch (t= 0.671, p=0.5100) did not vary with invasive to native plant ratio.

In summer, as the invasive thistle to native sunflower ratio increased, the indirect effect of sunflowers on thistle via shared pollinators in the network at the meadow scale as measured by the Müller index decreased (t=-2.547, p=0.0232) (Figure 2E), while the invasive thistle's indirect effects on the sunflower increased (t= 3.782, p=0.00202) (Figure 2F). Moreover, as the ratio of invasive to native plant increased, the individual contribution of sunflower to network nestedness did not significantly change (Figure 2G), but the individual contribution of yellow star-thistle to the network's nestedness increased (t= 3.747, p= 0.00217) (Figure 2H). In other words, as the amount of thistle relative to sunflower increased, the thistle exerted a stronger influence on the network's nested structure, becoming more of a hub for pollinators. In partial support of our fourth hypothesis about seasonality, these effects of invasive to native plant ratio on network indirect effects appeared stronger in the summer than spring based on Müller's index. In summer, as the ratio of invasive to native plant at the boundary increased, the betweenness centrality of sunflower did not significantly change (t=0.645, p=0.528), but the betweenness centrality of star-thistle significantly increased (t=2.794, p=0.0144). Closeness centrality for neither sunflower (t=-0.408, p=0.689660) nor star-thistle (t=-0.065, p=0.94948) varied with invasive to native plant ratio.

In spring, the ratio of invasive to native plant at the boundary did not significantly correlate with pollinator similarity between vetch and clover for both the Jaccard index of similarity (250 m: t=-1.670, p=0.111; 500 m: t=-1.423, p=0.170808; 1000m: t=-1.180, p=

0.252663) nor the Sorensen index (250 m: t=-1.566, p=0.134; 500m: t=-1.358, p=0.190308; 1000m: t=-1.136, p=0.270286)

In summer, as the ratio of invasive to native plant at the boundary increased, pollinator similarity as measured by the Jaccard index between sunflower and thistle increased marginally at 250 m (t=1.846, p=0.086142), significantly at 500 m (t=2.217, p=0.043692) but not at 1000 m (t=1.362, p=0.195). Likewise, as the ratio of invasive to native plant at the boundary increased, pollinator similarity as measured by the Sorensen index between sunflower and thistle increased marginally at 250 m (t=1 2.141, p=0.0504), significantly at 500 m (t= 2.398, p=0.030993) but not at 1000 m (t= 1.486, p=0.159).

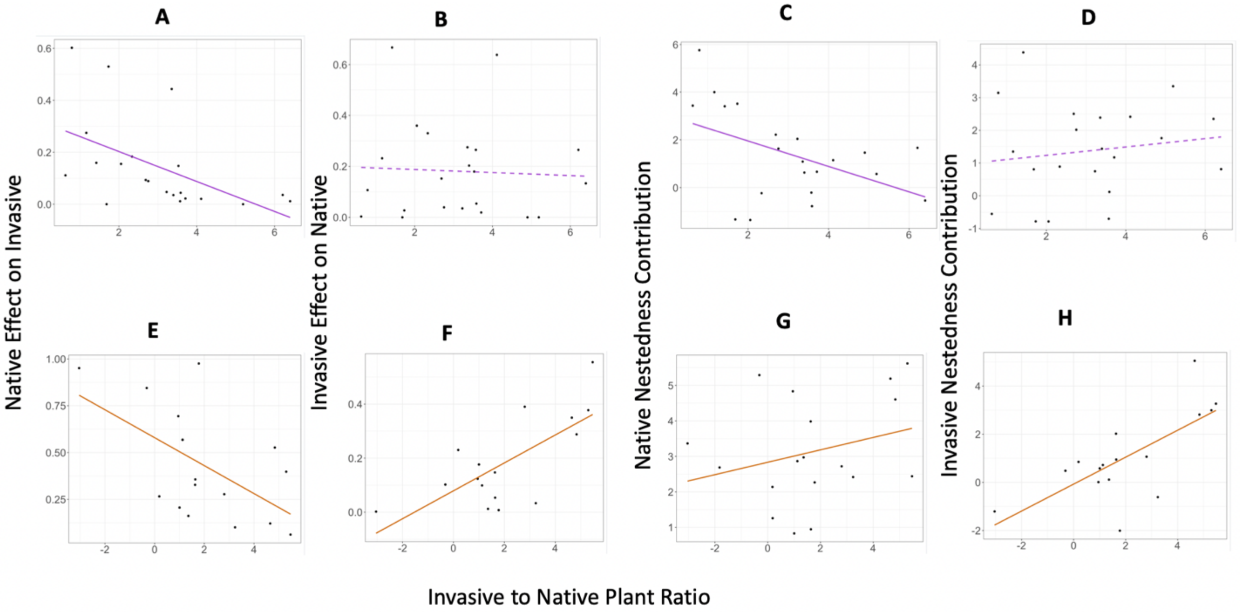

Figure 2. The relationship between the log ratio of invasive plant to native plant floral abundance at 250 M on network metrics for (A) the Mueller indirect effect of native plants on vetch, (B) the Mueller indirect effect of vetch on clover, (C) the individual contribution of clover to network nestedness, (D) the individual contribution of vetch to network nestedness, (E) the

Mueller indirect effect of sunflower on thistle, (F) the Mueller indirect effect of thistle on sunflower, (G) the individual contribution of sunflower to network nestedness and (H) the individual contribution of thistle to network nestedness. Each dot represents a network for a given meadow in a given season. Solid regression lines denote a statistically significant relationship at an alpha level of 0.05, while dashed regression lines denote a non-significant relationship.

Discussion

As the ratio of invasive non-serpentine flowers to native serpentine flowers at the boundary increased, pollinator visits and pollinator richness on native serpentine plants decreased along with seed set by native plants. Moreover, as the ratio of invasive flowers to native flowers increased, the structural role of the native plants in the network decreased as they exerted weaker indirect effects on the invasive plant species and contributed less to the nestedness and between centrality of the network. Network responses were more apparent in summer than spring networks, and in summer only, pollinator similarity between invasive thistles and native sunflowers increased with increasing invasive to native plant ratio. In accordance with our hypotheses, these findings suggest that invasive plant species can have indirect cross-boundary effects on the pollinator visitation, seed set and network role of native plant species through spillover competition for shared pollinators.

Pollinator richness and abundance on focal patches of native plants adjacent to boundaries decreased as the ratio of invasive plants to native plants increased. These results suggest that pollinators are foraging in response to the relative abundance of the two plant species at the boundary, favoring the relatively more abundant species rather than showing a species preference, consistent with theory that pollinators can adaptively track shifts in floral reward availability (Valdovinos et al. 2016, Johnson et al. 2022). Our findings from a wildland system are consistent with prior work showing that the abundance of agricultural crops at an agroecological boundary can affect pollinator spillover into non-crop habitats (Ekroos et al. 2008, Magrach et al. 2017). Although pollinator spillover was driven by pollinators moving from nesting habitat to foraging habitat at a swamp-prairie boundary (Artz and Waddington 2006), pollinator spillover effects in our system were driven by pollinators foraging for floral resources on both sides of the serpentine-non-serpentine boundary. In contrast, pollinators mostly foraged within their home patches within a naturally fragmented glade ecosystem (Harmon-Threatt and Anderson 2023). Evidence for spillover competition supports the idea that invasive species can alter the magnitude of spatial flows across ecological boundaries through affecting patterns of organism movement (Peller and Altermatt 2024). Non-serpentine invasive species may have cross-boundary effects on plant-pollinator diversity and serpentine plant pollination as an ecosystem function, consistent with prior work highlighting that spillover across ecological boundaries may alter patterns of biodiversity and ecosystem function in a neighboring community (Scherer-Lorenzen et al. 2022).

Native plant seed set decreased with increasing invasive to native plant ratio at the boundary. This finding suggests that increased spillover competition for shared pollinators across the boundary may decrease native plant fitness. Within single ecosystems, invasive plants were shown to have both positive and negative indirect effects on native plant fitness via indirect effects through facilitation of and competition for shared pollinators (Molina-Montenegro et al. 2008, Abdallah et al. 2021, Etter et al. 2022). Whether these indirect effects of shared pollinators are facilitative or competitive may further depend on spatial scale (Ekroos et al. 2008, Underwood et al. 2020). Consistent with prior studies focused on pollinators spillover in croplands (Tscharntke et al. 2005, Ekroos et al. 2008, Magrach et al. 2017), we found a relationship between the relative plant abundance at the boundary and plant fitness in a wildland system. Our finding of a negative relationship between seed set and invasive to native species abundance is consistent with decreases in native plant fitness with increasing crop density at an agroecological boundary (Magrach et al. 2017). The mechanisms, however, differed: we found with increasing invasive to native plant ratio, pollinator visitation (including that of honeybees) decreased as did seed set, while increasing crop density at the boundary increased honeybee spillover to native plants, decreasing seed set through decreased visit quality (Magrach et al. 2017).

The outcomes of pollinator spillover contrasted across the patch and individual flowerhead scale. In spring, while invasive to native plant ratio at the boundary decreased pollinator visitation to native plants at the patch scale, it did not affect visitation to individual flowerheads. This finding may suggest the need to account for differences in phenology across scales, as phenology can further shape plant-pollinator interactions (Ogilvie and Forrest 2017). While clover patches bloomed collectively for a few weeks, individual clover flowerheads typically bloomed for less than 48 hours before senescing. Phenological differences in scale did not pose as much as a factor in summer, where sunflower flowerheads bloomed for several days to weeks.

However, in summer, we still found differences in visitation across scale: patch scale visitation and seed set decreased, while individual flowerhead visitation increased with increasing invasive to native plant ratio. Theory suggests that pollinators adaptively track changes in floral reward plant abundances, so changes in the relative abundance of flowers may be a proxy for changing floral reward abundance (Sih and Baltus 1987, Valdovinos et al. 2016). At boundaries with a high invasive to native plant ratio, each individual native plant may receive more pollinator visits per capita because there are fewer native plants in total, while at boundaries with a low invasive to native plant ratio, each individual native plant may receive fewer pollinator visits per capita because there are more native plants in total. This increased visitation to individual flowerheads under increasing invasive to native plant ratio, however, did not translate to increased seed set, perhaps due to concomitant decreases in visit quality. As the invasive to native plant ratio increases, the probability of heterospecific pollen transfer from the invasive species may also increase, decreasing visit quality (Arceo-Gómez and Ashman 2016, Parra-Tabla et al. 2021). The extent of heterospecific pollen transfer may further depend on pollinator constancy vs switching behavior when foraging, which can be affected by plant spatial configuration (Bruninga-Socolar et al. 2022).

Pollinator type may further mediate the outcomes of spillover. European honeybees, which were one of the most abundant pollinators in our study, present a paradox for management, as they play a key role in provisioning pollination services to agriculture (DeGrandi-Hoffman 2003, Breeze et al. 2011) but can displace other bee species through apparent competition (Page and Williams 2023a, b) and can have a single-visit effectiveness that is less than that of other pollinator species (Page et al. 2021). As large, highly mobile, social bees, honeybees forage across ecosystem boundaries, often through spillover from croplands to wildland systems and can become invasive species when introduced to new areas (Gross 2001, Stanley et al. 2020). At crop-wildland boundary, honeybee spillover decreased native plant fitness through decreased visit quality (Magrach et al. 2017). However, we found that as invasive to native plant ratio at the boundary increased, honeybee visitation decreased at both the patch and individual flowerhead scale as did native plant seed set, raising questions about the role of honeybees as exotic pollinators in shaping spillover outcomes and plant fitness.

As the ratio of invasive to native plants increased at the boundary, the functional importance of native plant species as core hubs for pollinators in the plant-pollinator network decreased, as they exerted weaker apparent competition (as measured by the Müeller Index) on invasive plant species via shared pollinators, contributed less strongly to the nested structure of the network, and had decreased betweenness centrality in the network. Consequently, invasive plant species functionally replaced native plant species as core hubs in the network, exerting stronger apparent competition on native species via shared pollinators, contributing more strongly to the nested structure of the network, and having increased betweenness centrality in the network. This finding is consistent studies from within single ecosystems where invasive plant species became highly integrated into the plant-pollinator network, functionally replacing native species as core hubs for pollinators (Russo et al. 2019, Parra-Tabla and Arceo-Gómez 2021). Theory suggests that fewer, but higher quality pollinator visits can allow animal-pollinated species to establish as invaders, eventually becoming dominant network hubs (Valdovinos et al. 2009, 2023). Similar to our findings, pollinator spillover across an agricultural boundary altered network structure through decreasing pollinator niche breadth as a result of increased apparent competition between honeybees and other pollinators (Magrach et al. 2017). These findings raise questions as to how generalizable these network effects of spillover are across ecological systems and how to incorporate spillover into existing network theoretical models, which typically assumed a closed ecosystem in which pollinators do not enter or leave the area (Bascompte et al. 2003, Valdovinos et al. 2016). By incorporating spillover competition for pollinators across ecological boundaries into dynamical network models, future work could examine whether or not cross-boundary spillover maintains and stabilizes diverse mutualistic communities (Hale and Valdovinos 2021). The diversity begets diversity hypothesis would suggest that spatial heterogeneity can increase mutualistic diversity as pollinators forage across patchy habitat boundaries, contributing to positive diversity-stability relationships (Ponisio et al. 2016, Hatton et al. 2024).

We found evidence to partially support our hypothesis that spillover competition is stronger in less species-rich summer networks than spring networks. Although spring and summer plants showed similar patterns in patch level responses, the ratio of invasive plant species to native plant species affected network roles for summer more than for spring. The more diverse spring-blooming plant-pollinator network may be more robust to spillover effects than the less diverse, smaller network of summer-blooming plants. Flower community diversity and functional traits are important factors that govern plant-pollinator network structure (Valdovinos et al. 2018, Phillips et al. 2020, Lautenschleger et al. 2021). High species diversity and niche partitioning can confer biotic resistance to invasions (Levine and D'Antonio 1999, Levine and HilleRisLambers 2009). The spring legumes have long, deep flowers specialized to long-tongued pollinators. In contrast, the summer networks have less plant diversity and are dominated by asters with generalist, composite flowers that are accessible to a wider range of pollinator species. Thus, invasive species may more strongly modulate network structure for the summer network where, the floral community has higher niche overlap but lower plant diversity than spring. These temporal differences in spillover are consistent with the broader literature showing the timing of spatial flows can play an important role in modulating their cross-ecosystem effects (Yang et al. 2010, Peller and Altermatt 2024).

Consistent with findings of pollinator spillover in croplands (Ekroos et al. 2008, Tscharntke et al. 2012) and theory (Amarasekare 2004), our findings further suggest that spillover competition for shared pollinators can affect pollinator visitation and plant fitness in wildland systems. While prior work has shown that invasive species can have pervasive cross-ecosystem effects on food webs and nutrient cycling (Peller and Altermatt 2024), our work demonstrates that invasive plant species can have cross-ecosystem effects on plant-pollinator mutualisms in wildlands. Spillover competition may have downstream effects on the biodiversity, function and ecosystem services of plant-pollinator mutualisms (Magrach et al. 2017, Scherer-Lorenzen et al. 2022), raising the salience of accounting for cross-ecosystem effects when managing invasive species and restoring ecosystems. These results spark further questions about the extent to which anthropogenic global changes may alter pollinator spillover in ways that can affect plant reproduction. Examining whether cross-ecosystem spillover modulates community responses to anthropogenic perturbations remains an ongoing question that can inform the conservation of plant-pollinator diversity (Scherer-Lorenzen et al. 2022, Peller and Altermatt 2024).

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Acknowledgements

Thank you to Cathy Koehler, Paul Aigner, and Ben Amann at the McLaughlin Reserve for providing logistical support. The research was supported by the contributions of the following individuals as field and lab technicians: Bita Rostami, Alexis Grana, Isabel Mendoza, Nat Walts, Rebekah Shane, Kyle Bianchi, Izzie Wolter, KT Lynch, Seth Tantuico, Ngoc Thu Tat, Monica Rodriguez, Ryan Li, Anastasia Karp, Zach Schneider, and Alison Appelgate. Paul Havemann provided aerial drone images. At the Bohart Museum of Entomology, Thomas Zavortink for identified bee taxa and to Socrates Letana identified fly taxa. Thank you to the UC Davis Ecology Statistics Support Group and to members of the Valdovinos Lab for providing feedback on data analysis.

Author Contributions: Study Design & Conceptualization; Rebecca Nelson, Susan Harrison, Neal Williams, and Fernanda Valdovinos Data curation; Rebecca Nelson; Formal analysis; Rebecca Nelson, Neal Williams, Fernanda Valdovinos, Susan Harrison; Methodology; Rebecca Nelson, Neal Williams, Fernanda Valdovinos, Susan Harrison; Visualization; Rebecca Nelson; Writing - original draft; Rebecca Nelson; Writing - review & editing. Rebecca Nelson, Neal Williams, Fernanda Valdovinos, Susan Harrison.

Funding:

This research was funded with support from the California Native Grasslands Association GRASS scholarship, the University of California Reserve System Matthias Grant, the Davis Botanical Society, the UC Davis Jastro & Shields Fund, the Irene Brown Memorial Fund for Women in Environmental Science, and the Phi Beta Kappa Society.

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Chapter 2 Supplemental Appendix

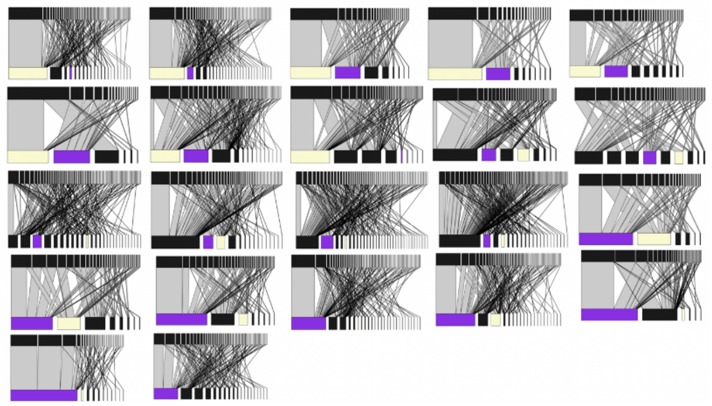

Figure S1. Plant-pollinator networks. Each network was constructed for a given meadow and year. The top panel of networks is for spring, and the bottom panel is for summer. The top rows are pollinators, and the bottom

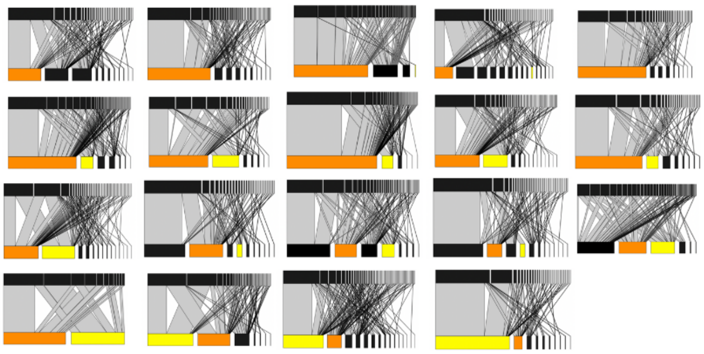	rows are plants. Each box represents a different species with the lines between them representing interactions. The thickness of each box and line is proportional the frequency with a species or interaction was observed in the network. For the spring networks, vetch is highlighted in purple and clover in beige. For the summer networks, star-thistle is highlighted in yellow and serpentine sunflower in orange.

Table S1 Meadows. List of unique meadows and years surveyed and the number of plant species and pollinator morphospecies recorded in the network.

Season	Year	Site	Number Pollinator Species	Number of Plant Species
Spring	2022	Bertha	79	21
Spring	2022	Rock	28	8
Spring	2022	Quarry	65	21
Spring	2022	Goatgrass	68	33
Spring	2022	Long	69	20
Spring	2022	Aikawa	65	26
Spring	2023	Bertha	58	19
Spring	2023	Quarry_1	81	27
Spring	2023	Rock	20	10
Spring	2024	Upper_Grid_2	18	6
Spring	2024	Felch	33	8
Spring	2024	Coyote	31	11
Spring	2024	Aikawa	36	9
Spring	2024	Goatgrass	54	15

Spring	2024	South Goatgrass	37	6
Spring	2024	Anu	54	14
Spring	2024	Quarry_1	42	8
Spring	2024	Quarry_2	39	8
Spring	2024	Quarry_3	23	6
Spring	2024	Quarry_4	21	9
Spring	2024	Quarry_5	47	9
Summer	2022	Bertha	38	12
Summer	2023	Bertha	61	15
Summer	2024	Bertha	43	6
Summer	2022	Pond	40	7
Summer	2023	Pond	50	13
Summer	2024	Pond	34	4
Summer	2022	Randy	29	10
Summer	2023	Randy	32	9
Summer	2022	Aikawa	38	15
Summer	2022	Vineyard	30	10
Summer	2024	Vineyard	41	8
Summer	2022	Lower_Banana	43	10
Summer	2022	Banana_Slug	38	11

Table S2. Plant Species List.

Abbreviation	Plant Species
AC	*Acmispon sp.*
ACMO	*Achyrachaena mollis*
ACMA	*Acmispon americanus*
ACMI	*Achillea millefolium*
ACBR	*Acmispon brachycarpus*
ACWR	*Acmispon wrangelianus*
AGHE	*Agoseris heterophylla*
ALAM	*Allium amplectens*
Allium	*Allium spp.*
AMME	*Amsinckia menziesii*
ANAR	*Anagallis arvensis*
ANFI	*Ancistrocarphus filagineus*
ASER	*Asclepias eriocarpa*
ASFA	*Asclepias fascicularis*
ASBR	*Astragalus breweri*
ASJE	*Astragalus rattanii jepsonii*
AS	*Astragalus*
BREL	*Brodiaea elegans*
CAEX	*Castilleja exserta*
CA	*Calochortus sp.*
CAPA	*Calycadenia pauciflora*
CAAM	*Calochortus amabilis*
CARU	*Castilleja rubicundula*
CADE	*Castilleja densiflora*
CAMI	*Castilleja minor*
CAME	*Calandrinia menziesii*
CALU	*Calochortus luteus*
CASU	*Calochortus superbus*
CAVE	*Calochortus vestae*
CL	*Clarkia gracilis_or_purpurea*
CLGR	*Clarkia gracilis ssp. tracyi*
CLPU	*Clarkia purpurea*
CAPY	*Carduus pycnocephalus*
CUCA	*Cuscuta californica*

COSP	*Collinsia sparsiflora*
CRHI	*Cryptantha hispidula*
DEHE	*Delphinium hesperium*
DEUL	*Delphinium uliginosum*
DUCY	*Dudleya cymosa*
DEVA	*Delphinium variegatum*
ERNU	*Eriogonum nudum*
DICA	*Dichelostemma capitatum*
ERLA	*Eriophyllum lanatum*
EPDE	*Epilobium densiflorum*
ERGU	*Erythranthe guttata*
ESCA	*Eschscholzia californica*
ERCI	*Erodium cicutarium*
ERBO	*Erodium botrys*
EUSP	*Euphorbia spathulata*
GEMO	*Geranium mole*
GEDI	*Geranium dissectum*
GICA	*Gilia capitate*
GRCA	*Grindelia camporum*
GITR	*Gilia tricolor*
HECU	*Heliotropium curassavicum*
HOCA	*Horkelia californica*
HECO	*Hemizonia congesta*
HOVI	*Holocarpha virgata*
HEAR	*Heteromeles arbutifolia*
HOMA	*Hoita macrostachya*
HIIN	*Hirschfeldia incana*
KECK	*Sidalcea keckii*
LACA	*Lasthenia californica*
LENI	*Lepidium nitidum*
LAMI	*Lagophylla minor*
LATSER	*Lactuca serriola*
LERE	*Lewisia rediviva*
LUAL	*Lupinus albifrons*
LULU	*Lupinus luteolus*
LERA	*Lessingia ramulosa*
LOHO	*Lomatium hooverii*

LASE	*Layia septentrionalis*
LIDI	*Linanthus dichotomus*
LUNA	*Lupinus nanus*
LUBI	*Lupinus bicolor*
LUMI	*Lupinus microcarpus*
LUSU	*Lupinus succulentus*
MEIN	*Melilotus indicus*
MICA	*Micropus californicus*
MIDO	*Minuartia douglassi*
MEPO	*Medicago polymorpha*
WYAN	*Wyethia angustifolia*
PLNO	*Plagiobothrys nothofulvus*
NONE	*NONE*
PEKE	*Perideridia kelloggii*
PE	*Penstemon spp.*
PHAQ	*Phalaris aquatica*
PRHE	*Primula hendersonii*
PLER	*Plantago erecta*
RILE	*Rigiopappus leptocladus*
RACA	*Ranunculus californicus*
SAVE	*Sairocarpus vexillocalyculatus*
STAL	*Stachys albens*
SCSI	*Scutellaria siphocampyloides*
SI	*Sidalcea sp.*
SEVU	*Senecio vulgaris*
SIBE	*Sisyrinchium bellum*
HEEX	*Helianthus exilis*
TOVE	*Toxicoscordion venenosum*
TOFR	*Toxicoscordion fremontii*
Toxicoscordion	*Toxicoscordion spp.*
TRAL	*Trifolium albopurpureum*
TRER	*Triphysaria eriantha*
TRBI	*Trifolium gracilentum_or_bifidum*
TRFU	*Trifolium fucatum*
TROB	*Trifolium obtusiflorum*
TRIWI	*Trifolium willdenovii*
TRHI	*Trifolium hirtum*
THCA	*Thermopsis californica*

TRLA-Summer	*Trichostema laxum*
TRLA-Spring	*Triteleia laxa*
UNK_PLANT	*Unknown plant*
VIDO	*Viola douglasii*
VIVI	*Vicia villosa*
CESO	*Centaurea solstitialis*
ZETR	*Zeltnera tricantha*
white-daisy	*Leucanthemum vulgare*
URLI	*Uropappus lindleyi*
VIAM	*Vicia americana*
yellow_lomatium	*Lomatium utriculatum*
CHGL	*Chaenactis glabriuscula*
smooth_catsear	*Hypochaeris glabra*
SOAS	*Sonchus asper*
VICSAT	*Vicia sativa*
LODA	*Lomatium dasycarpum*
PHTA	*Phacelia tanacetifolia*
WYGL	*Wyethia glabra*

Table S3. Pollinator morphospecies list.

Species Code	Species
ADEL	*Adela eldorada*
Adela	*Adela spp.*
ADFL	*Adela flammeusella*
ADPL	*Adrena plana*
ADTR	*Adela trigrapha*
ANBA	*Andrena baeriae*
ANDL	*Andrena sp.*
ANDI	*Andrena dissona*
ANLE	*Andrena lewisorum*
ANPE	*Andrena pensilis*
ANOR	*Andrena orthocarpi*
ANSU	*Andrena subchalybea*
Anthidium	*Anthidium sp.*

aphideater	*Eupeodes fumipennis*
anthophora_sp	*Anthophora crotchii*
ANCA	*Anthophora californica*
alfalfa_looper	*Autographa californica*
AGTE	*Agapostemon texanus*
Anth_Syr	*Scaeva affinis*
APME_SYR	Syrphidae sp.
Andrena	*Andrena sp.*
Aphoebantus	*Aphoebantus* sp.
APME	*Apis mellifera*
BF1	Small black fly (Diptera)
blister_beetle	Meloidae
big_black_beetle	*Brachysomida californica*
big_yellow_wasp	*Polistes sp.*
black_bee	*Panurginus sp.*
black_ovi_wasp	Braconidae
big_black_osmia	*Osmia sp.*
Big_Nomada	*Nomada edwardsii*
black_white_beetle	*Anthrenus sp.*
black_weevil	Curculionidae
big_pale_fuzzy_andrena	*Andrena sp.*
black geen red beetle	*Malachius sp.*
black_andrena	*Andrena sp.*
black_green_beetle	Coleoptera
black_lh_beetle	*Anastrangalia laetifca*
black_muscid	Muscidae
black_red_lep	*Annaphila decia*
black_red_wasp	*Sphex asmeadii*
black_robber_fly	Asilidae
black_fuzzy_osmia	*Osmia sp.*
big_gray_listrus	*Listrus sp.*
big_dark_andrena	*Andrena sp.*
black_yellow_ornate_beele	*Trichodes ornatus*
black_small_beetle	*Listrus sp.*
BY_SYR	Syrphidae
Beetle_1	*Listrus sp.*
bluish_moth	Lepidoptera
brown_moth	Lepidoptera

BOCA	*Bombus californicus*
BOCR	*Bombus crotchii*
BOED	*Bombus melanopygus ssp. edwardsii*
BOMA	*Bombylius major*
BOMB	*Bombyliidae*
Bombus	*Bombus spp.*
BOVO	*Bombus vosnesenskii*
BRCA	*Brachymelecta californica*
carder	*Anthidium illustre*
click_beetle	Elateridae
clark_sphinx	*Proserpinus clarkiae*
CAOB	*Calliopsis obscurella*
CAAN	*Calypte anna*
CEPU	*Ceratina punctigena*
Chrysid	*Chrysidae*
COEU	*Colias eurytheme*
Cobalt_Beetle	*Chrysochus cobaltinus*
COFE	*Conophorus fenestratus*
coppery_hairstreak	*Tharsalea xanthoides*
convergent_ladybug	*Hippodamia convergens*
Cone_Bomb	*Conophorus fenestratus*
COTU	*Coenonympha tullia*
CYNA	*Cylindromyia nana*
DAPA	*Dalmannia pacifica*
DIBI	*Diadasia bituberculata*
DIDU	*Dianthidium dubium*
PHMY	*Phyciodes mylitta*
cucumber_beetle	*Diabrotica umdecimpuncata*
DIDU	*Dianthidium dubium*
DINI	*Diadasia nigrifrons*
dung_fly	*Scathophaga stercoraria*
diadasia	*Diadasia sp.*
duskywing	*Erynnis sp.*
dark_eristolis	*Eristolia hirta*
dark_gray_bomb	Bombyliidae
dark_green_fly	*Lucilia sp.*

dark_duskywing	*Erynnis propertius*
EMPID	Empididae
ERHI	*Eristalis hirta*
ERST	*Eristalis stipator*
ERTE	*Eristalis tenax*
EUAC	*Eucera actuosa*
Eucera	*Eucera spp.*
EUAL	*Eucera frater ssp. albopilosa*
Euodynerus	*Euodynerus hidalgo*
Eupeodes	*Eupeodes sp.*
EUPH	*Euphydryas spp.*
EXFA	*Exoprosopa fascipennis*
fritillary	Lepidoptera
flower_beetle	*Acmaeodera sp.*
fuzzy_nomad	*Geminaria c.f. canalis*
Geron	*Geron sp.*
green_cuckoo_bee	*Chrysis angolensis*
green_gold_andrena	*Andrena sp.*
green_osmia	*Osmia sp.*
green_lacewing	Chrysopidae
gold_Osmia	*Osmia gabrielis*
GLLY	*Glaucopsyche lygdamus*
Habropoda sp.	*Habropoda sp.*
HALI	*Halictus ligatus*
HALI_LATI	*Halictus ligatus or Lassioglossum titusi*
HATR	*Halictus tripartitus*
HATR_DIAL	*Halictus tripartitus or Lasioglossum incompletum*
HEDI	*Heliothodes diminutiva*
Ochlodes sp.	*Hesperiidae sp.*
HOHY	*Hoplitis hypocrita*
ICAC	*Icaricia acmon*
JUCO	*Junonia coenia ssp. grisea*
LAIN	*Lasioglossum incompletum*
LATI	*Lasioglossum titusi*
ladybug	Coccinellidae
lh_andrena	*Andrena sp.*

MERO	*Melissodes robustior*
Melissodes sp.	*Melissodes sp.*
MEGA	*Megachile apicalis and parallela*
Megachile sp.	*Megachile sp.*
Melanosmia	*Melanosmia sp.*
MELU	*Melissodes lupina*
DAPL	*Danaus plexippus*
N_white_skipper	*Heliopetes ericetorum*
Muscid	Muscidae
Nomada	*Nomad asp.*
NOHE	*Nomada Hesperia*
NONE	NONE
NOOB	Nomada obscurella
Nomada_Syr	Syrphidae
Ochlodes	*Ochlodes sp.*
OSCA	*Osmia cara*
Osmia	*Osmia sp.*
orange_beetle	Coleoptera
orange_ab_syrpid	Syrphidae
PAHA	Paragus haemorrhous
PANUR	Panurginus spp.
pomonabee	*Peponapis sp.*
VAVI	*Vanessa virginienis*
plume_moth	Pterophoridae
Pseudomaris	*Pseudomaris sp.*
pale_brown_bomb	Bombyliidae
pink_brown_moth	*Heliolonche modicella*
PHBU	*Physocephala burgessi*
PIRA	*Pieris rapae*
POAU	*Polistes aurifer*
red_blister_beetle	Meloidae
rocky_mountain_clearwing	*Hemaris thetis*
Rhamphomyia	*Rhamphomyia sp.*
SAB	Anthophila
SAB_UNSFL	Syrphidae
SACA	*Satyrium californica*
SPHAE	*Sphaerophoria sp.*

STME	*Strymon melinus*
skinny_black_bee	Anthophila
small_black_bee	Anthophila
skinny_gray_fly	Diptera
small_gray_bomb	Bombyliidae
skinny_gray_moth	Lepidoptera
skinny_beetle	Mordellidae
silver_moth	Lepidoptera
straw_moth	*Heltiothis phloxiphaga*
snakefly	Mecoptera
small_muscid	Muscidae
small_gray_moth	Lepidoptera
small_dark_Andrena	*Andrena sp.*
small_brown_moth	Lepidoptera
small_skinny_syr	Syrphidae
small_stripe_SYR	Syrphidae
small_paper_wasp	Vespidae
shiny_moth	Lepidoptera
Toxophora	*Toxophora sp.*
Triepeolus	*Triepeolus utahensis*
trla_bee	*Lasioglossum sp.*
tiny_green_halicitid	*Halictus sp.*
tiny_thrips	Thysanoptera
UNHYM	Hymenoptera
UNK_BOM	Bombyliidae
UNKBEE	Anthophila
UNKBUTTERFLY	Lepidoptera
UNKFLY	Diptera
UNKLEP	Lepidoptera
UNMOTH	Lepidoptera
UNSFL	Syrphidae
UNWASP	Hymenoptera
unwasp_small	Braconidae
Villa	*Villa spp.*
XYTA	*Xylocopa tabaniformis*
XYSO	*Xylocopa sonorina*
Xylocopa	*Xylocopa sp.*
unkblkfly	Diptera

variable_checkerspot	*Euphydryas chalcedona*
wasp_fly	Diptera
white_butterfly	Lepidoptera
white_moth	Lepidoptera
orange_tip	*Anthocharis sara*

Chapter 3: Forb diversity globally is harmed by nutrient enrichment but can be rescued by large mammalian herbivory

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Abstract

Forbs ("wildflowers") are important contributors to grassland biodiversity and services, but they are vulnerable to environmental changes that affect their coexistence with grasses. In a factorial experiment at 94 sites on 6 continents, we tested the global generality of several broad predictions arising from previous studies: (1) Forb cover and richness decline under nutrient enrichment, particularly nitrogen enrichment, which benefits grasses at the expense of forbs. (2) Forb cover and richness increase under herbivory by large mammals, especially when nutrients are enriched as grazing will release forbs from grass competition under fertilization. (3) Forb richness and cover are less affected by nutrient enrichment and herbivory in more arid climates, because water limitation reduces the impacts of competition with grasses. (4) Different forb families will respond differently to nutrient enrichment and mammalian herbivory due to

differences in nutrient requirements and tolerances. We found strong evidence for the first, partial support for the second, no support for the third, and support for the fourth prediction. Forb richness and cover are reduced by nutrient addition, with nitrogen having the greatest effect; forb cover is enhanced by large mammal herbivory, although only under conditions of nutrient enrichment and high herbivore intensity; and forb richness is lower in more arid sites, but is not affected by consistent climate-nutrient or climate-herbivory interactions. We also found that nitrogen enrichment disproportionately affects forbs in certain families (Asteraceae, Fabaceae). Our results underscore that anthropogenic nitrogen addition is a major threat to grassland forbs and the ecosystem services they support, but grazing under high herbivore intensity can offset these nutrient effects.

Introduction

Forbs, or non-graminoid herbaceous angiosperms [1], play essential roles in maintaining grassland diversity, structure and function through supporting pollinator populations [2–5]. Anthropogenic global change drivers, however, have altered forb-rich communities, such as grasslands, in ways not yet fully understood. Most field studies to date have been performed at the level of an individual site or single stressor, even though grassland forbs are widespread, diverse and threatened by multiple, interacting anthropogenic drivers [6–9]. Nutrient enrichment [10,11], changes in large mammalian herbivores through the loss of native megafauna and/or the addition of livestock[10,12,13], and climate change [14] are major contributors to losses in forb biodiversity. Based on predictions from coexistence theory [15–18], fertilization [19–23] and the loss of large mammalian herbivores [24–28] can decrease the diversity and abundance of shorter, slower-growing forbs through increasing competition from taller, faster-growing grasses [29–32] for light [33,34], even for initially dominant species[35]. Aridity, which tends to decrease forb richness and abundance[14], may dampen these fertilization and herbivore effects as water limitation reduces the impacts on forbs from competition with grasses[36–38]. However, empirical support from single site studies for the importance of grass-forb competition as a mediator of global change effects on forb biodiversity remains equivocal due to the contingencies that arise with biogeographic, ecological and climatic variation[5,39–43]. Fertilization effects may depend upon which and how many nutrients are added as nitrogen enrichment, in particular, favors grasses at the expense of forbs[10] due to nitrogen's role as a more prevalent limiting nutrient than potassium or phosphorus[44–46]. Studying the combined effects of nutrient enrichment, changes in large herbivores, and aridity gradients as interacting drivers of global change rather than isolating them fills a critical knowledge gap [9,39] and more accurately reflects real world scenarios of anthropogenic change [6–8].

In a factorial experiment at 94 grassland sites on 6 continents, we tested the global generality of several broad predictions arising from previous studies. We thus predict that (1) Forb cover and richness will decline under nutrient enrichment, particularly nitrogen enrichment, benefitting grasses at the expense of forbs. (2) Forb cover and richness will increase under herbivory by large mammals, especially when nutrients are enriched, as grazing will offset the effects of increased grass competition on forbs under fertilization. (3) Forb richness and cover will be less affected by nutrient enrichment and herbivory in more arid climates. (4) Different forb families will respond differently to nutrient enrichment and mammalian herbivory due to differences in nutrient requirements and tolerances. We found strong evidence for the first, partial support for the second, no support for the third prediction, and strong evidence for the fourth prediction. Forb richness and cover are reduced by nutrient addition, with nitrogen having the greatest effect; forb cover is enhanced by large mammal herbivory, although only under conditions of nutrient enrichment and high herbivore intensity; and forb richness is lower in more arid sites regardless of nutrient level or the presence of herbivores. We also found that nitrogen enrichment disproportionately affects forbs in certain families including Asteraceae and Fabaceae, two large families that are essential for pollination, biomass production, and nutrient cycling [5,10]. In contrast, Gerianaceae and Apiaeae did not respond to nitrogen enrichment, while nitrogen enrichment increased Polygonaceae cover. Our results underscore that eutrophication, especially nitrogen addition, is a major threat to grassland forbs and the ecosystem services they support, but large mammalian herbivory can offset these effects.

Results

Nutrient Effects

In support of our first prediction, NPKμ fertilization contributed to forb declines (Figure 1, Table 1). Fertilization via combined nitrogen, phosphorus, potassium with micronutrient enrichment (NPKμ treatment) decreased forb species richness by 27% (see Methods) (t=-8.11, p< 0.001), forb family richness by 19% (t=-6.04, p<0.001), and forb cover by 13% (t=-2.07, p=0.038) (Table 1). NPKμ fertilization also decreased grass species richness by 8%, (t=-2.50, p=0.012) but increased total grass cover by 22% (t=3.27, p= 0.001).

As predicted, nitrogen was the strongest contributor to declines in forb richness and cover compared to phosphorus and potassium with micronutrients (Figure 2, Table 2). Forb species richness decreased in response to nitrogen by 14% (t=-6.12, p<0.001), to phosphorus by 6% (t=-2.32, p=0.020), and potassium and micronutrients by 6% (t=-2.59, p=0.010) with no interactions among nutrients (Table 2). Likewise, forb family richness and forb cover in the fertilization factorial experiment decreased in response to nitrogen by 7% and 7%, respectively (family richness: t=-3.49, p<0.001; forb cover: t=-3.18, p=0.001), but did not respond to phosphorus addition nor potassium and micronutrients (Table 2).

Responses of common forb families further supported this trend and our prediction that fertilization effects vary by forb family. Asteraceae species richness decreased more in response to nitrogen than phosphorous and potassium with micronutrients (Figure 3, Table S1), and Fabaceae cover decreased in response to nitrogen, while increasing in response to phosphorus and potassium with micronutrients (Figure 3, Table S1) However, nitrogen enrichment did not affect Geraniaceae species richness, which instead declined under phosphorus enrichment (t=-

3.17, p=0.002), nor Apiaceae cover and richness (Table S1). In contrast, Polygonaceae cover increased with nitrogen enrichment (t=3.62, p<0.001) but declined with added potassium with micronutrients (Table S1) . Grass cover increased with nitrogen addition (t=4.44, p<0.001) and phosphorus addition. (t=5.29, p<0.001).

Herbivore Effects

In partial support of our second prediction, large mammalian herbivore exclusion under fertilization and high herbivore intensity, as measured by the difference in live biomass between the control and the fenced treatments, contributed to forb declines (Figure 1, Table 1). Herbivore exclusion via fencing did not directly affect forb or grass richness and cover estimates (Figure 1, Table 1). High herbivore intensity, however, alleviated the suppression of forb species richness by NPKμ fertilization (t=-1.96, p=0.0499) such that forb richness no longer was negatively affected by NPKμ when herbivore intensity was high. For sites with high herbivore intensity, herbivory alleviated the suppression of forb cover under fertilization, although forb cover was not strongly affected by the exclusion of herbivores under ambient conditions (t=3.06, p=0.002) (Figure S1, Table 1). Herbivore effects further varied considerably among common forb families (Figures S1-S5, Table S2). Asteraceae richness and cover was highest at sites with high herbivore intensity, but was strongly suppressed under fertilized conditions when herbivores were removed from the high intensity sites (Figure S2, Table S2). In contrast, Fabaceae and Apiaceae richness and cover were not affected by herbivore exclusion nor intensity (Table S2). Geraniaceae cover was enhanced by the interaction between herbivore exclusion and fertilization, while Polygonaceae richness was positively associated with herbivore intensity but dampened by interactions between fertilization and fencing (Table S2).

Aridity Effects

Contrary to our third prediction, potential evapotranspiration (PET), a measure of aridity, did not interact with herbivore exclusion and nutrient enrichment to modify forb or grass richness or cover (Table 1). Forb species and family richness both decreased with increasing PET (Figure 1, Table 1). PET was negatively associated with forb family richness ($t=-3.88$, $p<0.001$), forb species richness ($t=-3.36$, $p=0.001$) but not with forb cover, grass richness, nor grass cover (Table 1).

Discussion

Across diverse grasslands spanning a global range of climate conditions, nutrient enrichment, particularly nitrogen, reduced forb richness, while increasing grass cover. Herbivory by large mammals, however, can rescue forb diversity declines from these detrimental nutrient effects especially at sites where herbivore intensity was high. Large mammalian herbivores at naturally occurring densities play an essential role in offsetting these negative effects of fertilization on forbs by decreasing competition from grasses through consuming grasses. Forb richness declined with increasing potential evapotranspiration (PET), but PET did not interact with herbivore exclusion and fertilization. Our findings highlight that fertilization, especially nitrogen enrichment, is a major threat to grassland forb diversity. Furthermore, nutrient enrichment especially threatened richness and cover of Asteraceae and Fabaceae, two of the largest plant families that are key providers of pollinator support and strongly contribute to food security, nutrient cycling and productivity [5,10,47].

In support of our first prediction regarding nutrients, forb richness decreased under fertilization, while grass cover increased, producing functional shifts from a more diverse forb-dominated community to a less forb-rich, more grass-dominated system. These findings support past studies that have found fertilization increases grass dominance at the expense of forbs [10,20,22,33,46,48] including for initially dominant forb species[35]. Taller, faster-growing grasses favored under nutrient enrichment may shade out more light-demanding, shorter, slower-growing forbs [20,33], altering grass-forb community assembly and coexistence [17,38,49]. Losses in forb richness under fertilization, as in this study, suggest that extinctions of forb species, likely those of smaller stature with lower competitive ability for light [34,50], can drive the negative impact of nutrient enrichment on overall community diversity. Losses in forb diversity can have consequences for the provisioning of ecosystem services such as pollination, food security, and medicinal plants[5].

In further support of our first prediction, nitrogen addition, more than phosphorus or potassium with micronutrients, strongly contributed to declines in forb cover and forb richness, while increasing grass cover. This suggests that nitrogen addition may have more severe consequences for forb declines than the addition of other nutrients by giving grasses a competitive advantage [10,45,51]. Outcomes of grass versus forb competition may thus depend on type of nutrient [52]. In general, nitrogen enrichment is likely to have a pervasive impact on forbs because of its mobility and widespread inputs through airborne nutrient deposition [44,53]. While at a global scale nitrogen is typically a growth-limiting nutrient, these effects can vary regionally as under heavy nitrogen fertilization, other nutrients like phosphorus and potassium can become the limiting nutrients instead with nutrient colimitation more widespread than single-nutrient limitation[52,54].

In partial support of our second prediction, herbivory offset the negative effects of fertilization on forb cover for sites with high herbivore intensity, where the effects of herbivores on biomass were strong. Differences in herbivore diet and density across our study sites produce a gradient in the potential site-level control of forbs by herbivores [26,28,32]. Herbivore diet, for example, can determine how herbivory modulates grass-forb competition [26,28]. Herbivores that predominantly consume grasses may benefit forbs via relaxing light competition between grasses and forbs; the loss of these large grazers can negatively affect forb abundance and diversity [8,26,31,32]. Alternatively, as some forbs are palatable and are preferred forage for many herbivores [55,56], the loss of forb-consuming herbivores can instead increase forb diversity and abundance [25,32]. Our results provide evidence for these impacts: at sites with greatest herbivore effects on total biomass, we find that herbivores increase both richness and cover of abundant forb families, while suppressing grass cover. Variation in herbivore intensity may modulate the outcomes of grass-forb competition and coexistence [25,57,58].

In contrast to our third prediction and some prior findings [38,59], PET did not interact with fertilization and herbivore exclusion. Instead, increasing PET decreased forb richness, but did not affect grass cover and richness. This finding suggests that the increase in grass cover under fertilization may be consistent across climatic variation in aridity, while aridity may further drive forb declines. This result is supported by prior work that found that forb functional diversity decreased under increasing aridity, and that variation in rainfall modulated grass-forb coexistence [14,60]. In contrast, other studies have found forbs to be more resilient under dry conditions than grasses and that increased precipitation increased grass biomass[46], perhaps due to the differing ways in which these studies have measured aridity[37,59,61]. Our results from 94 grasslands across six continents suggest, however, that aridity generally has negative effects on forb diversity.

In support of our fourth prediction, fertilization effects further varied by forb taxonomic family. Fabaceae and Asteraceae declined under nitrogen enrichment, while Geraniaceae declined under phosphorus enrichment, Polygonaceae declined under enrichment of potassium with micronutrients, and Apiaceae did not respond to nutrient effects, consistent with past findings [10,62]. While Fabaceae increased under enrichment of phosphorus and potassium with micronutrients, Polygonaceae increased under nitrogen enrichment. Forb families differ in floral traits and floral rewards, suggesting that compositional shifts in forb families under fertilization could have impacts upon communities of pollinators that depend on these forbs[63].

Declines in forbs under nutrient enrichment may have consequences for ecosystem functions and services, as forbs constitute a large portion of functional diversity in grasslands [5,60]. Forbs play a critical role in contributing to grassland species richness, ecosystem functions and ecological stability, such as nutrient cycling and provision of food for pollinators [60]. A greater diversity of forbs may provide both more functional redundancy and niche complementarity that could make forb communities more resilient to disturbance [64,65] and benefit pollinators [66]. A loss in forb richness due to nutrient enrichment may thus reduce the resiliency of grassland forb communities to further perturbations under anthropogenic global change, for example, due to climate change. Restoration efforts could consider maximizing the phylogenetic and functional diversity of forbs, while mitigating nutrient enrichment and reintroducing or protecting the local, native large mammalian herbivores [3,67–69].

Forb declines under anthropogenic change can have downstream effects on plant-pollinator mutualisms [39,41,70,71]. The shift from forb to grass-dominated, less forb-rich ecosystems likely has negative functional consequences for pollinators that require a diversity and abundance of forbs as floral resources [4,72,73]. Our findings of strong nutrient enrichment effects on Fabaceae and Asteraceae are especially concerning, giving that these two abundant families respectively provide critical early and late season resources for pollinators[47]. Fertilization can alter the quality [74,75] and quantity [41,76,77] of floral resources that forbs provide to pollinators and can shift floral phenology [78,79]. As decreases in forb richness may decrease pollination services essential for food production and agriculture [41,80], future research could examine how nutrient-driven forb declines affect pollinators and whether grazing can rescue these forb-pollinator mutualisms. These widespread fertilization effects on forbs, modulated by herbivory may be pivotal in explaining pollinator declines at a global scale [80–82]. This research supports past findings that large herbivores can rescue forbs from the negative effects of nutrient enrichment and that nitrogen enrichment, in particular, is detrimental to forbs[28,46,58], and further suggests that these responses are generalizable to the global scale and that forb response varies by taxonomic family with nitrogen enrichment strongly decreasing Fabaceae and Asteraceae but not Apiaceae or Gerianaceae, and increasing Polygonaceae.

Methods

Study Design

This study used data from 94 sites in the Nutrient Network (https://nutnet.org/), an experimental study of nutrient enrichment and herbivore exclusion in grasslands that is globally replicated[8]. At each site, 5x5 m plots included a factorial combination of nutrient additions of nitrogen (N), phosphorus (P), potassium (K) and micronutrients (Kμ) or no nutrients (control) and fencing or not to yield 10 treatment plots per block (for more details on experimental design see Borer et al. 2014). Most sites contained three replicate blocks. The following nutrients were added annually to the fertilized plots: 10 g N $\mathrm{m}^{-2}\mathrm{y}^{-1}$as slow release urea $((\mathrm{NH}_2)_2\mathrm{CO}))$, 10 g P $\mathrm{m}^{-2}\mathrm{y}^{-1}$as triple-super phosphate $(\mathrm{Ca}(\mathrm{H}_2\mathrm{PO}_4)_2)$, 10 g K $\mathrm{m}^{-2}\mathrm{y}^{-1}$ as potassium sulfate $(\mathrm{K}_2\mathrm{SO}_4)$. The plots receiving the potassium treatment received a one-time addition of other micronutrients and macronutrients in the first year: 100 gm-2 of a mixture of 15% iron (Fe), 14% sulfur (S), 1.5% magnesium (Mg), 2.5% manganese (Mn), 1% copper (Cu), 1% zinc (Zn), 0.2% boron (B) and 0.05% molybdenum (Mo). The control plots were left untreated. Herbivore exclusion fences were up to 2.3 m high with the goal of excluding all aboveground large mammalian herbivores more than 50 grams, including rabbits, hares and marsupials [8,28,32]. Sites varied in their climate [8], soil fertility[44], species richness and composition, and grazing history [28].

We analyzed data from these 10 experimental plots in two combinations. First, "fertilization factorial" plots (n= 89 sites) applied different factorial combinations of nitrogen, phosphorus, and potassium to experimental plots: control, N, P, Kμ, NP, PKμ, NKμ, and NPKμ. with the control plots left unfertilized [8]. Second, "fencing by fertilization" plots (n = 82 sites) combined large herbivore exclusion using fencing with NPKμ fertilization resulting in four treatments: unfenced control, fenced control, unfenced NPKμ, and fenced NPKμ [8].

Vegetation Sampling

Sampling at all sites followed a standardized sampling protocol [8]: all plots were 5x5 m, and all sites collected at least one year of pre-treatment data and at least two years of post-treatment data[8]. The plots were non-destructively sampled for vegetative cover; a 1x1 m quadrat was used to estimate aerial vegetative cover within each plot for each plant species. Summed cover may exceed 100% if vegetation contains multiple layers. Site scientists provided information on functional lifeform (ex: graminoid, forb, woody etc). For sites where cover was assessed multiple times each year, species were assigned their maximum cover across the different dates. Annual peak season live biomass was measured as the aboveground live biomass of all plants rooted within two 10 x 100 cm strips per plot [8,32]. Clipped vegetation was dried at at 60 °C for 48 hour, and then weighed to the nearest 0.01 gram [8,32].

Data analysis

All data analyses were performed in R [83] using the “nlme”[84], “lme4”[85], “sjPlot”[86], and “Rmisc” [87] packages. We examined responses for combined legume and forb functional groups (hereafter referred to as forbs) and combined grasses and graminoids (hereafter referred to as grasses). We first calculated the following diversity metrics: forb and grass species richness (total number of species present), and forb and grass family richness (total number of families present) for each plot and year per site. We calculated the total normalized forb vegetative cover per plot for each year (total forb cover/total plot vegetative cover) and normalized grass cover for each plot in each year (total grass cover/total plot vegetation cover) for a given year at each site. For the five most abundant taxonomic families of forbs across our dataset, Asteraceae (n=89 sites), Fabaceae (n=85 sites), Geraniaceae (n=28 sites), Apiaceae (n=46 sites), and Polygonaceae (n=54 sites), we calculated response variables of species richness within a family and total normalized forb cover within a family (cover of family in plot/total plot vegetative cover) for a given year at each site.

For all response variables, we calculated a log response ratio (LRR) that accounted for differences in pre- (i.e., year 0) and post-treatment data. We used the formula ln(Experimental Treatment data/Pretreatment data) for each plot sampled each year. We used pretreatment data in the denominator to account for initial site variation prior to treatments. We then calculated the percent change using the formula $100 \times (e^{LRR} - 1)$ where the LRR was the model estimate, since the natural log was used to calculate LRR. An alpha level of p=0.05 was the threshold for statistical significance.

To test our predications, we used separate Gaussian linear mixed effects models for all response variables with global change treatments as fixed effects (described below). Our models included a random intercept of block nested within site. To account for autocorrelation of responses within plots since treatments began, we included a corAR1 autocorrelation-moving average temporal correlation structure of years since treatments began nested by site, block, and plot.

To test our first prediction about type of fertilization, we ran these Gaussian linear mixed effects models for all response variables with N, P, and Kμ as fixed effects. Interactions between climate and fertilization were nonsignificant, so we did not include climate in these models. We took advantage of our full factorial experimental design to explore all interactions of our experimental treatment fixed effects (e.g. N*P*Kμ) for our models with richness and cover as response variables. In these models, these nutrient predictor variables tended to show significant two- or three-way interactions for models of richness, but not for models of cover with the exception of f Fabaceae and Polygonaceae (see Appendix). However, there is still information to be gained from the data despite non-significant interactions for our predictor variables. Thus, for our models where cover was our response variable, we dropped all interactions and focused on additive models of our predictor variables (N, P and Kμ. and fencing). This backward selection approach allowed us to leverage our experimental design to more fully quantify the controls on forb and grass richness and abundance.

To test our second and third predictions about herbivory and aridity, we ran Gaussian linear mixed effects models for all response variables with fixed effects of fencing and NPKμ treatments, herbivore intensity, and potential evapotranspiration (PET). We included interactions between NPKμ fencing, and herbivore intensity as well as interactions between NPKμ, fencing, and PET. We calculated site-level herbivore intensity as the mean difference in live biomass between unfenced and fenced controls (Unfenced Control Live Biomass – Fenced Control Live Biomass) in year 1 of treatments for each block and then took the mean herbivore intensity across blocks at the site level [32], such that increasingly negative values of this metric indicate greater reduction of herbivore intensity under herbivore exclusion, reflecting the greater impact of herbivore-exclusions on site-level biomass. We extracted average yearly PET data from the CRU climate dataset for each year through 2016 [88], a measure of aridity. We selected PET because it combines Mean Annual Precipitation (MAP) and Mean Annual temperature (MAT). We scaled PET for each year.

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Figures

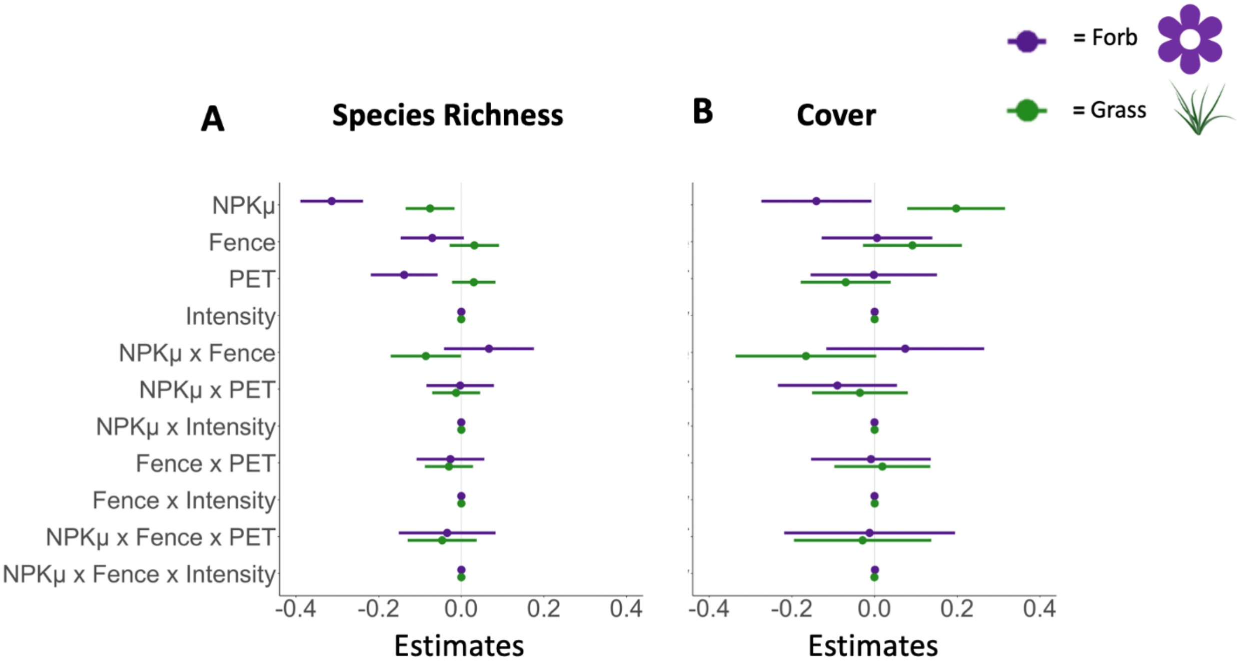

Figure 1. The effect of the herbivore exclusion via fencing treatment, fertilization treatment (fencing by fertilization experiment), potential evapotranspiration (PET) and herbivore intensity on (A) species richness and (B) cover for forbs (purple) and grasses (green). Model estimates of log response ratios for the effect of different treatments are shown relative to the control treatment (estimate =0). Binary response variables were converted to log response variables to account for the change from pre-treatment to current data and cover data were normalized relative to maximum plot cover. Fence refers to herbivore exclusion fencing. NPK□ refers to the nitrogen, phosphorus and potassium with micronutrients treatment. Error bars show the 89% confidence interval.

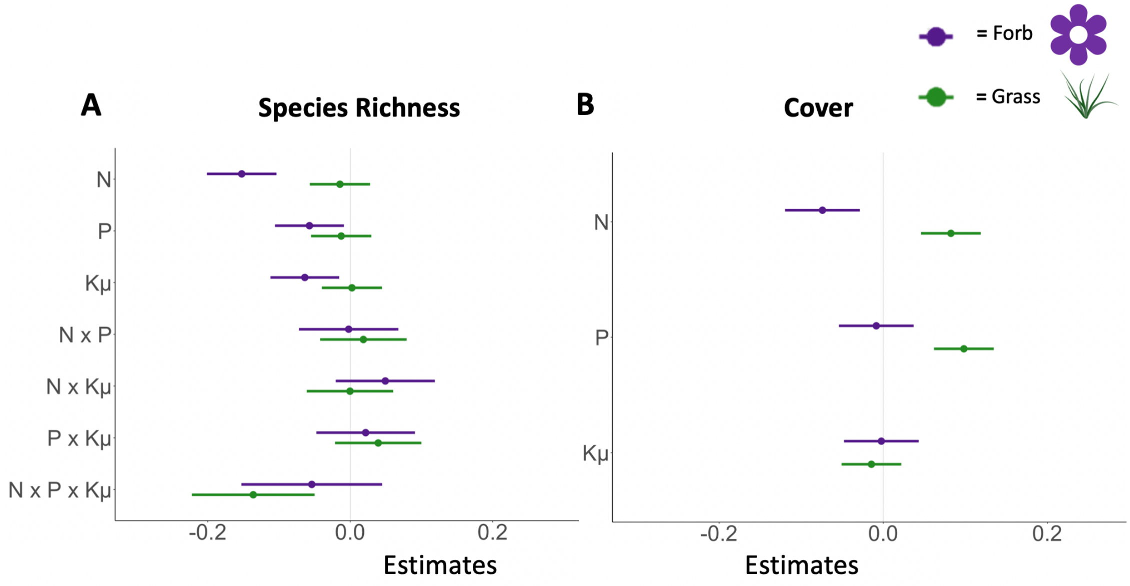

Figure 2. The effect of different nutrients (fertilization factorial experiment) on the fertilization factorial experiment on (A) species richness and (B) cover for forbs (purple) and grasses (green). Model estimates for log response ratios are shown relative to the control treatment (estimate = 0). Response variables were converted to log response variables to account for the change from pretreatment to current data with cover data normalized by maximum plot cover. Multiple nutrient interactions are included for richness. N refers to nitrogen, P refers to phosphorus, and K to potassium with micronutrients. Error bars show the 89% confidence interval.

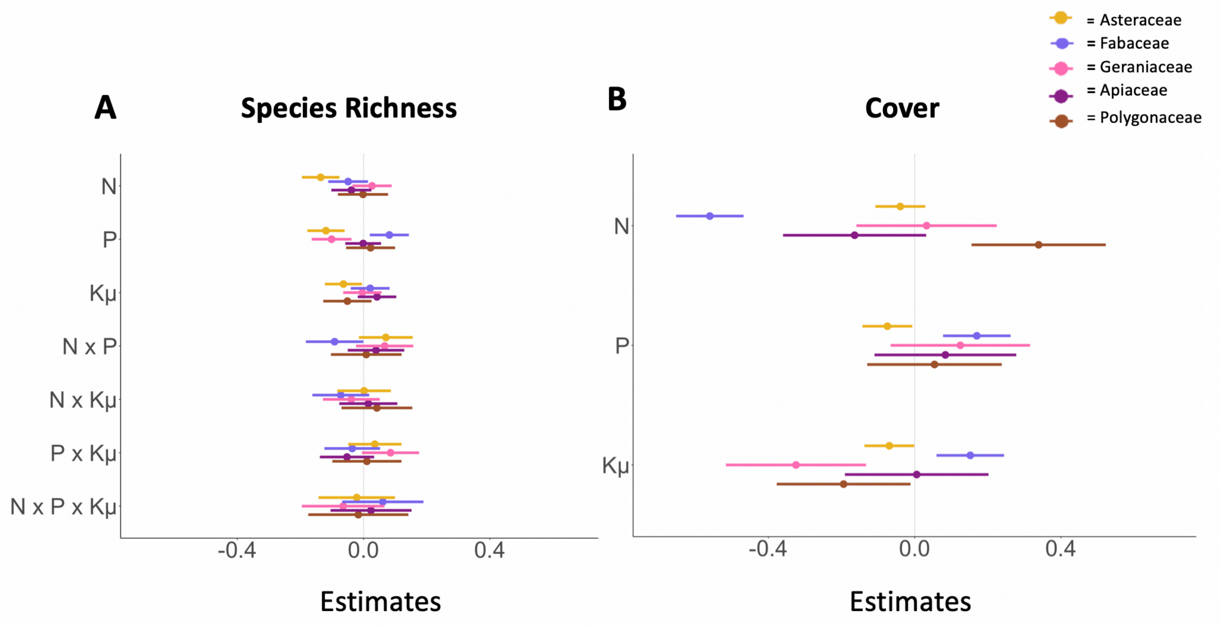

Figure 3. The effect of different nutrients (fertilization factorial experiment) on on (A) species richness and (B) family-level cover for Asteraceae (yellow), Fabaceae (indigo), Geraniaceae (pink), Apiaceae (magenta) and Polygonaceae (brown). Model estimates are shown relative to the control treatment (estimate = 0). Response variables were converted to log response variables to account for the change from pretreatment to current data with cover data normalized by maximum plot cover. Multiple nutrient interactions are included for richness. Multiple nutrient interactions are included for richness. N refers to nitrogen, P refers to phosprus, and K refers to potassium with micronutrients. Error bars show the 89% confidence interval.

Tables

Table 1. Mixed effects model results for the effects of fertilization by fencing, herbivore intensity, and potential evapotranspiration (PET) on Forb Family Richness, Forb Species Richness, Normalized Forb Cover, Grass Species Richness and Normalized Grass Cover. All response variables were calculated using LRRs. The intercept is the mean value of the unfenced and unfertilized control plots. The parenthetical numbers are the confidence interval. * p<0.05   ** p<0.01   *** p<0.001

	Forb Species Richness		**Forb Family Richness**		**Forb Cover**		**Grass Species Richness**		**Grass Cover**	
Predictors	*Estimates*	*t-value; p-value*	*Estimates*	*t-value; p-value*	*Estimates*	*t-value; p-value*	*Estimates*	*t-value; p-value*	*Estimates*	*t-value; p-value*
Intercept	-0.00 (-	-0.08;	-0.02 (-	-0.39;	0.09 (-	0.75;	-0.04 (-	-1.18;	-0.11 (-	-1.49;

	0.11 – 0.10)	0.933	0.10 – 0.07)	0.699	0.14 – 0.31)	0.454	0.11 – 0.03)	0.239	0.26 – 0.04)	0.136
NPK□	-0.31 *** (-0.39 – -0.24)	**-8.11; <0.001**	-0.21 *** (-0.28 – -0.14)	**-6.04; <0.001**	-0.14 * (-0.27 – -0.01)	**-2.07; 0.038**	-0.08 * (-0.13 – -0.02)	**-2.50; 0.012**	0.20 ** (0.08 – 0.32)	**3.27; 0.001**
Fence	-0.07 (-0.15 – 0.01)	-1.80; 0.072	0.01 (-0.06 – 0.07)	0.16; 0.870	0.01 (-0.13 – 0.14)	0.09; 0.932	0.03 (-0.03 – 0.09)	1.04; 0.300	0.09 (-0.03 – 0.21)	1.50; 0.133
PET	-0.14 *** (-0.22 – -0.06)	**-3.36; 0.001**	-0.14 *** (-0.21 – -0.07)	**-3.88; <0.001**	-0.00 (-0.15 – 0.15)	-0.02; 0.982	0.03 (-0.02 – 0.08)	1.13; 0.259	-0.07 (-0.18 – 0.04)	-1.25; 0.210
Intensity	0.00 (-0.00 – 0.00)	0.07; 0.945	0.00 (-0.00 – 0.00)	0.14; 0.892	0.00 (-0.00 – 0.00)	0.30; 0.762	-0.00 * (-0.00 – -0.00)	**-2.60; 0.013**	0.00 (-0.00 – 0.00)	0.19; 0.854

NPK□ × Fence	0.07 (-0.04 – 0.18)	1.21; 0.228	0.04 (-0.06 – 0.14)	0.80; 0.425	0.07 (-0.12 – 0.27)	0.76; 0.447	-0.09 * (-0.17 – -0.00)	**-1.98; 0.048**	-0.17 (-0.34 – 0.00)	-1.91; 0.056
NPK□ × PET	-0.00 (-0.08 – 0.08)	-0.06; 0.949	0.01 (-0.07 – 0.08)	0.17; 0.865	-0.09 (-0.23 – 0.05)	-1.22; 0.221	-0.01 (-0.07 – 0.05)	-0.42; 0.676	-0.04 (-0.15 – 0.08)	-0.60; 0.549
NPK□ × Intensity	-0.00 * (-0.00 – -0.00)	**-1.96; 0.050**	-0.00 (-0.00 – 0.00)	-1.03; 0.305	-0.00 (-0.00 – 0.00)	-1.74; 0.082	0.00 (-0.00 – 0.00)	0.30; 0.767	0.00 (-0.00 – 0.00)	0.15; 0.879
Fence × PET	-0.03 (-0.11 – 0.06)	-0.63; 0.528	-0.03 (-0.10 – 0.05)	-0.68; 0.494	-0.01 (-0.15 – 0.14)	-0.12; 0.907	-0.03 (-0.09 – 0.03)	-1.00; 0.315	0.02 (-0.10 – 0.14)	0.32; 0.750

Fence × Intensity	0.00 (-0.00 – 0.00)	0.11; 0.909	0.00 (-0.00 – 0.00)	1.57; 0.116	-0.00 (-0.00 – 0.00)	-1.13; 0.257	0.00 (-0.00 – 0.00)	0.65; 0.514	0.00 (-0.00 – 0.00)	1.14; 0.255
NPK□ × Fence × PET	-0.03 (-0.15 – 0.08)	-0.57; 0.566	0.01 (-0.09 – 0.12)	0.27; 0.787	-0.01 (-0.22 – 0.19)	-0.11; 0.909	-0.05 (-0.13 – 0.04)	-1.09; 0.277	-0.03 (-0.20 – 0.14)	-0.34; 0.732
NPK□ × Fence × Intensity	0.00 (-0.00 – 0.00)	0.95; 0.340	-0.00 (-0.00 – 0.00)	0.76; 0.449	0.00 ** (0.00 – 0.00)	**3.06; 0.002**	0.00 (-0.00 – 0.00)	0.23; 0.821	-0.00 (-0.00 – 0.00)	-1.64 0.101
Random Effects										
σ^2	0.28		0.23		0.91		0.16		0.64	
τ_{00}	0.30 block		0.24 block		0.67 block		0.17 block		0.41 block	
	0.14 site_code		0.11 site_code		0.29 site_code		0.08 site_code		0.21 site_code	
N	6 block		6 block		6 block		6 block		6 block	
	46 site_code		46 site_code		46 site_code		48 site_code		48 site_code	

Observations	3500	3500	3500	3535	3535
					**** $p<0.05$ ** $p<0.01$ *** $p<0.001$***

Table 2. Mixed effects model results for the effects of fertilization by nutrient type on Forb Family Richness, Forb Species Richness, Normalized Forb Cover, Grass Species Richness and Normalized Grass Cover. . The intercept is the mean value of the unfenced and unfertilized control plots. All response variables were calculated using LRRs. The parenthetical numbers are the confidence interval. * p<0.05   ** p<0.01   *** p<0.001

	Forb Species Richness		**Forb Family Richness**		**Forb Cover**		**Grass Species Richness**		**Grass Cover**	
Predictors	Estimates	*t-value; p-value*	Estimates	*t-value; p-value*	Estimates	*t-value; p-value*	Estimates	*t-value; p-value*	Estimates	*t-value; p-value*
Intercept	0.01 (-0.06 – 0.08)	0.38; 0.704	-0.01 (-0.07 – 0.05)	-0.19; 0.850	0.04 (-0.11 – 0.19)	0.53; 0.594	-0.01 (-0.07 – 0.05)	-0.40; 0.689	-0.03 (-0.14 – 0.07)	-0.64; 0.525

N	-0.15 *** (-0.20 – -0.10)	**-6.12; <0.001**	-0.08 *** (-0.13 – -0.04)	**-3.49; <0.001**	-0.07 ** (-0.12 – -0.03)	**-3.18; 0.001**	-0.01 (-0.06 – 0.03)	-0.67; 0.503	0.08 *** (0.05 – 0.12)	**4.44; <0.001**
P	-0.06 * (-0.11 – -0.01)	**-2.32; 0.020**	-0.01 (-0.06 – 0.03)	-0.54; 0.592	-0.01 (-0.05 – 0.04)	-0.36; 0.715	-0.01 (-0.05 – 0.03)	-0.59; 0.557	0.10 *** (0.06 – 0.13)	**5.29; <0.001**
K□	-0.06 ** (-0.11 – -0.02)	**-2.59; 0.010**	-0.04 (-0.09 – 0.00)	-1.78; 0.075	-0.00 (-0.05 – 0.04)	-0.10 0.921	0.00 (-0.04 – 0.04)	0.11; 0.911	-0.01 (-0.05 – 0.02)	-0.78; 0.438
N × P	-0.00 (-0.07 – 0.07)	-0.06; 0.951	-0.03 (-0.09 – 0.04)	-0.80; 0.421			0.02 (-0.04 – 0.08)	0.60; 0.552		

N × K□	0.05 (-0.02 – 0.12)	1.39; 0.165	0.05 (-0.02 – 0.11)	1.44; 0.149			-0.00 (-0.06 – 0.06)	-0.01; 0.991		
P × K □	0.02 (-0.05 – 0.09)	0.61; 0.540	-0.03 (-0.09 – 0.04)	-0.83; 0.405			0.04 (-0.02 – 0.10)	1.27; 0.204		
N × P × K□	-0.05 (-0.15 – 0.04)	-1.07; 0.285	-0.03 (-0.12 – 0.06)	-0.67; 0.500			-0.14 ** (-0.22 – -0.05)	**-3.10; 0.002**		
Random Effects										
σ2	0.26		0.22		0.86		0.17		0.49	
τ00	0.28 block		0.23 block		0.63 block		0.24 block		0.45 block	
	0.12 site_code		0.11 site_code		0.25 site_code		0.09 site_code		0.23 site_code	
N	6 block		6 block		6 block		6 block		6 block	
	84 site_code		84 site_code		84 site_code		87 site_code		87 site_code	

Observations	13686	13684	13686	14259	14259
*** p<0.05 ** p<0.01 *** p<0.001**					

Chapter 3 Supplemental Appendix

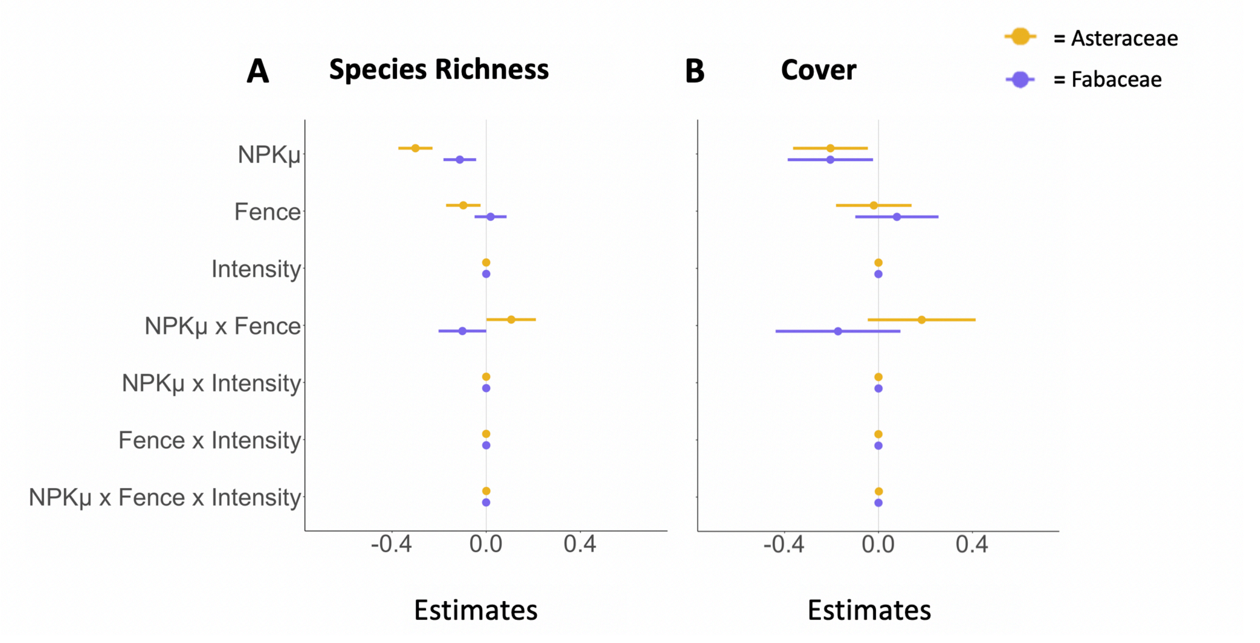

Figure S1. The effect of the fencing x fertilization experiment and herbivore intensity on (A) Species Richness and (B) Cover for Asteraceae (yellow) and Fabaceae (indigo). Model estimates of log response ratios for the effect of different treatments are shown relative to the control treatment (estimate =0). Binary response variables were converted to log response variables to account for the change from pre-treatment to current data and cover data were normalized relative to maximum plot cover. Fence refers to herbivore exclusion fencing. NPK*μ* refers to the nitrogen, phosphorus and potassium with micronutrients treatment.

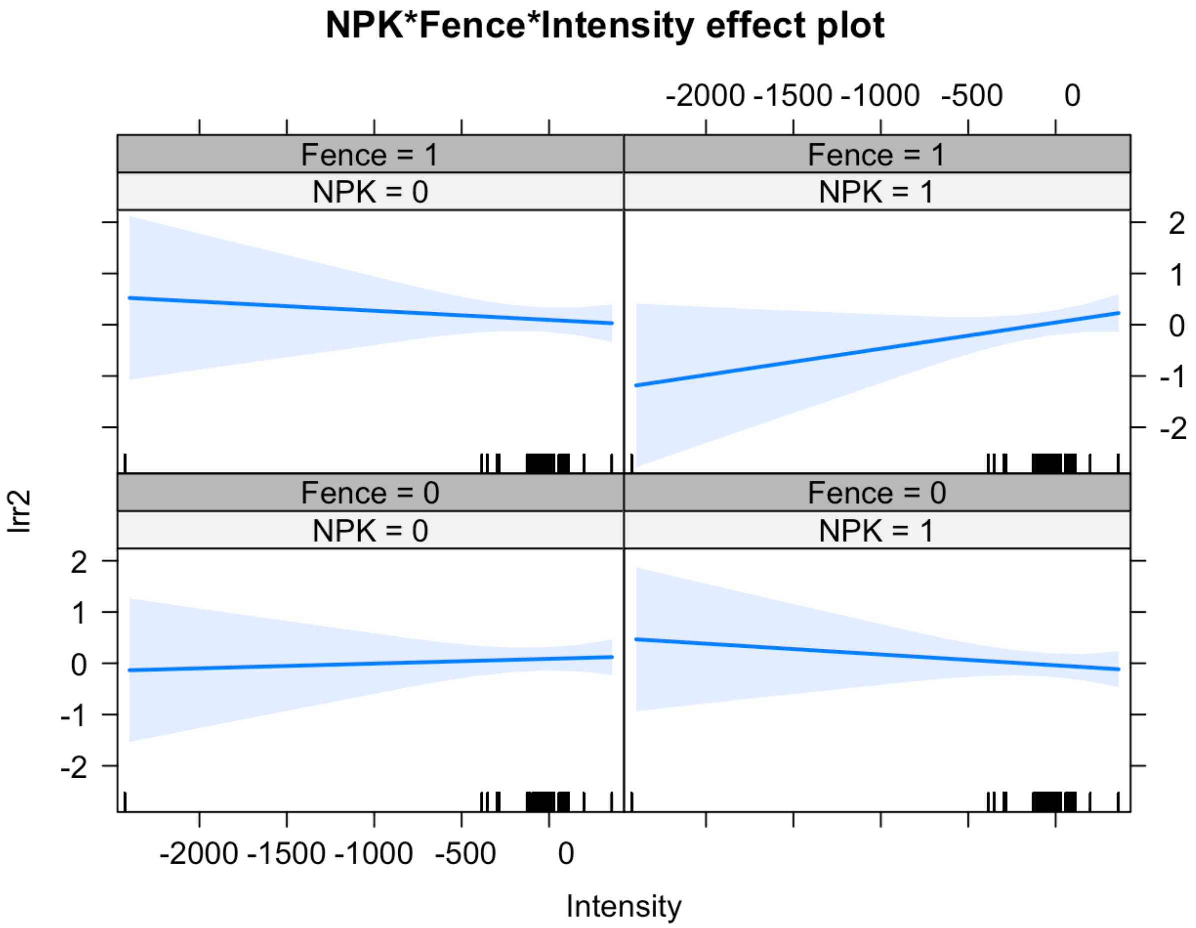

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Figure S2. Three-way-interaction plots for the effect of fencing, NPKμ fertilization, and herbivore intensity on forb cover log response ratio. A more negative value for herbivore intensity corresponds to greater herbivore intensity.

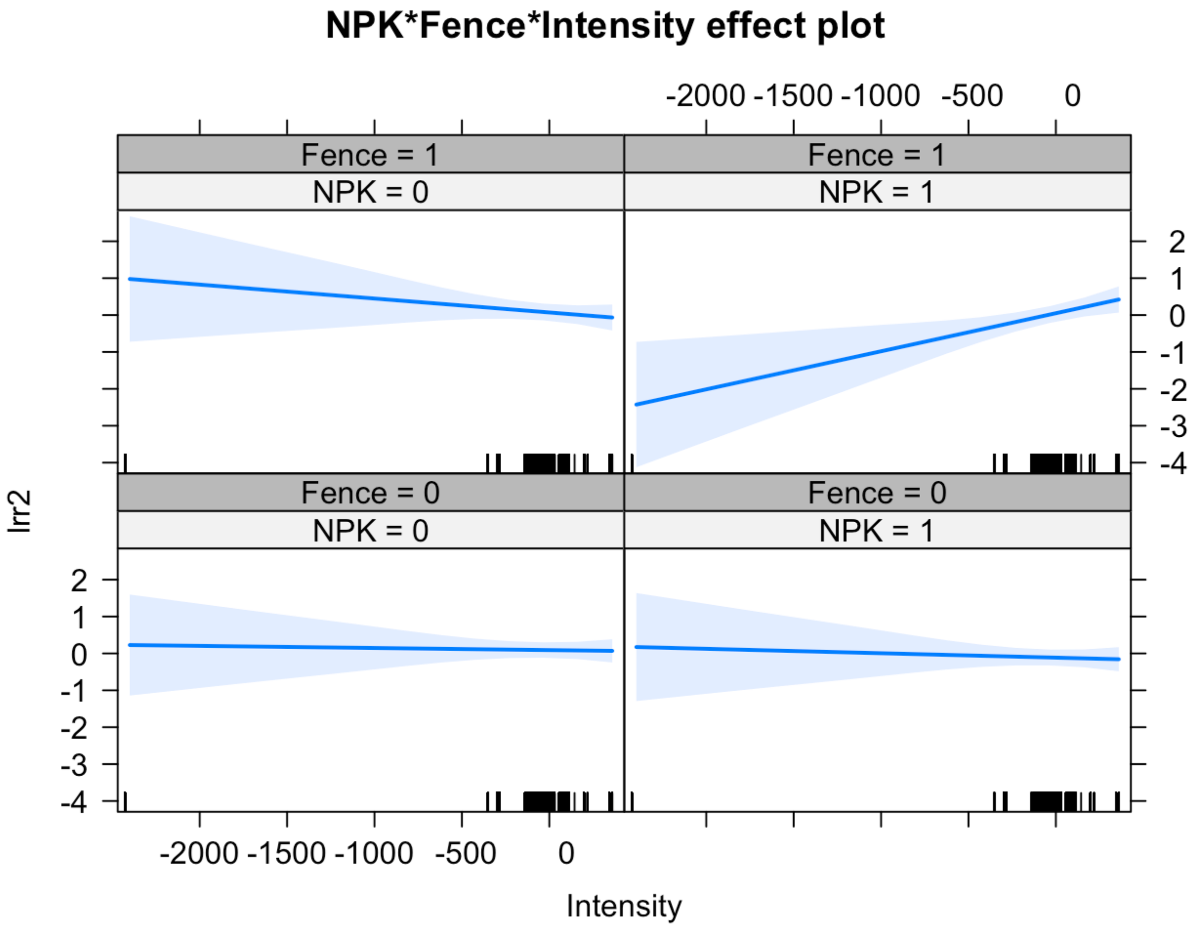

Figure S3. Three-way-interaction plots for the effect of fencing, NPKμ fertilization, and herbivore intensity on Asteraceae cover log response ratio. A more negative value for herbivore intensity corresponds to greater herbivore intensity.

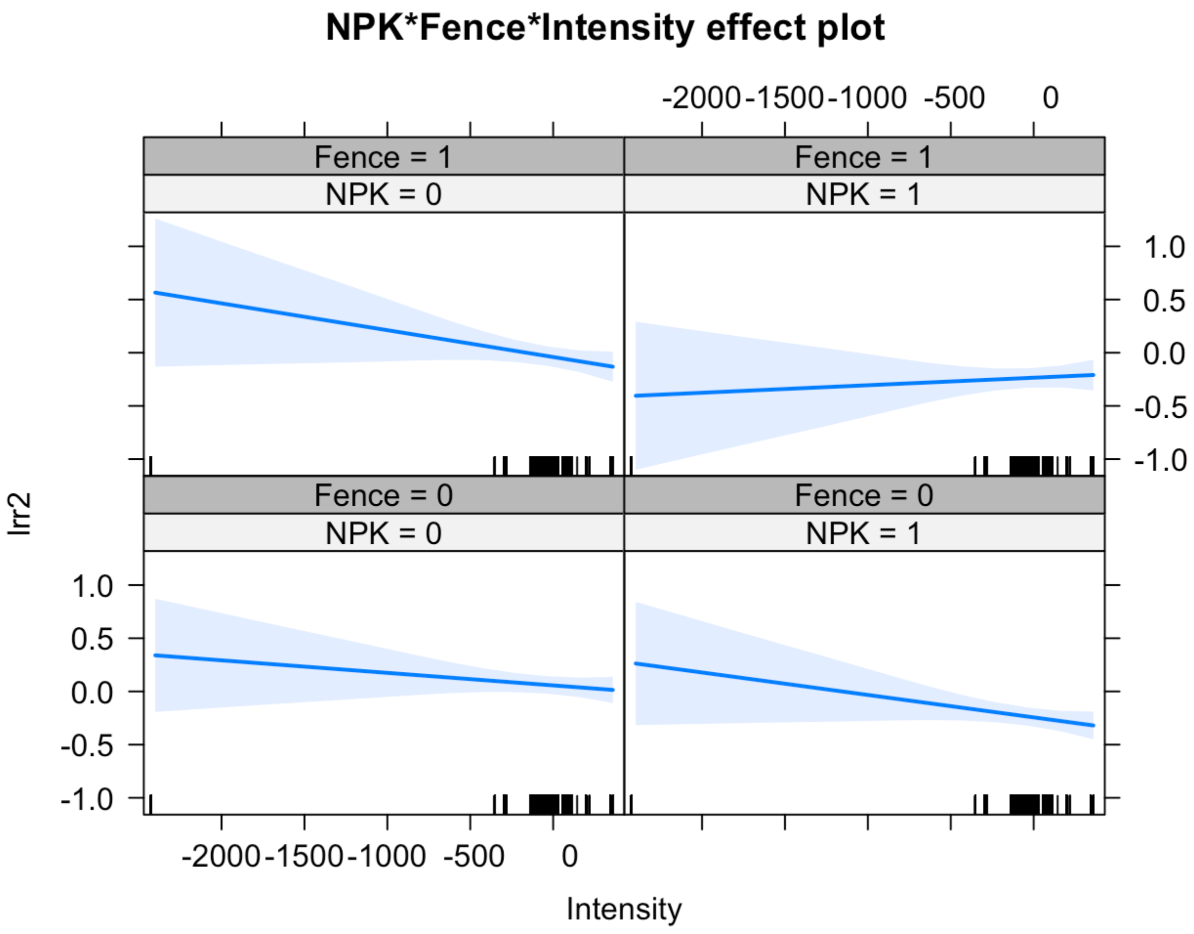

Figure S4. Three-way-interaction plots for the effect of fencing, NPKμ fertilization, and herbivore intensity on Asteraceae richness log response ratio. A more negative value for herbivore intensity corresponds to greater herbivore intensity.

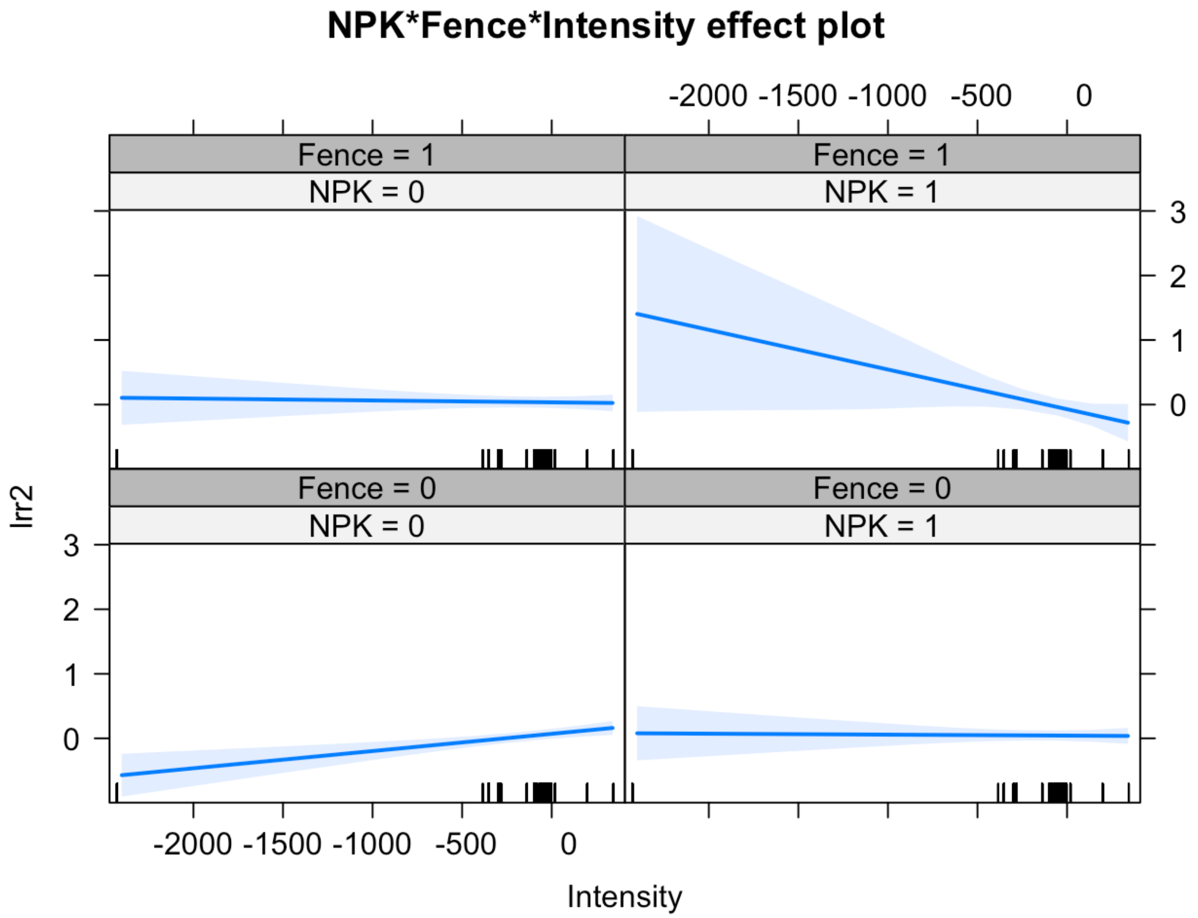

Figure S5. Three-way-interaction plots for the effect of fencing, NPKμ fertilization, and herbivore intensity on Polygonaceae richness log response ratio. A more negative value for herbivore intensity corresponds to greater herbivore intensity.

Table S1. Effects of fertilization on cover and richness of key floral families. Mixed effects model results for the effects of fertilization by nutrient type on Asteraceae Richness, Asteraceae Cover, Fabaceae Richness, Fabaceae Cover, Geraniaceae Richness, Geraniaceae Cover, Apiaceae Richness, Apiaceae Cover, Polygonaceae Richness, Polygonaceae Cover. All response variables were calculated using LRRs. The intercept is the mean value of the unfenced and unfertilized control plots. The parenthetical numbers are the confidence interval. * $p<0.05$ ** $p<0.01$ *** $p<0.001$

	Asteraceae Richness		**Asteraceae Cover**		**Fabaceae Richness**		**Fabaceae Cover**		**Geraniaceae Richness**		**Geraniaceae Cover**	
Predictors	*Estimates*	*t-value; p-value*	*Estimates*	*t-value; p-value*	*Estimates*	*t-value; p-value*	*Estimates*	*t-value; p-value*	*Estimates*	*t-value; p-value*	*Estimates*	*t-value; p-value*
Intercept	0.05 (-0.02 – 0.11)	1.36; 0.174	0.05 (-0.11 – 0.20)	0.56; 0.574	-0.04 (-0.09 – 0.02)	-1.27; 0.204	0.20 * (0.01 – 0.40)	**2.09; 0.037**	0.07 (-0.01 – 0.15)	1.64; 0.101	0.19 (-0.27 – 0.66)	0.81; 0.417
N	-0.14 *** (-0.20 – -0.08)	**-4.49; <0.001**	-0.04 (-0.11 – 0.03)	-1.13; 0.258	-0.05 (-0.11 – 0.01)	-1.53; 0.127	-0.56 *** (-0.65 – -0.47)	**-11.90; <0.001**	0.03 (-0.04 – 0.09)	0.84; 0.401	0.03 (-0.16 – 0.22)	0.33; 0.739
P	-0.12 *** (-0.18 – -0.06)	**-3.96; <0.001**	-0.07 * (-0.14 – -0.01)	**-2.15; 0.032**	0.08 ** (0.02 – 0.14)	**2.58; 0.010**	0.17 *** (0.08 – 0.26)	**3.61; <0.001**	-0.10 ** (-0.16 – -0.04)	**-3.17; 0.002**	0.12 (-0.07 – 0.32)	1.28; 0.199
Kμ	-0.06 * (-0.12 – -0.01)	**-2.13; 0.033**	-0.07 * (-0.14 – -0.00)	**-1.99; 0.046**	0.02 (-0.04 – 0.08)	0.66; 0.508	0.15 ** (0.06 – 0.24)	**3.23; 0.001**	-0.00 (-0.06 – 0.06)	-0.13; 0.900	-0.32 *** (-0.52 – -0.13)	**-3.32; 0.001**
N × P	0.07 (-0.01 – 0.16)	1.62; 0.106			-0.09 * (-0.18 – -0.00)	**-1.98; 0.047**			0.07 (-0.02 – 0.16)	1.44; 0.149		
N × Kμ	0.00 (-0.08 – 0.09)	0.04; 0.971			-0.07 (-0.16 – 0.02)	-1.57; 0.116			-0.04 (-0.13 – 0.05)	-0.85; 0.395		
P × Kμ	0.04 (-0.05 – 0.12)	0.82; 0.410			-0.04 (-0.12 – 0.05)	-0.80; 0.424			0.09 (-0.00 – 0.18)	1.85; 0.064		
N × P × Kμ	-0.02 (-0.14 – 0.10)	-0.35; 0.726			0.06 (-0.07 – 0.19)	0.93; 0.354			-0.06 (-0.20 – 0.07)	-0.97; 0.332		

Random Effects						
σ^2	0.26	1.23	0.17	1.54	0.07	2.01
τ_{00}	0.22 $_{\text{block}}$	0.60 $_{\text{block}}$	0.13 $_{\text{block}}$	0.66 $_{\text{block}}$	0.14 $_{\text{block}}$	0.93 $_{\text{block}}$
	0.13 $_{\text{site\_code}}$	0.34 $_{\text{site\_code}}$	0.10 $_{\text{site\_code}}$	0.30 $_{\text{site\_code}}$	0.08 $_{\text{site\_code}}$	0.24 $_{\text{site\_code}}$
N	6 $_{\text{block}}$	6 $_{\text{block}}$	6 $_{\text{block}}$	6 $_{\text{block}}$	5 $_{\text{block}}$	5 $_{\text{block}}$

	81 site_code	81 site_code	70 site_code	70 site_code	21 site_code	21 site_code
Observations	10951	10951	5900	5900	1774	1774

	Apiaceae Richness		**Apiaceae Cover**		**Polygonaceae Richness**		**Polygonaceae Cover**	
Predictors	*Estimates*	*t-value; p-value*	*Estimates*	*t-value; p-value*	*Estimates*	*t-value; p-value*	*Estimates*	*t-value; p-value*
Intercept	0.02 (-0.03 – 0.07)	0.80; 0.424	0.33 * (0.03 – 0.63)	**2.18; 0.029**	0.01 (-0.05 – 0.08)	0.44; 0.663	0.13 (-0.12 – 0.37)	0.99; 0.321
N	-0.04 (-0.10 – 0.02)	-1.20; 0.231	-0.16 (-0.36 – 0.03)	-1.65; 0.100	-0.00 (-0.08 – 0.08)	-0.05; 0.963	0.34 *** (0.16 – 0.52)	**3.62; <0.001**
P	-0.00 (-0.06 – 0.06)	-0.05; 0.962	0.08 (-0.11 – 0.28)	0.85; 0.396	0.02 (-0.06 – 0.10)	0.56; 0.576	0.05 (-0.13 – 0.24)	0.58; 0.563
Kμ	0.04 (-0.02 – 0.10)	1.36; 0.173	0.01 (-0.19 – 0.20)	0.06; 0.956	-0.05 (-0.13 – 0.03)	-1.32; 0.189	-0.19 * (-0.38 – -0.01)	**-2.08; 0.037**
N × P	0.04 (-0.05 – 0.13)	0.86; 0.390			0.01 (-0.10 – 0.12)	0.15; 0.884		
N × Kμ	0.01 (-0.08 – 0.11)	0.32; 0.752			0.04 (-0.07 – 0.15)	0.73; 0.463		
P × Kμ	-0.05 (-0.14 – 0.03)	-1.20; 0.231			0.01 (-0.10 – 0.12)	0.19; 0.852		
N × P × Kμ	0.02 (-0.10 – 0.15)	0.36; 0.719			-0.02 (-0.18 – 0.14)	-0.21; 0.836		

Random Effects				
σ^2	0.05	1.76	0.10	2.06
τ_{00}	0.07 block	0.58 block	0.09 block	0.40 block
	0.08 site_code	0.34 site_code	0.06 site_code	0.36 site_code
N	5 block	5 block	5 block	5 block
	35 site_code	35 site_code	33 site_code	33 site_code
Observations	1888	1888	2354	2354

Table S2. Effects of fertilization by fencing and herbivore intensity on cover and richness of key floral families. Mixed effects model results for the effects of fertilization by herbivore exclusion via fencing on Asteraceae Richness, Asteraceae Cover, Fabaceae Richness, Fabaceae Cover, Geraniaceae Richness, Geraniaceae Cover, Apiaceae Richness, Apiaceae Cover, Polygonaceae Richness, Polygonaceae Cover. . The intercept is the mean value of the unfenced and unfertilized control plots. All response variables were calculated using LRRs. The parenthetical numbers are the confidence interval. * $p<0.05$ ** $p<0.01$ *** $p<0.001$

	Asteraceae Richness		**Asteraceae Cover**		**Fabaceae Richness**		**Fabaceae Cover**		**Geraniaceae Richness**		**Geraniaceae Cover**	
Predictors	*Estimates*	*t-value; p-value*	*Estimates*	*t-value; p-value*	*Estimates*	*t-value; p-value*	*Estimates*	*t-value; p-value*	*Estimates*	*t-value; p-value*	*Estimates*	*t-value; p-value*
Intercept	0.06 (-0.02 – 0.14)	1.40; 0.161	0.09 (-0.11 – 0.29)	0.88; 0.379	-0.02 (-0.08 – 0.05)	-0.52; 0.604	0.03 (-0.25 – 0.31)	0.22; 0.828	0.03 (-0.05 – 0.10)	0.68; 0.495	-0.06 (-0.55 – 0.43)	-0.24; 0.809
NPK µ	-0.30 *** (-0.37 – -0.23)	**-8.13; <0.001**	-0.20 * (-0.36 – -0.05)	**-2.52; 0.012**	-0.11 ** (-0.18 – -0.04)	**-3.18; 0.002**	-0.21 * (-0.39 – -0.02)	**-2.21; 0.027**	-0.03 (-0.11 – 0.05)	-0.80; 0.424	-0.22 (-0.63 – 0.19)	-1.06; 0.289
Fence	-0.10 ** (-0.17 – -0.02)	**-2.60; 0.009**	-0.02 (-0.18 – 0.14)	-0.24; 0.807	0.02 (-0.05 – 0.09)	0.53; 0.593	0.08 (-0.10 – 0.26)	0.87; 0.386	-0.01 (-0.09 – 0.06)	-0.28; 0.782	-0.17 (-0.56 – 0.22)	-0.84; 0.403
Intensity	-0.00 (-0.00 – 0.00)	-1.03; 0.308	-0.00 (-0.00 – 0.00)	-0.19; 0.847	0.00 (-0.00 – 0.00)	0.05; 0.962	-0.00 (-0.00 – 0.00)	-1.03; 0.308	-0.00 (-0.00 – 0.00)	-0.17; 0.871	-0.00 (-0.01 – 0.00)	-0.34; 0.740
NPK µ × Fence	0.11 * (0.00 – 0.21)	**1.99; 0.046**	0.18 (-0.05 – 0.41)	1.57; 0.116	-0.10 (-0.20 – 0.00)	-1.96; 0.051	-0.17 (-0.44 – 0.09)	-1.27; 0.204	-0.01 (-0.12 – 0.11)	-0.11; 0.915	0.95 ** (0.37 – 1.54)	**3.22; 0.001**
NPK µ × Intensity	-0.00 (-0.00 – 0.00)	-0.84; 0.402	-0.00 (-0.00 – 0.00)	-0.26; 0.799	-0.00 (-0.00 – 0.00)	-0.59; 0.554	0.00 (-0.00 – 0.00)	0.41; 0.680	-0.00 (-0.00 – 0.00)	-0.75; 0.451	0.00 (-0.00 – 0.01)	1.67; 0.094

Fence × Intensity	-0.00 (-0.00 – 0.00)	-0.97; 0.331	-0.00 (-0.00 – 0.00)	-1.04; 0.296	0.00 (-0.00 – 0.00)	0.64; 0.521	-0.00 (-0.00 – 0.00)	-0.53; 0.594	0.00 (-0.00 – 0.00)	0.18; 0.861	0.00 (-0.00 – 0.01)	1.41; 0.159
NPK µ × Fence × Intensity	0.00 * (0.00 – 0.00)	**2.14; 0.033**	0.00 *** (0.00 – 0.00)	**3.44; 0.001**	-0.00 (-0.00 – 0.00)	-1.35; 0.176	-0.00 (-0.00 – 0.00)	-0.11; 0.910	0.00 (-0.00 – 0.00)	0.18; 0.857	-0.01 (-0.01 – 0.00)	-1.62; 0.105
σ^2	0.27	1.26			0.17		1.35		0.07		1.89	
τ_{00}	0.22 $_{\text{block}}$	0.60 $_{\text{block}}$			0.07 $_{\text{block}}$		0.78 $_{\text{block}}$		0.09 $_{\text{block}}$		0.72 $_{\text{block}}$	
	0.15 $_{\text{site\_code}}$	0.41 $_{\text{site\_code}}$			0.18 $_{\text{site\_code}}$		0.64 $_{\text{site\_code}}$		0.07 $_{\text{site\_code}}$		0.49 $_{\text{site\_code}}$	
N	6 $_{\text{block}}$	6 $_{\text{block}}$			6 $_{\text{block}}$		6 $_{\text{block}}$		5 $_{\text{block}}$		5 $_{\text{block}}$	
	63 $_{\text{site\_code}}$	63 $_{\text{site\_code}}$			55 $_{\text{site\_code}}$		55 $_{\text{site\_code}}$		18 $_{\text{site\_code}}$		18 $_{\text{site\_code}}$	
Observations	4223	4223			2336		2336		778		778	

	Apiaceae Richness		**Apiaceae Cover**		**Polygonaceae Richness**		**Polygonaceae Cover**	
Predictors	*Estimates*	*t-value; p-value*	*Estimates*	*t-value; p-value*	*Estimates*	*t-value; p-value*	*Estimates*	*t-value; p-value*
Intercept	0.01 (-0.06 – 0.09)	0.40; 0.689	0.19 (-0.33 – 0.70)	0.27; 0.476	0.07 (-0.01 – 0.15)	1.73; 0.084	-0.03 (-0.46 – 0.39)	-0.16; 0.872
NPKµ	0.02 (-0.04 – 0.08)	0.62; 0.533	0.23 (-0.25 – 0.70)	0.93; 0.354	-0.03 (-0.12 – 0.07)	-0.56; 0.574	0.23 (-0.25 – 0.71)	0.95; 0.344
Fence	0.00 (-0.06 – 0.06)	0.05; 0.959	0.40 (-0.09 – 0.88)	1.60; 0.109	-0.04 (-0.14 – 0.07)	-0.68; 0.495	-0.30 (-0.80 – 0.20)	-1.18; 0.238

Intensity	-0.00 (-0.00 – 0.00)	-0.20; 0.843	-0.00 (-0.01 – 0.00)	-0.49; 0.632	0.00 ** (0.00 – 0.00)	**3.60; 0.001**	0.00 (-0.00 – 0.00)	0.35; 0.729
NPKµ × Fence	-0.00 (-0.10 – 0.09)	-0.07; 0.941	-0.39 (-1.10 – 0.32)	-1.07; 0.284	-0.08 (-0.23 – 0.07)	-1.02; 0.309	0.98 * (0.23 – 1.73)	**2.56; 0.011**
NPKµ × Intensity	0.00 (-0.00 – 0.00)	0.07; 0.945	-0.00 (-0.01 – 0.00)	-1.28; 0.201	-0.00 ** (-0.00 – -0.00)	**-2.75; 0.006**	-0.00 (-0.00 – 0.00)	-0.17; 0.866
Fence × Intensity	-0.00 (-0.00 – 0.00)	-0.65; 0.513	-0.00 (-0.01 – 0.00)	-0.50; 0.619	-0.00 ** (-0.00 – -0.00)	**-2.92; 0.004**	-0.00 (-0.00 – 0.00)	-1.12; 0.263
NPKµ × Fence × Intensity	0.00 (-0.00 – 0.00)	0.29; 0.773	0.00 (-0.00 – 0.01)	0.86; 0.390	-0.00 (-0.00 – 0.00)	-0.86; 0.390	0.00 (-0.00 – 0.01)	1.74; 0.082
σ^2	0.04		1.94		0.10		2.36	
τ_{00}	0.12_{block}		0.81_{block}		0.09_{block}		0.55_{block}	
	$0.04_{\text{site\_code}}$		$0.00_{\text{site\_code}}$		$0.07_{\text{site\_code}}$		$0.53_{\text{site\_code}}$	
N	5_{block}		5_{block}		5_{block}		5_{block}	
	$22_{\text{site\_code}}$		$22_{\text{site\_code}}$		$26_{\text{site\_code}}$		$26_{\text{site\_code}}$	
Observations	788		788		954		954	

Table S3. Mixed effects model results for the effects of fertilization by nutrient type on Forb Normalized Forb Cover and Normalized Grass Cover with full interactions. The intercept is the mean value of the unfenced and unfertilized control plots. All response variables were calculated using LRRs. The parenthetical numbers are the confidence interval. * $p<0.05$ ** $p<0.01$ *** $p<0.001$

	Forb Cover			**Grass Cover**		
Predictors	*Estimates*	*T-value*	*p*	*Estimates*	*T-value*	*p*
Intercept	0.03 (-0.13 – 0.18)	0.33	0.742	-0.04 (-0.15 – 0.07)	-0.63	0.530
N	-0.01 (-0.10 – 0.08)	-0.14	0.888	0.07 (-0.00 – 0.14)	1.90	0.057
P	-0.01 (-0.10 – 0.08)	-0.19	0.848	0.10 ** (0.03 – 0.17)	2.80	**0.005**
K□	0.00 (-0.09 – 0.09)	0.05	0.962	-0.02 (-0.09 – 0.06)	-0.45	0.656
N × P	-0.07 (-0.20 – 0.06)	-1.10	0.271	0.02 (-0.08 – 0.13)	0.45	0.655
N × K□	-0.08 (-0.21 – 0.05)	-1.24	0.214	0.04 (-0.07 – 0.14)	0.67	0.500
P × K□	0.05 (-0.08 – 0.18)	0.80	0.424	0.00 (-0.10 – 0.10)	0.03	0.979
N × P × K□	0.04 (-0.15 – 0.22)	0.40	0.690	-0.07 (-0.21 – 0.08)	-0.88	0.379
Random Effects						
σ^2	0.86			0.49		
τ_{00}	0.63 $_{\text{block}}$			0.45 $_{\text{block}}$		
	0.25 $_{\text{site\_code}}$			0.23 $_{\text{site\_code}}$		
N	6 $_{\text{block}}$			6 $_{\text{block}}$		
	84 $_{\text{site\_code}}$			87 $_{\text{site\_code}}$		
Observations	13686			14259		
						***** p<0.05 ** p<0.01 *** p<0.001***

Table S4. Effects of fertilization on cover of key floral families with full interactions. Mixed effects model results for the effects of fertilization by nutrient type on Asteraceae Cover, Fabaceae Cover, Geraniaceae Cover, Apiaceae Cover, Polygonaceae Cover. All response variables were calculated using LRRs. The intercept is the mean value of the unfenced and unfertilized control plots. The parenthetical numbers are the confidence interval. * p<0.05 ** p<0.01 *** p<0.001

	Asteraceae Cover			**Fabaceae Cover**			**Geraniaceae Cover**			**Apiaceae Cover**			**Polygonaceae Cover**		
Predictors	*Estimates*	*T-value*	*p*	*Estimates*	*T-value*	*p*	*Estimates*	*T-value*	*p*	*Estimates*	*T-value*	*p*	*Estimates*	*T-value*	*p*
Intercept	0.04 (-0.13 – 0.21)	0.50	0.617	0.10 (-0.11 – 0.31)	0.92	0.357	0.08 (-0.41 – 0.56)	0.31	0.760	0.27 (-0.06 – 0.61)	1.61	0.108	-0.07 (-0.37 – 0.23)	-0.46	0.648
N	-0.01 (-0.14 – 0.12)	-0.16	0.873	-0.34 *** (-0.51 – -0.16)	-3.66	**<0.001**	0.29 (-0.07 – 0.65)	1.57	0.116	-0.02 (-0.41 – 0.37)	-0.10	0.924	0.41 * (0.05 – 0.78)	2.22	**0.026**
P	-0.10 (-0.24 – 0.03)	-1.53	0.125	0.37 *** (0.19 – 0.55)	4.12	**<0.001**	0.18 (-0.19 – 0.54)	0.95	0.340	0.16 (-0.19 – 0.51)	0.90	0.369	0.44 * (0.09 – 0.80)	2.44	**0.015**
K□	-0.00 (-0.14 – 0.13)	-0.06	0.952	0.22 * (0.04 – 0.39)	2.45	**0.014**	-0.12 (-0.48 – 0.24)	-0.66	0.509	0.25 (-0.13 – 0.62)	1.28	0.202	0.10 (-0.25 – 0.45)	0.57	0.566
N × P	0.00 (-0.19 – 0.20)	0.05	0.961	-0.41 ** (-0.67 – -0.15)	-3.12	**0.002**	-0.06 (-0.59 – 0.47)	-0.24	0.813	-0.17 (-0.73 – 0.38)	-0.62	0.536	-0.14 (-0.65 – 0.37)	-0.53	0.594
N × K□	-0.19 (-0.38 – 0.00)	-1.93	0.053	-0.13 (-0.38 – 0.13)	-0.97	0.332	-0.38 (-0.90 – 0.15)	-1.40	0.162	-0.48 (-1.05 – 0.08)	-1.67	0.095	0.05 (-0.47 – 0.56)	0.17	0.862
P × K□	-0.07 (-0.26 – 0.12)	-0.73	0.465	-0.09 (-0.34 – 0.16)	-0.70	0.481	0.05 (-0.48 – 0.58)	0.18	0.854	-0.36 (-0.89 – 0.17)	-1.32	0.186	-0.59 * (-1.09 – -0.09)	-2.30	**0.021**
N × P × K□	0.25 (-0.02 – 0.53)	1.82	0.069	0.16 (-0.21 – 0.52)	0.84	0.403	-0.21 (-0.97 – 0.56)	-0.54	0.592	0.70 (-0.09 – 1.49)	1.73	0.084	-0.12 (-0.84 – 0.61)	-0.31	0.754
Random Effects															
σ^2	1.23			1.54			2.00			1.76			2.03		
τ_{00}	0.60_{block}			0.66_{block}			0.93_{block}			0.58_{block}			0.40_{block}		
	$0.33_{\text{site\_code}}$			$0.30_{\text{site\_code}}$			$0.25_{\text{site\_code}}$			$0.34_{\text{site\_code}}$			$0.36_{\text{site\_code}}$		
N	6_{block}			6_{block}			5_{block}			5_{block}			5_{block}		
	$81_{\text{site\_code}}$			$70_{\text{site\_code}}$			$21_{\text{site\_code}}$			$35_{\text{site\_code}}$			$33_{\text{site\_code}}$		

Observations	10951	5900	1774	1888	2354

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

Table S5. List of forb taxonomic families present in Nutrient Network data and their within family species richness. Pretreatment data were used to generate this list. Families used in main analysis are shaded in gray and were the five most abundant families by aboveground biomass.

Family	Number of Species Present Within Family
Acanthaceae	9
Adiantaceae	2
Agavaceae	5
Alismataceae	1
Alstroemeriaceae	3
Amaranthaceae	17
Amaryllidaceae	13
Anacardiaceae	1
Apiaceae	56
Apocynaceae	15
Araceae	2
Araliaceae	2
Asparagaceae	5
Asphodelaceae	2
Aspleniaceae	1
Asteraceae (Compositae)	393
Bataceae	1
Blechnaceae	1
Boraginaceae	29
Boryaceae	2
Brassicaceae	42

Bromeliaceae	3
Cactaceae	2
Calceolariaceae	1
Campanulaceae	16
Cannabaceae	1
Caprifoliaceae	4
Caryophyllaceae	53
Chenopodiaceae	1
Cistaceae	6
Colchicaceae	1
Commelinaceae	10
Convolvulaceae	14
Cornaceae	1
Crassulaceae	9
Cucurbitaceae	1
Dennstaedtiaceae	1
Dipsacaceae	1
Droseraceae	1
Dryopteridaceae	1
Ephedraceae	1
Equisetaceae	6
Ericaceae	1
Eriocaulaceae	1
Euphorbiaceae	31
Fabaceae	197
Gentianaceae	24
Geraniaceae	21
Goodeniaceae	3
Hyacinthaceae	6
Hymenophyllaceae	1
Hypericaceae	6

Hypoxidaceae	7
Iridaceae	23
Juncaginaceae	1
Lamiaceae	32
Lentibulariaceae	1
Liliaceae	6
Linaceae	7
Loganiaceae	1
Lycopodiaceae	5
Lythraceae	3
Malvaceae	18
Mazaceae	1
Melanthiaceae	3
Melastomataceae	1
Molluginaceae	3
Montiaceae	8
Nyctaginaceae	4
Onagraceae	29
Ophioglossaceae	1
Orchidaceae	19
Orobanchaceae	10
Oxalidaceae	11
Pontederiaceae	1
Papaveraceae	3
Parnassiaceae	2
Phyllanthaceae	2
Plantaginaceae	19
Plumbaginaceae	3
Poaceae	1
Polemoniaceae	23
Polygalaceae	11

Polygonaceae	31
Polypodiaceae	1
Portulacaceae	3
Primulaceae	8
Proteaceae	1
Ranunculaceae	34
Rosaceae	58
Rubiaceae	30
Salicaceae	1
Santalaceae	3
Saxifragaceae	4
Schoepfiaceae	1
Scrophulariaceae	58
Selaginellaceae	2
Smilacaceae	1
Solanaceae	12
Thelypteridaceae	1
Thymelaeaceae	2
Ulmaceae	1
Urticaceae	2
Valerianaceae	1
Verbenaceae	7
Violaceae	19
Xanthorrhoeaceae	1
Xyridaceae	2
Zygophyllaceae	1
Family Not Given	5

Supplemental Methods to Tables S6-S7

To further account for differences in control vs treatment data, we used the method from Lind et al. 2017 *Ecology Letters*, to calculate a difference in Log Response Ratio (LRR) between the LRRs for the control and treatment data for a given site, plot and year for each response variable:

$$
\begin{aligned}
Difference\ in\ LRR \\
&= \left(\frac{ln(Treatment\ Response\ Variable_{Post-Treatment})}{ln(Treatment\ Response\ Variable_{Pre-Treatment}}\right) \\
&- \frac{ln(Control\ Response\ Variable_{Post-Treatment})}{ln(Control\ Response\ Variable_{Pre-Treatment}})
\end{aligned}
$$

For the control treatment, the difference in LRR is 0. We then ran linear mixed models with a Gaussian distribution with our response variables as difference in LRR and the same fixed effects as described in the paper and a random effect of block nested within site to test.

Table S6. Model outputs for the effects of the fencing by fertilization experiment on Difference in LRR between treatment and control.

	Forb Species Richness			**Forb Family Richness**			**Forb Cover**			**Grass Species Richness**			**Grass Cover**		
Predictors	*Estimates*	*Statistic*	*p*	*Estimates*	*Statistic*	*p*	*Estimates*	*Statistic*	*p*	*Estimates*	*Statistic*	*p*	*Estimates*	*Statistic*	*p*
Intercept	-0.01 (-0.07 – 0.05)	-0.33	0.743	-0.02 (-0.08 – 0.04)	-0.74	0.458	-0.07 (-0.21 – 0.07)	-0.97	0.331	0.00 (-0.04 – 0.04)	0.11	0.915	0.05 (-0.07 – 0.16)	0.81	0.416
NPK µ	-0.32 *** (-0.36 – -0.27)	-13.31	**<0.001**	-0.20 *** (-0.24 – -0.15)	-8.73	**<0.001**	-0.14 ** (-0.22 – -0.05)	-3.12	**0.002**	-0.09 *** (-0.12 – -0.05)	-4.55	**<0.001**	0.20 *** (0.13 – 0.27)	5.42	**<0.001**
Fence	-0.06 * (-0.10 – -	-2.41	**0.016**	0.02 (-0.02 – 0.07)	1.03	0.305	0.00 (-0.08 – 0.09)	0.06	0.954	0.02 (-0.02 – 0.06)	1.17	0.240	0.08 * (0.01 – 0.15)	2.22	**0.026**

	0.01)														
PETs	-0.01 (-0.07 – 0.05)	-0.30	0.762	-0.02 (-0.07 – 0.04)	-0.61	0.542	-0.09 (-0.21 – 0.04)	-1.36	0.173	0.01 (-0.03 – 0.05)	0.39	0.693	0.01 (-0.08 – 0.10)	0.22	0.822
Intensity	-0.00 (-0.00 – 0.00)	-0.16	0.872	-0.00 (-0.00 – 0.00)	-0.09	0.928	0.00 (-0.00 – 0.00)	0.27	0.786	-0.00 (-0.00 – 0.00)	-0.26	0.793	-0.00 (-0.00 – 0.00)	-0.19	0.853
NPKµ × Fence	0.05 (-0.01 – 0.12)	1.57	0.115	0.02 (-0.05 – 0.08)	0.54	0.588	0.10 (-0.03 – 0.22)	1.55	0.122	-0.08** (-0.13 – -0.02)	-2.87	**0.004**	-0.16** (-0.26 – -0.06)	-3.09	**0.002**
NPKµ × PETs	-0.01 (-0.06 – 0.05)	-0.24	0.810	0.01 (-0.04 – 0.06)	0.41	0.680	-0.08 (-0.18 – 0.01)	-1.67	0.094	-0.03 (-0.07 – 0.01)	-1.42	0.156	-0.01 (-0.09 – 0.06)	-0.27	0.785
NPKµ × Intensity	-0.00** (-0.00 – -0.00)	-3.13	**0.002**	-0.00 (-0.00 – 0.00)	-1.34	0.181	-0.00** (-0.00 – -0.00)	-2.92	**0.003**	0.00 (-0.00 – 0.00)	1.01	0.315	0.00 (-0.00 – 0.00)	0.19	0.848
Fence × PETs	-0.07* (-0.12	-2.50	**0.012**	-0.06* (-0.11	-2.50	**0.012**	-0.05 (-0.15 – 0.04)	-1.12	0.265	-0.05* (-0.09	-2.55	**0.011**	0.06 (-0.02 – 0.13)	1.51	0.130

	– -0.01)			– -0.01)						– -0.01)					
Fence × Intensity	0.00 (-0.00 – 0.00)	0.83	0.405	0.00*** (0.00 – 0.00)	3.34	**0.001**	-0.00 (-0.00 – 0.00)	-1.73	0.085	0.00 (-0.00 – 0.00)	1.82	0.069	0.00* (0.00 – 0.00)	2.38	**0.017**
NPK μ × Fence × PETs	-0.02 (-0.10 – 0.05)	-0.63	0.530	0.05 (-0.02 – 0.12)	1.29	0.196	0.01 (-0.12 – 0.15)	0.20	0.840	-0.04 (-0.10 – 0.02)	-1.34	0.180	-0.11* (-0.22 – -0.00)	-2.03	**0.042**
NPK μ × Fence × Intensity	0.00 (-0.00 – 0.00)	1.27	0.203	-0.00* (-0.00 – -0.00)	-2.16	**0.031**	0.00*** (0.00 – 0.00)	5.10	**<0.001**	0.00 (-0.00 – 0.00)	0.32	0.752	-0.00** (-0.00 – -0.00)	-2.70	**0.007**
Random Effects															
σ^2	0.23			0.21			0.79			0.16			0.57		
τ_{00}	0.09 $_{\text{block}}$ 0.28 $_{\text{site\_code}}$			0.06 $_{\text{block}}$ 0.27 $_{\text{site\_code}}$			0.33 $_{\text{block}}$ 0.46 $_{\text{site\_code}}$			0.04 $_{\text{block}}$ 0.18 $_{\text{site\_code}}$			0.29 $_{\text{block}}$ 0.34 $_{\text{site\_code}}$		
ICC	0.61			0.61			0.50			0.58			0.53		
N	6 $_{\text{block}}$ 46 $_{\text{site\_code}}$			6 $_{\text{block}}$ 46 $_{\text{site\_code}}$			6 $_{\text{block}}$ 46 $_{\text{site\_code}}$			6 $_{\text{block}}$ 48 $_{\text{site\_code}}$			6 $_{\text{block}}$ 48 $_{\text{site\_code}}$		
Observations	3729			3681			3743			3648			3798		

* *p<0.05* ** *p<0.01* *** *p<0.001*

Table S7. Model outputs for the effects of the fencing by fertilization experiment on Difference in LRR between treatment and control.

	Forb Species Richness	**Forb Family Richness**	**Forb Cover**	**Grass Species Richness**	**Grass Cover**

Predictors	*Estimates*	*Statistic*	*p*	*Estimates*	*Statistic*	*p*	*Estimates*	*Statistic*	*p*	*Estimates*	*Statistic*	*p*	*Estimates*	*Statistic*	*p*
Intercept	-0.00 (-0.04 – 0.04)	-0.07	0.945	-0.00 (-0.04 – 0.04)	-0.20	0.841	-0.01 (-0.10 – 0.07)	-0.33	0.744	0.00 (-0.03 – 0.03)	0.06	0.956	0.04 (-0.03 – 0.10)	1.08	0.282
N	-0.15*** (-0.19 – -0.12)	-9.26	**<0.001**	-0.07*** (-0.10 – -0.04)	-4.60	**<0.001**	-0.07*** (-0.10 – -0.04)	-4.27	**<0.001**	-0.01 (-0.04 – 0.02)	-0.48	0.628	0.08*** (0.05 – 0.10)	6.52	**<0.001**
P	-0.07*** (-0.10 – -0.03)	-3.98	**<0.001**	-0.02 (-0.05 – 0.01)	-1.54	0.123	-0.03* (-0.06 – -0.00)	-2.06	**0.039**	-0.02 (-0.05 – 0.01)	-1.49	0.135	0.08*** (0.06 – 0.10)	6.88	**<0.001**
Kμ	-0.04** (-0.08 – -0.01)	-2.70	**0.007**	-0.04* (-0.07 – -0.01)	-2.38	**0.017**	0.03 (-0.00 – 0.06)	1.81	0.070	-0.01 (-0.04 – 0.02)	-0.78	0.436	-0.03** (-0.05 – -0.01)	-2.64	**0.008**
N × P	0.00 (-0.04 – 0.05)	0.13	0.896	-0.04 (-0.08 – 0.01)	-1.60	0.109				0.03 (-0.01 – 0.07)	1.53	0.126			
N × Kμ	0.03 (-0.02	1.09	0.278	0.04 (-0.00	1.93	0.054				0.02 (-0.02	0.87	0.382			

	– 0.07)			– 0.09)						– 0.06)					
P × Kμ	0.01 (-0.04 – 0.05)	0.25	0.805	-0.03 (-0.07 – 0.02)	-1.18	0.237				0.06 ** (0.01 – 0.10)	2.68	**0.007**			
N × P × Kμ	-0.03 (-0.09 – 0.04)	-0.74	0.457	-0.03 (-0.09 – 0.03)	-0.97	0.333				-0.18 *** (-0.24 – -0.12)	-6.21	**<0.001**			
Random Effects															
σ^2	0.29			0.26			1.00			0.22			0.58		
τ_{00}	0.07 $_{\text{block}}$			0.05 $_{\text{block}}$			0.25 $_{\text{block}}$			0.02 $_{\text{block}}$			0.18 $_{\text{block}}$		
	0.27 $_{\text{site\_code}}$			0.26 $_{\text{site\_code}}$			0.50 $_{\text{site\_code}}$			0.23 $_{\text{site\_code}}$			0.39 $_{\text{site\_code}}$		
ICC	0.54			0.55			0.43			0.53			0.49		
N	6 $_{\text{block}}$			6 $_{\text{block}}$			6 $_{\text{block}}$			6 $_{\text{block}}$			6 $_{\text{block}}$		
	84 $_{\text{site\_code}}$			84 $_{\text{site\_code}}$			84 $_{\text{site\_code}}$			87 $_{\text{site\_code}}$			87 $_{\text{site\_code}}$		
Observations	16447			16251			16809			16093			17742		

* *p<0.05* ** *p<0.01* *** *p<0.001*