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Invasive Plant Impacts Preventing Community Recovery

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Plant Biology

by

Stuart T Schwab

September 2023

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Dr. Loralee Larios, co-chairperson
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ABSTRACT OF THE DISSERTATION

Invasive Plant Impacts Preventing Community Recovery

by

Stuart T Schwab

Doctorate of Philosophy, Plant Biology
University of California, Riverside, September, 2023
Dr. Loralee Larios, Dr. G. Darrel Jenerette co-chairpersons

Ecosystems are undergoing unprecedented rates of change with severe consequences for biodiversity loss, yet natural resilience and restoration of ecosystems provides hope to mitigate these losses. Despite the potential for natural recovery, there are thresholds and feedback mechanisms that inhibit recovery that are often driven by invasive species. Consequently, understanding how invasive species interact with their surrounding communities and how they respond to restoration efforts is crucial information for effective management and successful restoration. My dissertation seeks to understand how the impact of invasive plants prevents effective restoration through a combination of field experiments with paired greenhouse components to capture robust processes in the field and disentangle the underlying mechanisms in a controlled greenhouse setting. My first two chapters investigate how a local and regionally obnoxious novel invasive forb, *Oncosiphon pilulifer*, responds to the common management technique of prescribed fire, and how *Oncosiphon* interacts with soil biota to inhibit native plant growth. My final chapter focuses on how the multiple factors of seed limitation, invasive litter accumulation, and soil symbiont depletion constrain

restoration success in a Northern Californian annual grassland. Taken together, my dissertation projects aim to both address fundamental questions in community ecology and produce actionable science for land management practitioners.

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CHAPTER 3: Managing in the face of multiple constraints: the impacts of seed availability, litter accumulation, and symbiont depletion.

General Introduction

Elucidating the impacts of invasive species, and how they reduce the efficacy of management efforts is a significant challenge for the practice of restoration. Plant invaders have negative impacts on recipient communities as they are typically better competitors and can exclude other species in recipient communities (Vila et al. 2011). Managing for invasive plants is complicated by non-target impacts on natives, where strategies to reduce invasive plants may not benefit native plants (Skurski et al. 2013, Sherrill et al. 2021). Further, invasive plants alter the biotic and abiotic surroundings beyond competition, which can drive resilience of invaded community states returning communities back to invader dominance after restoration efforts (Suding et al. 2004).

Californian grasslands contain a high biodiversity of flora and fauna, providing important social-ecological ecosystem services. Unfortunately, grasslands are also very heavily invaded by a suite of nonnative annual grasses that can form dense monocultures, reducing the habitat quality and social-ecological services provided by grasslands (DiTomaso 2000). Additionally, within this system, native seedbanks are depleted and invaders can overwhelm seedbanks to exacerbate the paucity of native seed (Cox & Allen 2008; Schneider & Allen 2012). Grasslands have also been invaded by a suite of forbs, where both non-native grasses and non-native forbs maintain dominance and create invaded dominated states through key feedbacks - litter and soil - that are resilient to management. The litter feedback re-enforces nonnative annual grass dominance as other species cannot grow in thick litter (Mariotte et al. 2017). The most common soil feedback for invaders is the reduction of symbionts that native plants need causing a mutualism disruption (Vogelsang & Bever 2009; Grove et al. 2017). Both litter and soil feedback mechanisms contribute to resilience of the invaded community, but the ecological mechanisms and management actions for each feedback are distinct requiring careful consideration of when a particular feedback should be addressed to maximize native recovery.

Indigenous peoples engaged in cultural burning practices for a large variety of purposes across California for centuries (Kimmerer & Lake 2001) and have made fire an integral component of land management in America (Lake et al. 2017). In fact, there were so many cultural burns and reasons for these burns that determining which fires were natural and which were initiated by indigenous people is nearly impossible (Hammet 1991; Martin and Sapsis 1992). Prescribed fires are performed commonly today to manage litter buildup from invasive plants (Valkó et al. 2021), but it is important to note that prescribed fires are distinct from cultural fire, where prescribed fires have a ‘command and control’ perspective adopted from the United States military and do not have the same cultural values and may not carry the same ecological benefits as indigenous burning practices (Pyne et al. 2016; Long et al. 2021). Prescribed fires are effective at mitigating invasive grass impacts because they remove litter and kill grass seeds, which are two major components of how nonnative grasses alter communities and maintain their dominance (Reynolds et al. 2003). Despite the efficacy of prescribed burns on invasive grasses, how invasive forbs will respond to prescribed fire is unclear and has been largely unstudied (Tomat-Kelly & Flory 2022). Invasive forbs may not be consumed as readily as the invasive grasses, and thus leave behind stands of partially consumed plants with their seeds post burn. These stands retain litter and seed, which may function as refugia as these stands have greater invader seed in a favorable microclimate and are spread out across the post burn landscape. Managing invasive plants is complicated by the range of responses between functional types of invaders, but successful restoration may be even further constrained by the impact invaders have on other ecosystem properties.

Invasive plants interact with soil communities in ways that alter the invader performance, and native performance. These interactions with soil biota include beneficial symbionts and harmful pathogens which can alter how plants grow (Bever et al. 1997). One of the most common plant-microbe symbiosis is the association with arbuscular mycorrhizal fungi (AMF) (Lee et al. 2013), where AMF can intercept water for plant hosts (Allen 2007) and increase nutrient access for plant hosts with the reciprocal benefit of carbohydrates for the symbiont (Kiers et al. 2011). Californian native forb and grass species evolved under low resource conditions and formed obligate mutualisms with soil symbionts. Unfortunately, invasive forbs can kill fungal partners that native species depend upon (Wakefield et al. 2023; Vogelsang & Bever 2009). Additionally, invasive plants interact with pathogens and can increase the amount of pathogens present to reduce their own growth and the growth of natives (Diez et al. 2010, Kelly et al. 2009). Invader driven changes in soil biota, for both AMF and pathogens, have dynamic development over the course of invasion and can change in strength from neutral to strongly negative impacts on native plant and invader growth (Grove et al. 2017; Flory & Clay 2013). Determining how invaders alter soil biota over the course of invasion can be useful information for prioritizing which invaders to target, and to determine which soil amendment strategies are going to be the most effective. Ultimately restoring native communities is mediated by not only circumventing these feedbacks but having seed available to take advantage of management actions.

Native plant recruitment is constrained by the many impacts of invasive plants (Suding et al. 2004; Corbin & D'Antonio 2012). The widespread cover of invasive plants and low native cover results in dispersal limitation as native seeds cannot establish or disperse into new areas (Gioria et al. 2019). Native plant recruitment is severely inhibited by depleted seedbanks (Cox & Allen 2008), but recruitment is even further constrained by the impacts of grass litter (Marriotte et al. 2017), and symbiont depletion (Vogelsang & Bever 2009). While integrated strategies to address all constraints is an enticing idea, engaging in all actions can have unnecessary costs and management budgets are typically limited (Kimball et al. 2016). Determining which constraints are preventing desired management outcomes can both enhance the efficacy of management practices but also elucidate how strongly these constraints are limiting native recovery.

My dissertation seeks to enhance restoration success by elucidating how invaders respond to prescribed fire, how an invasive forb creates soil legacies via a plant soil feedback (PSF), and how singular vs integrated management strategies can achieve management goals in Californian grassland ecosystems. My first chapter investigates how heterogeneity in the post burn environment in the form of partly consumed stands of an invasive forb *Oncosiphon pilulifer* alters post burn recruitment. My second chapter assesses how invasive forb *Oncosiphon pilulifer* interacts with soil biota over the course of invasion to impact native plant performance and its own performance. My third chapter seeks to understand how management actions addressing the constraints of native dispersal limitation, invader litter buildup, and depletion of soil symbionts differentially achieve management goals and restructure the plant community. Specifically, I compared single vs integrated management strategies of adding native seeds, removing litter, and inoculating the soil to infer how these constraints are limiting native recruitment and maintaining the dominance of invaders. Taken together, my dissertation highlights the dynamic and complex nature of invasive plant impacts preventing recovery and restoration in Californian grasslands and aims to produce actionable science for managers as well as fundamental advances in our understanding of ecology.

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Chapter 1: Prescribed burning may produce refugia for invasive forb, *Oncosiphon pilulifer*

Abstract:

Prescribed burning is a common management technique to reduce non-native grass cover and seed availability in temperate forests and grasslands; however, its effectiveness in reducing non-native forbs is unclear. Litter of invasive forbs like *Oncosiphon pilulifer* are not consumed by fire like invasive grass litter is, resulting in residual singed stands and high heterogeneity in the post-burn landscape. We investigated the potential for this incomplete burning to alter post fire establishment of native and non-native plant species by conducting a field experiment in a prescribed burn in Lake Perris State Park, CA. We investigated the role of microclimate and seed availability on establishment for two years following a prescribed burn in both singed stands and completely burned patches by adding or removing litter and adding native seed in a factorial design. Litter presence reduced soil temperatures and light availability, while singed stands had lower soil moisture and temperature. Litter present treatments had 5.6±5.9%SE greater *Oncosiphon* cover yet doubled *Oncosiphon* viable seeds in the seedbank. Singed stands had 22.6±4.9% SE greater *Oncosiphon* cover and more than doubled *Oncosiphon* viable seeds. Native seed addition did not influence native or *Oncosiphon* cover. These results suggest that residual singed stands within the prescribed burn landscape can create a favorable microclimate and allow *Oncosiphon* to retain seed, increasing re-invasion. Our experiment suggests that litter increased establishment of non-species as these species may better utilize post burn establishment opportunities impacting overall community

recovery. Management of invasive forbs with prescribed burns may require secondary treatments to reduce re-invasion.

Introduction

Prescribed burns are a multi-faceted management tool used within many temperate grasslands and forests to mitigate biodiversity loss (Valkó et al. 2020; Valkó & Deák 2021). In North American grasslands and forests, prescribed burning techniques have been used for centuries by traditional practices of indigenous people and are a common modern practice for a variety of goals including the reduction of non-native plant cover and increasing establishment success of native plants (Kimmerer & Lake 2001). When this tool is applied to invasive forbs, the post burn landscape may not confer these management benefits due to differences in fuel type compared to the invasive grasses that are more commonly the targets of prescribed burning (Pyke et al. 2010; Padullés Cubino et al. 2018); however, there is a substantial research gap investigating the relationships between fire and forb invasion (Tomat-Kelly & Flory 2022). Fire is a heterogenous disturbance and does not consume fuel evenly across the landscape causing patch-based differences within the burns (Platt & Connell 2003). This heterogeneity can result in refugia for plant species present pre-burn to spread within the burn area. For invaded landscapes, these refugia might facilitate re-establishment of invaders or promote establishment of different resident non-native species rather than increase establishment of native species (Larios et al. 2013). Increasing the success of prescribed burns thus requires an understanding of the mechanisms that mediate post-burn establishment within a heterogeneous landscape.

Post-burn establishment can be mediated by the stressful environmental situations present post-burn such as low water availability and increased temperatures (Iverson & Hutchinson 2002; Kuppinger et al. 2010), yet naturally occurring litter from singed stands might act as microclimate refugia, alleviating these stressful environmental conditions. Dryland restoration has demonstrated that installing shelters or debris can simulate nurse plant effects ameliorating stressful local environmental conditions to promote seedling establishment (Fick et al. 2016). Establishment and early growth are the most sensitive stages of seedling establishment (Grubb 1977; Fenner & Michael 2005) and singed stands may similarly provide a favorable microclimate reducing mortality during early life stages by functioning like artificial shelters. While native species may benefit from these differences in microclimate and have improved establishment (Okin et al. 2015; Fick et al. 2016), increased competitive interactions from non-native species could impede these outcomes. Non-native species with their fast resource acquisition strategies may more quickly use resources, limiting native performance (Wainwright et al. 2011). Thus, the presence of litter (i.e., singed-stands or artificial shelters) post-burn can mediate recovery, differentially favoring invasive or native species depending on the interaction of microclimate effects and post burn seed availability.

In post burn settings for annual communities, seed availability is a key driver of system recovery (Connell & Slatyer 1977), and similarly within prescribed burns in annual communities, any residual seed availability will mediate management trajectories to favor species with remnant seeds. Systems with persistent seedbanks that contain native species experience a flush of native establishment after prescribed burns; however, non-native species may re-establish within a few years due to surrounding propagule pressure (Dickens & Allen 2009; Alba et al. 2014). The establishment of non-native species may be accelerated if incomplete consumption results in singed stands of non-native vegetation that still have seeds present either on the plant or in the understory, thus, forming refugia from which a plant invader can spread (Moody & Mack 1988; Weston et al. 2019). These refugia combined with the high seed output of invasive species could therefore result in high cover and reinvasion of treated areas (Fenner & Michael 2005; Colautti et al. 2006). While prescribed burns are effective at reducing transient invader seedbanks (Reynolds et al. 2001; Keeley et al. 2008), incompletely burned patches that provide refugia for invader seed may thwart achieving management goals such as invader removal and native establishment.

Invader legacies may create additional establishment barriers that prevent the successful establishment of native species in post burn landscapes, necessitating additional management efforts (Larios & Suding 2013). In heavily invaded areas, native seedbanks are often depleted (Cox & Allen 2008; Gioria & Pyšek 2015). Consequently, native seed addition and planting native seedlings are an essential management action for increasing native establishment (Nolan et al. 2021). However, broadscale seed addition often does not translate to high native establishment, high post emergence seedling mortality results from environmental conditions and competitive pressures from invasive species (Shackelford et al. 2021). While construction of artificial shelters can help ameliorate stressful conditions (Okin et al. 2015; Fick et al. 2016; Abella & Chiquoine 2019), successful native establishment in these conditions likely needs to be paired with seed addition (Havrilla et al. 2020). Therefore, achieving native management goals post-prescribed burn may require selectively adding native seeds to areas with litter.

Such combined management activities are widely used for managing the negative effects of non-native annual grasses in Californian grasslands, which are heavily invaded semi-arid ecosystems (Mack 1981; D'Antonio & Vitousek 1992; DiTomaso 2000; Reynolds et al. 2001). However, success of this technique on the small seeded invasive forbs that are increasingly invading these systems is unclear. *Oncosiphon pilulifer,* stinknet, is one such invasive forb that is spreading in southern California grasslands. *Oncosiphon* is emerging as a highly problematic species, as it produces many seeds and can grow under a variety of environmental conditions (Riefner & Boyd 2008). As a new invasive species in North America, management practitioners have less knowledge of the ecology of *Oncosiphon* within the invaded area and limited experience with successful management and eradication efforts. Prescribed burns do not fully consume this invader, leaving behind singed (incompletely burned) stands after burn events, which may facilitate *Oncosiphon* re-establishment, making this a model system to evaluate how post burn heterogeneity may create refugia that mediates post burn recovery.

To address this uncertainty, we asked how do *Oncosiphon* singed stands influence the post-burn establishment of native and non-native species? To elucidate the relative contributions of litter and seed availability post burn, we conducted a factorial field experiment to investigate the role of burn completion, litter effects, and native seed addition on post burn community recovery. We hypothesized 1) that post burn litter will function like artificial shelters to alleviate environmental stress resulting in a greater number of species and greater cover within litter treatments post burn and 2) that singed stands will harbor viable seeds from the transient seedbank and provide more favorable microclimates in which *Oncosiphon* can establish post burn, effectively acting as a refugia within burns. Additionally, we hypothesized 3) that native species are seed limited, and the addition of native seeds will increase native cover and establishment will be enhanced by the interaction between the ameliorating effects of litter and seed addition. Despite potential increases in native cover, if these singed stands promote re-invasion, we would predict communities would exhibit a divergent and undesirable trajectory away from native establishment and towards invasive dominance as invaders can outperform and spread faster than natives if native seeds are not present to increase native recruitment.

Methods

Study area and Design

Our study took place in the Lake Perris State Recreation Park (LPSP; 33.87934073, -117.1431129) in Riverside County California in 2020 and 2021. LPSP experiences a Mediterranean-type climate with warm dry summers, and cooler wet winters. The total precipitation at LPSP during the first year growing season (October through June) was 316 mm, and 125 mm in the second year, and the average temperatures were 15.3 ºC in the first year and 16.0 ºC in the second year (PRISM Climate Group). LPSP contains several dominant annual invasive forbs (e.g., *Brassica tournefortii, Oncosiphon pilulifer, Sisymbrium irio*) and annual grasses (e.g., *Avena fatua, Bromus madritensis, Schismus barbatus*) as well as annual native forb species (e.g., *Amsinckia intermedia, Calandrinia menziesii, Lasthenia platyglossa*). *Oncosiphon* is an *Asteraceae* originally from South Africa and was accidentally introduced to LPSP in the 1980s, where it remained at low densities until the last several years. At the time of the study, *Oncosiphon* had become largely dominant across the reserve.

The LPSP prescribed burn practices are intended to remove invasive plants and create a more favorable habitat for the threatened, Stephens' kangaroo rat, *Dipodomys stephensi*. Fire return intervals are determined by visual observation of biomass accumulation, to ensure enough fuel is present to carry fire and to maximize efficacy of burns by waiting until visual thresholds of accumulation are surpassed. Fires are performed in the morning with temperatures below $29.5^{\circ C}$ and with winds from the South West with humidity between 20-60%. Fires are executed utilizing drip torches from perimeters, and fire flares into the interior of the fire to assist with developing heat. This study was performed in a 0.914 km^2 area previously burned in 2015 and burned again in June 2019. The burn occurred in the morning and lasted between three and four hours. (K. Kietzer 2023, California State Parks, CA, personal communication). The cover of *Oncosiphon* singed stands varied across the post-burn landscape from 50-100% cover, and we aimed to account for this variation by selecting ten *Oncosiphon* stands within the prescribed burn area with an even number of mid-level cover (50-80%) and high-level cover (80-100%) stands to implement a factorial experiment and monitor plant recovery dynamics (Figure 1A). Burn completion was determined in August 2019 when live vegetation was minimal. We found a binary distribution of either completely bare ground or intact but singed stands of *Oncosiphon* litter and used this distinction to determine if plots were incompletely or completely burned.

We set up an experiment to tease apart the role of burn completion (i.e., complete burn or singed stand), litter presence (i.e., litter or no litter), and native seed availability (i.e. seed, unseed) on native and non-native establishment post prescribed burn. We set up four burn and litter treatments: 1) complete burn + no litter, 2) complete burn + litter, 3) singed stand + no litter, and 4) singed stand + litter and crossed these with a native seed addition (i.e., seed or unseed) for a total of eight plots within each of the ten sites identified above, for a total of 80 plots. The singed stands contained only *Oncosiphon* litter, which retained its vertical structure from the previous year's growth, while more flammable non-native grasses were removed by the fire. We paired litter removal with the litter additions, by taking singed *Oncosiphon* litter from the singed stand + litter removed treatment and staking them in the same arrangement and density in the complete burn + litter treatment (Figure 1B). We removed any seeds remaining on the litter to isolate litter effects. In the singed stand + no litter, we removed any aboveground singed litter to isolate just the effects of post burn seed availability. The plots were 0.5m x 0.5m. Each stand of singed *Oncosiphon* formed the basis of a block that was roughly 15 m^2. Within these blocks we set up the 8 plots so that each complete burn or singed stand plot was placed at least 2 m away from the next treatment plot of complete burn or singed stands, respectively. The complete burn plots were placed at least 5m away from the edge of *Oncosiphon* stands. Plots were placed haphazardly, where within the singed stand plot locations were focusing on consistency of *Oncosiphon* singed litter cover, and the surrounding complete burn plots were haphazardly placed on bare ground representative of the surrounding complete burn landscape. Our seeding treatment was composed of a diverse mix of dominant and rare native plant species (*Camisoniopsis bistorta, Escscholzia california, Layia platyglossa, Salvia columbariae, Stipa pulchra, Uropappus lindelyii*). All species except for *Stipa pulchra* are annual species that are representative of the dominant ecological strategy in this system. The perennial *Stipa pulchra* was included as it is often included in restoration seed palettes under the assumption that it historically occurred in these areas (Bartolome & Gemmill 1981). The mix was selected in consultation with the LPSP Senior Environmental Scientist and seed was purchased to use locally sourced populations except for *Uropapus lindelyii,* which was collected from a local preserve, Motte Rimrock Reserve, Riverside, CA (33.8005747, -117.2553159). We sowed the native seed mix at a rate of 8g/m^2 in November of 2019 before the growing season rains began with each species sowed at 1.33 g/m2.

Data collection

We measured plant composition and environmental conditions during peak biomass (April) for two years (2020-2021) following the Jepson Flora species names and classifications of origin (Jepson 2022). To address the impacts of singed stands on growing conditions, we measured soil volumetric water content (VWC) with a soil moisture probe (Campbell Scientific, Hydrosense II), soil surface temperature with a digital thermometer (Carolina Digital Pocket Thermometer), and light with a photosynthetically active radiation meter (AccuPAR LP-80, Meter Group), within a week of plant composition measures (April). For the VWC and soil temperature measures, three points were measured in each plot and averaged together. To measure photosynthetically active radiation (PAR), we simultaneously measured PAR above the canopy level and at the ground level between 11:30AM and 1:30PM, to calculate the proportion of light reaching the ground. We replicated our PAR measures twice for each plot, then averaged the proportions to have one measure per plot.

To measure plant community responses, we made visual estimates of species composition, where we recorded percent cover for each vascular plant species within a plot. All layers of the canopy were included in our visual estimates of percent cover, and thus the plot cover totals can be greater than 100%. To identify differences in surface seedbank composition, we additionally performed a five-month seedbank study from November 2021 to May 2022 in a University of California Riverside greenhouse. In each experimental plot, we scraped the top 250 ml of soil (top 1 cm) from each whole plot in April 2021 as this was the end of the growing season with peak seed production and thus the time to have the most representative seed availability at the end of our experiment. Each soil sample was spread across a 25 cm by 25 cm tray and covered with a thin layer of sand. The trays were placed in stratified greenhouse blocks to ensure no replicates from the same field block were included in the same greenhouse block. Ten greenhouse blocks were randomized biweekly to minimize greenhouse effects. All seedlings were identified and removed to prevent double counting or transplanted and then grown to the point of flower to ensure identification. Our *Oncosiphon* focal response variables were percent cover from visual field estimates, as well as total number of viable seeds in our greenhouse seedbank study. To address native species responses, we focused on total native cover, seeded species cover, seeded species richness from the field study, and total native viable seeds for all native plants and seeded species viable seeds from the greenhouse study.

Analyses

Due to large differences in precipitation between years that strongly regulated plant performance, we performed separate models for 2020 and 2021. This approach allowed us to decipher the impacts of our experimental treatments without the effect of precipitation overwhelming treatment effects; however, as the data from 2021 is dependent upon treatments imposed in 2020, these data should be interpreted as a continuation of the first analysis rather than independent analyses. To quantify differences in microclimate, we performed a linear mixed effects model on VWC, soil surface temperature, and proportion available light with the fixed effects of burn completion, litter treatment, and seed treatment as well as all interactions, and block with nested factor of initial *Oncosiphon* singed stand density as the random effect.

We performed a linear mixed effects model with the random effect of block with initial *Oncosiphon* singed stand density nested within block, and fixed effects of burn completion, litter treatment, and seed treatment as well as all interactions for *Oncosiphon* field cover, total native cover, seeded species cover, and seeded species richness responses. For viable seed responses, including *Oncosiphon,* total natives, and seeded natives, we performed a linear mixed effects model with the fixed effects of burn completion, litter treatment, seed treatment, as well as all interactions and greenhouse block and field block as the random effects. While we seeded a total of six species, *Stipa* *pulchra* had zero percent cover in the field and none germinated in our seedbank study. Due to the substantial number of plots with no seeded species we utilized a linear mixed effects model on ln(x +1) transformed data for seeded species cover and total number of seeded species present.

To address community level responses, we focused on univariate and multivariate responses. We calculated Shannon Weiner diversity (H) using the 'vegan' package (v.2.6-2, Oksanen 2022), and calculated species richness as the sum of all species within a plot. For both H and richness, we performed a linear mixed effects model with the fixed effects of burn completion, litter treatment, and seed treatment as well as all interactions and block with nested factor of initial *Oncosiphon* singed stand density as the random effect. To address how burn completion, litter and seed treatments influence overall community composition over time, we performed a PERMANOVA with the fixed effects of burn completion, litter treatment, and seed treatment and all interactions and block with nested factor of initial *Oncosiphon* singed stand density as a random effect and the response variable was the matrix of cover for each species present in a plot. Statistically significant differences between factors from the PERMANOVA are visualized with a principal coordinates analysis (PCoA).

All analyses were performed in R (v.4.0.2). All linear mixed effects models were performed using the 'lme4' package (Bates et al. 2015). Post hoc tests were performed using the emmeans function in the 'emmeans' package (Russell 2022), and any letters present in figures are derived from TukeyHSD. PcoA was performed using the 'mass' package (Venables & Ripley 2002) and PERMANOVA was performed using the adonis function in the ‘vegan’ package (v.2.6-2, Oksanen et al. 2022). Data were transformed as needed to achieve normality in model residual distributions, where soil moisture, *Oncosiphon* cover, total native cover, total seeded cover, *Oncosiphon* viable seeds, total native viable seeds, and total seeded species viable seeds were ln(x+1) transformed and photosynthetically active radiation was cube transformed.

Results

Over our two-year study, we observed 27 vascular plant species in our field plots -- 13 non-native and 14 native species (Table S1). Native cover ranged from 0% to 53% with an average of 7.3±0.8%SE, and non-native cover ranged from 5% to 102.5% with an average of 60.2±1.9% SE cover in both years combined. Within our seedbank study we found 32 species, 14 non-native species and 18 native species (Table S2). The total native viable seeds --pooled for all species -- ranged from 2 to 515 with an average of 92.5±9.8 SE, and total non-native viable seeds ranged from 9 to 498 with an average of 133.1±10.7 SE.

Microclimate response

The microclimate was significantly different in key aspects among treatment groups in both years. The singed stands had lower VWC than complete burn, and had lower soil temperature (Table 1; Figure 2 A,C). Singed stands did not have different amounts of light reaching the ground in 2020 but had less light reaching the ground in 2021 (Table 1; Figure 2E). Litter presence did not influence soil moisture in either year (Table 1; Figure 2B); however, the litter treatment was cooler at the surface and had less light reaching the ground (Table 1; Figure 2 D,F). Our seeding treatment did not influence soil moisture, soil surface temperature, or the proportion of light reaching the ground (Table 1).

Oncosiphon response

The percent *Oncosiphon* cover was significantly higher in the incomplete burn (Table 2; Figure 3A) where singed stands had 36.8±7.66% SE greater *Oncosiphon* cover than the complete burn in the first year, and 8.33±4.15% SE greater cover in 2021. The litter treatment in 2020 had 11.4±10.0% SE greater *Oncosiphon* cover; however, litter presence did not significantly influence *Oncosiphon* cover in 2021 (Table 2; Figure 3B). There was consistently greater *Oncosiphon* cover within singed stands; however, there was an interaction between burn completion and litter treatment in 2020 where in the complete burn with litter present, *Oncosiphon* cover increased 11.88±4.81% SE compared to no litter (Table 2). The interaction between burn completion and litter was not significant in 2021 (Table 2). The addition of native seeds did not have an observed impact on *Oncosiphon* cover (Table 2).

There were more viable *Oncosiphon* seeds in the soil from the singed stands, and with litter present (Table 2, Figure 3C). Singed stands had more than double the amount of viable *Oncosiphon* seeds with 123±15 SE viable seeds vs 47±8 SE in the complete burn. The litter present plots had 102±12 SE viable seeds compared to 68±15 SE in the no litter plots. The difference between litter treatments was greatest in the complete burn, where litter in complete burn had 71±14 SE viable seeds compared to 23±7 SE viable seeds in the no litter complete burn (Table 2, Figure 3C).

Native species responses

Total native cover was higher in complete burn than singed stands in 2020, but not 2021 (Table 3). Total native cover was not influenced by litter treatment or seed addition (Table 3). Our native seed addition increased the total cover, and number of seeded species established in plots in both 2020 and 2021 (Table 3; Figure S1). The percent cover remained low across all species, with the greatest cover in any plot of all seeded species being 5% in 2020 and 2021 with only a maximum of two seeded species establishing in the same plot in both years. Seeded species cover and species richness of the seeded subset were similar between burn treatment and litter treatment in both years (Table 3). Individually, our seeded species were consistently low in cover, where the highest cover of an individual species was *Layia platyglossa* with an average of 0.51±0.16% SE in the seeded plots in 2020, and 0.40±0.10% SE in 2021, and 0.13±0.13% SE in the unseeded plots in 2020 and 0% in the unseeded 2021 plots. Overall, establishment of seeded native species ranged from 0% to 0.45% cover in the seeded treatment, and species averaged between 0% and 0.02% cover in the unseeded treatment (Table S3).

A greater number of native seeds germinated in our seedbank study in the complete burn with an average of 105±16 SE total native seeds compared to 75±10 SE total native seeds in the singed stands (Table 4). The litter treatment and seed treatments did not influence the number of native seeds that germinated (Table 4). The number of viable seeds of our seeded species present in our greenhouse was consistently low with a range from 0 to 15 individuals. There were no differences in how many seeded species germinated in complete burn (3.05±0.39 SE) and singed stands (3.95±1.02 SE) (Table 4). More seeded species were present in the seed treatment (Table 4), with an average of 4.1±0.6 SE seeded species present in seed treatment and 0.7±0.11 SE present in unseeded plots. Within the seeded treatment, there were more seeded species present with litter than with no litter (Table 4).

Community response

We did not observe a change in diversity (H) between singed stands and complete burn in 2020 (0.74±0.05 SE vs 0.78±0.06 SE; Table 5) but singed stands had greater diversity in 2021 (0.80±0.06 SE vs 0.55±0.05 SE; Table 5). Litter treatment did not influence diversity in 2020 (litter: 0.81±0.06 SE vs no litter: 0.70±0.05 SE; Table 5) or 2021 (litter: 0.72±0.06 SE vs no litter: 0.64±0.06 SE; Table 5). The seed addition did not statistically influence diversity in 2020 (seed 0.77±0.06 SE vs unseed 0.74±0.06 SE; Table 3) or 2021 (Seed: 0.65±0.06 SE vs unseed: 0.70±0.06 SE; Table 5).

Singed stands had statistically similar species richness in 2020 (complete: 5.35±0.29 SE vs singed stand: 5.55±0.24 SE; Table 5) but had greater richness in 2021 (complete 4.35±0.25 SE vs singed stand: 5.30±0.2 SE; Table 5). The litter treatment did not statistically influence species richness in 2020 (litter: 5.3±0.26 SE vs no litter: 0.56±0.27 SE) or 2021 (litter 4.9±0.24 SE vs no litter 4.8±0.29 SE; Table 5); however, within the no litter treatment the complete burn area had lower species richness (3.85±0.30 SE) compared to the singed stands with no litter (5.7±0.41 SE) (Table 5).

The plots with native seed addition had greater species richness in both years (2020 seed: 5.9±0.27 SE vs unseed: 5.0±0.24 SE; 2021 seed:5.3±0.27 SE vs unseed: 4.4±0.2 SE; Table 5).

For our whole community level analysis, the PcoA loadings indicate three main species driving differences in community types: *Erodium cicutarium, Oncosiphon pilulifer*, and *Bromus madritensis,* which are all non-native (Figure 4). The direction of loadings shifts between years, but *Oncosiphon* remained ordinal to *Erodium* in both years and the singed stand dynamics had similar trends. Burn completion significantly influenced community composition in both years (Figure 4 A,B; Table 5). The completely burned plots were mostly dominated by *Erodium* regardless of year, and the singed stand plots were associated with more *Oncosiphon* in year one or was split dominated by *Oncosiphon* or *Bromus* in 2021 (Figure 4 A, B). Litter also influenced community composition in both years (Table 5), where litter plots were slightly more dominated by *Bromus* and *Oncosiphon* than *Erodium* in 2020 (Figure 4C) and were split between *Bromus, Oncosiphon* and three other species in 2021 (Figure 4D). The addition of native seeds did not influence community composition in 2020 but seed addition did statistically influence community composition in 2021, where seeded plots were more dominated by *Erodium* than unseeded plots which were split between *Bromus, Oncosiphon*, and three other species (Table 5; Figure S2), notably none of the seed addition species were significant vectors of plant dominance in the ordination.

Discussion

Our study demonstrates that post burn heterogeneity in the form of singed forb stands impacts both invader and native establishment, with stronger benefits for the invader thus increasing the risk of reinvasion. The focal invader *Oncosiphon* had greater percent cover and a greater amount of viable seeds in the seedbank when litter was present, likely a result of the more favorable microclimate created by litter. Additionally, there was greater *Oncosiphon* cover within singed stands (i.e. incomplete burn) in both years and double the amount of viable seeds in singed stands, implying that singed stands were contributing to the retention of *Oncosiphon* seeds post burn. The addition of native seeds did not influence native cover, indicating that additional barriers limit the establishment of native species, beyond seed availability. At a community level, we found three main dominant invasive species: *Erodium cicutarium* (forb), *Bromus madritensis* (grass), and *Oncosiphon pulilifer* (forb) structured post-management trajectories. The presence of litter altered the composition of the community to favor *Bromus* and *Oncosiphon,* suggesting that these two invasives are the most successful at taking advantage of ameliorated microclimates post burn. The complete burn area was dominated by *Erodium,* while singed stands were either dominated by *Bromus* or *Oncosiphon*¸ suggesting a difference in invader seed availability results in two trajectories based on burn completion. These results support the hypotheses that heterogeneity in the post burn landscape can alter establishment via litter-microclimate effects and seed availability and can drive recovery trajectories towards re-invasion.

The post burn landscape is heterogenous, and differences in microclimate can facilitate invasive establishment post-burn. Our findings suggest that litter (whether naturally remaining in singed stands or additions) may act like shelters to form islands of greater establishment for invasive species in arid systems via nurse plant effects (Fick et al. 2016; Abella & Chiquoine 2018). The singed stands had lower soil moisture and soil temperatures compared to the complete burn, and litter reduced soil temperatures as well as proportion of light. Invasive species often exhibit priority effects, where they may draw down resources earlier in the growing season that can have drastic impacts on community structuring and composition (Fukami 2015). In our experiment we had high invasive cover, and the environmental conditions that were measured during peak biomass likely reflect the higher resource demands of the plants that survived the full growing season. Taking measurements earlier in the growing season may help elucidate to what extent differential resource use may impact the germination and establishment of other species. Although native species may benefit from ameliorated microclimate stresses through litter retention, the competitive interactions from non-native species with faster resource acquisition strategies may limit native performance (Amatangelo et al. 2008, Wainwright et al. 2011). Alternatively, litter can cause strong selection pressure to favor taller species due to light limitation (Harpole & Tilman 2007; Amatangelo et al. 2008). Within California grasslands, grass litter has been shown to reduce native establishment (Molinari & D’Antonio 2020). *Oncosiphon* litter may similarly facilitate the dominance of *Oncosiphon* and *Bromus,* two fast growing species with vertical growth forms, while increasing native seedling mortality due to increased competitive pressures

(Fenner & Michael 2005; Harpole & Tilman 2007; Amatangelo et al. 2008). Additionally, litter presence exacerbates competitive differences and can negatively impact both resource acquisitive and resource conservative native species (LaForgia 2021). Thus, the potential beneficial impacts of litter on native species establishment in xeric areas post burn may be dependent upon the density of litter and the environmental or biotic stressors driving seedling mortality.

In addition to the microclimate differences, seed availability in the post burn landscape is a major driver of post-burn establishment. Initial flushes of native species post fire are often overwhelmed by reinvasion of well-established invaders coming from surrounding areas (Dickens & Allen 2009; Alba et al. 2014). We observed that remaining singed stands acted as reservoirs of invasive seed, forming an in-situ source of invader seed within burn areas. Increased seed availability post fire has been demonstrated to facilitate invader spread and alter recovery trajectories of grasslands (Dickens & Allen 2009; Gioria et al. 2012). In our study, singed stands had both greater *Oncosiphon* cover and more than double the amount of *Oncosiphon* seeds. More work is needed to explore whether any residual *Oncosiphon* seed benefits from increased germination rates post-burn, which may exacerbate refugia dynamics. In our community level analysis, *Bromus* and *Oncosiphon* both had greater dominance with litter and in singed stands. The high cover of non-native species like *Oncosiphon* and *Bromus* within singed stands and with litter added suggests that these species were able to better capitalize on the ameliorated conditions of the litter and singed stands and both benefitted from retention of seed. Such establishment of invaders post control efforts (i.e., secondary invasions) have been demonstrated as a key factor limiting management outcomes (O'Loughlin & Green 2017) as non-native species are often more able to take advantage of increases in resource availability (Wainwright et al. 2011) in the post management landscape (D'Antonio & Meyerson 2002). The singed stands might provide a refuge for invaders to form in situ satellite sub-populations (Moody & Mack 1988) causing the reinvasion of burn areas to occur more rapidly than from surrounding seed rain alone. In this study, the singed stands within the post-burn landscape had a small spatial footprint, taking up less than 20% of the full burn area in this experiment, and are highly visible within the burn area; therefore, we suggest that they could be a target for further restoration activities. Notably, the removal of satellite populations of the invader *Pennisetum ciliare* was found to reduce the doubling time of the invasion (Weston et al. 2019). Selectively spraying pre-emergent herbicide may further reduce the potential for re-invasion (Lazarus & Germino 2022). The source of invader seed as either surrounding propagule pressure or in situ survival of seeds in singed stands may be best elucidated by remote sensing, as this technique allows for spatial analysis of spread within treatment areas (Park et al. 2018; Dash et al. 2019). Our study demonstrates the importance of post burn heterogeneity and a mechanism from which invaders may spread within a prescribed burn; however, long term studies as well as remote sensing may be required to determine the rate and extent of this mechanism contributing to post-burn recovery and the full impacts on native establishment.

We predicted that native species would be seed limited, but as in many other studies (e.g. Suding & Gross 2006; Tognetti & Chaneton 2012; James et al. 2013) we observed a limited response from our native seed addition efforts. A lack of symbiotic partners (Vogelsang & Bever 2009), combined with competitive pressures from invasives (Pearson et al. 2016), and anthropogenic changes like nitrogen deposition (Vallano et al. 2012) might be too great of an obstacle for native species establishment and persistence. Moreover, these factors may interact to create barriers to native species establishment (Sigüenza et al. 2006; Larios & Suding 2015). Barriers to native establishment such as the lack of symbionts (Vogelsaang & Bever 2009) may require inoculations to improve native establishment as other studies have shown (e.g. Middleton & Bever 2010; Koziol & Bever 2017). Invasive allelopathic litter can further drive reductions in native performance through preventing germination (Loydi et al. 2015); however, there have not been any published studies on the potential allelopathic effects of *Oncosiphon* litter. Despite the general low native species cover, even with seed addition, our study highlights the need for additional management actions beyond seeding to increase native establishment.

Prescribed burns are an important vegetation management tool (Kimmerer & Lake 2001; Valkó et al. 2020; Valkó & Deák 2021) and, in many cases, can be effective for intended purposes of removing litter and invader seedbanks while increasing native establishment (Reynolds et al. 2001; Keeley et al. 2008; Dickens & Allen 2009). However, in the case of *Oncosiphon,* an invasive forb, burning leads to extensive heterogeneity that may result in rapid invader re-establishments. Future work should explore the flammability of this species and other invasive forbs to better understand the mechanisms that may result in more singed stands. Spatial heterogeneity can have substantial impacts on restoration outcomes (Baer et al. 2005; Suding 2011), and we have demonstrated that heterogeneity created by management actions can also drive substantial differences in recovery trajectories. The variation in restoration outcomes remains a major challenge (Brudvig et al. 2017); however, this challenge also provides an opportunity to elucidate ecological mechanisms mediating the efficacy of management efforts (Eviner & Hawkes 2008). Litter and seed consumption during fire are the underlying mechanisms driving the efficacy of prescribed burns, and forbs are not consumed in the same way as grasses invasive forbs (Pyke et al. 2010). Our study highlights the potential for prescribed burning of invasive forbs to create a network of residual stands that facilitate reinvasion.

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Figures & Tables

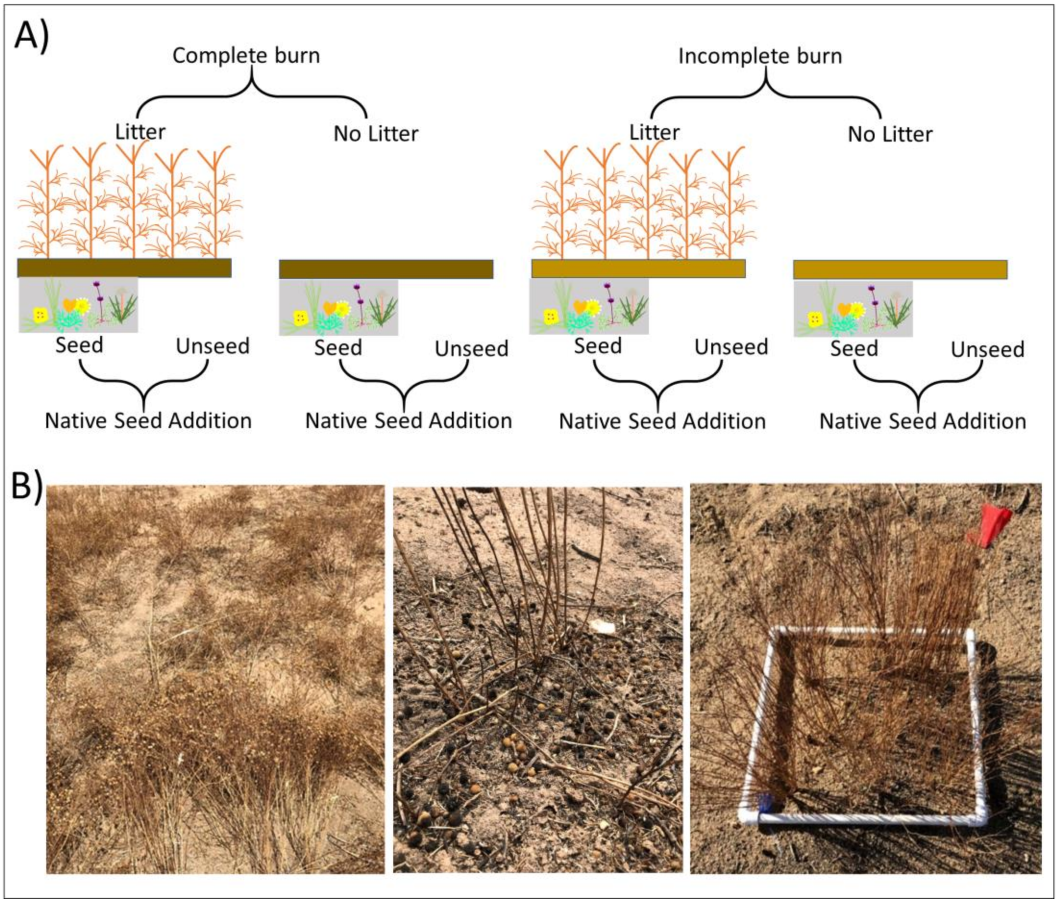


Figure 1) A) Diagram of experimental groups and B) Images of incompletely consumed *Oncosiphon* singed stands and litter manipulation. A) Experimental groups include factorial combination of burn completion (complete burn vs incomplete burn/singed stands), litter treatment (litter vs no litter), and native seed addition treatments (seed vs unseeded). B) The leftmost picture is singed *Oncosiphon* litter, the center picture is of singed inflorescences, and the right image is a litter addition plot. The singed litter were taken from litter removal in singed stands and staked into complete burn + litter plots to create a similar vertical litter structure and total litter cover.

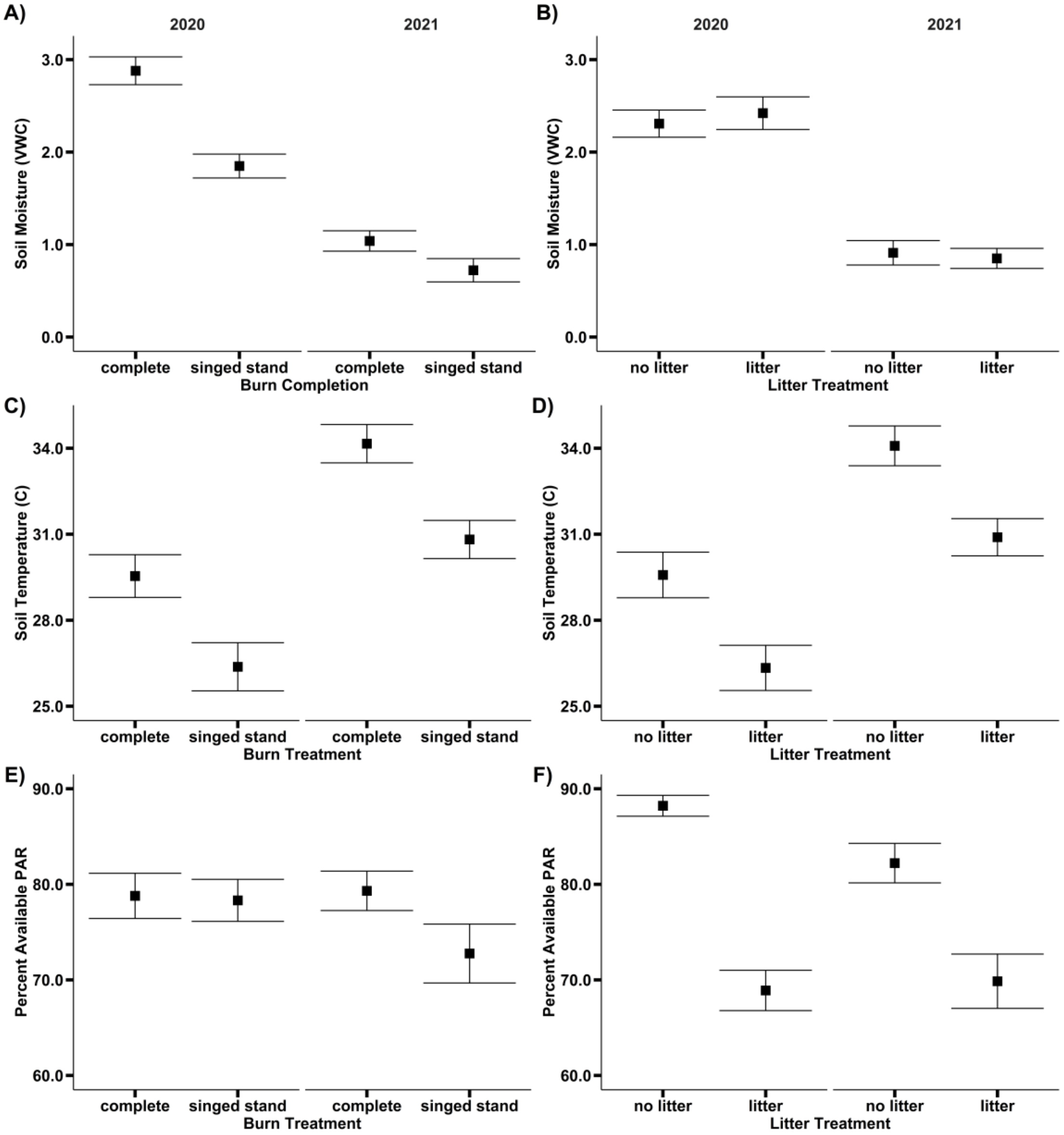


Figure 2) Environmental differences between burn completion treatments (A,C,E) and litter treatments (B,D,F). Averages of soil moisture (A,B), soil temperature (C,D), and percent of available photosynthetically active radiation (E,F) with standard error bars are shown. The first year of data collection in 2020 is on the left half of each graph, and the second year in 2021 is on the right half of each graph.

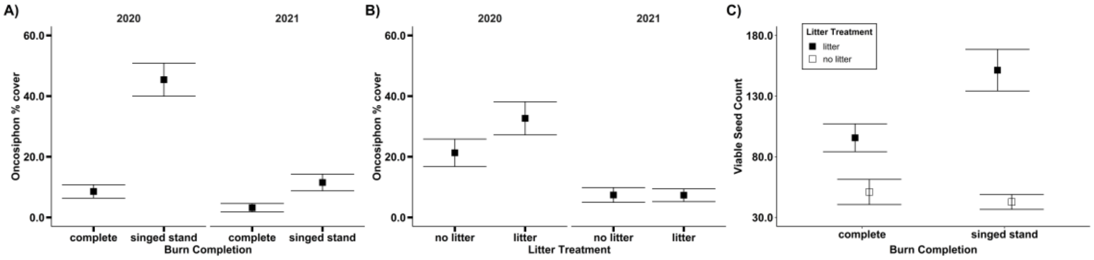


Figure 3) The effect of burn completion (A) and litter treatment (B) on *Oncosiphon pilulifer* percent cover in two consecutive years in the field and on their viable seeds (C), counted during a seedbank experiment in 2021. Analyses were performed using natural log transformed data, and graphed data display averages of raw data, with standard error bars.

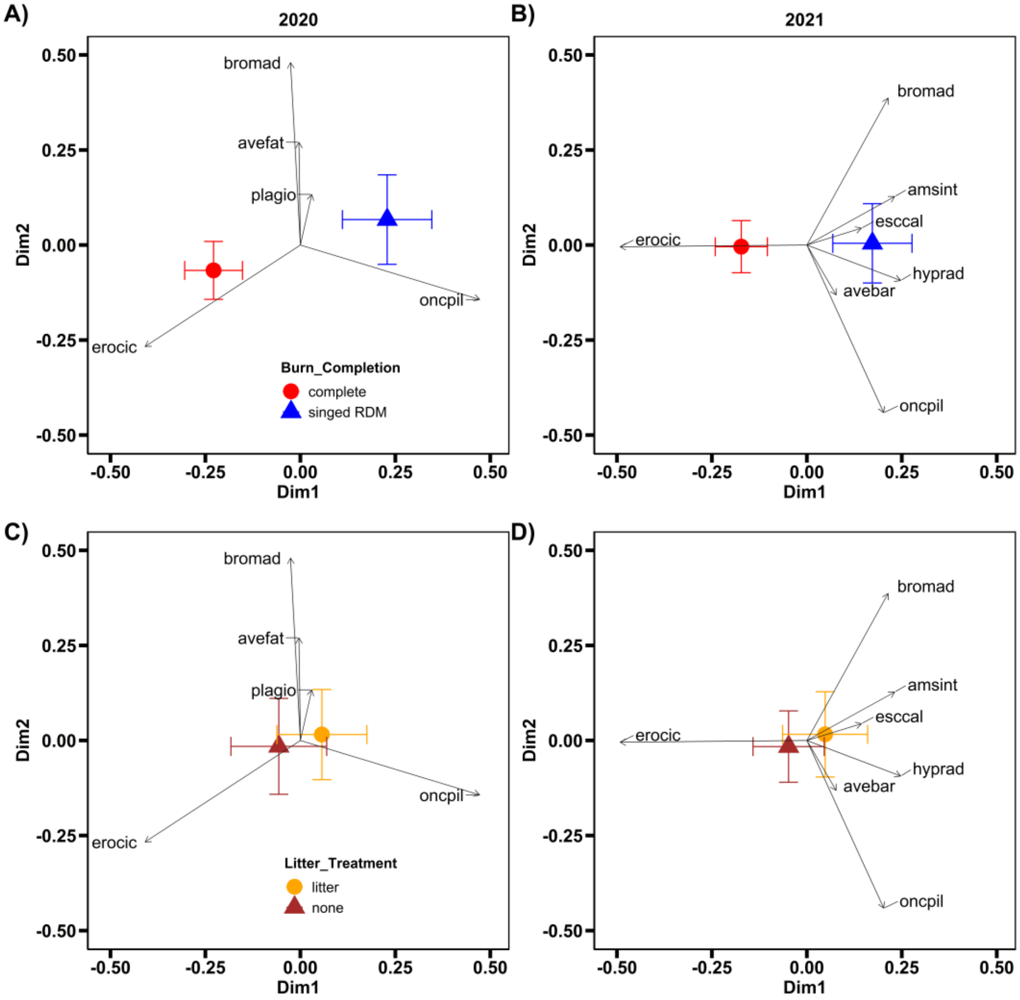


Figure 4) Principal Coordinates Analysis visualization of community level differences indicated by permutational analysis of variance (PERMANOVA). Points are average scores of every plot, and bars are 95% confidence intervals. Species codes, full names, years present, and common names are listed in table S1. A) Effects of burn completion on community composition in 2020 B) Effects of burn completion on community composition in 2021 C) Effects of litter treatments on community composition in 2020 D) Effect of litter treatment on community composition in 2021.

Table 1) Summary statistics for environmental metrics. The first number is the F statistic, the following numbers in subscript are Sattherwaite approximations of the degrees of freedom with numerator and denominator separated by a comma, with p values afterwards. Soil moisture was measured by volumetric water content, and ln(x+1) transformed, soil surface temperature was not transformed, and percent available PAR was cube transformed.

	2020	**2021**
Soil Moisture	F stat $_{\text{DF}}$ P value	F stat $_{\text{DF}}$ P value
Burn Completion	37.99 $_{1,61.217}$ <0.0001	9.46 $_{1,63.127}$ 0.003
Litter Treatment	0.12 $_{1,61.217}$ 0.735	0.11 $_{1,63.127}$ 0.743
Seed Treatment	0.04 $_{1,61.217}$ 0.847	0.29 $_{1,63.127}$ 0.592
Burn X Litter	0.01 $_{1,61.217}$ 0.932	0.17 $_{1,63.127}$ 0.680
Burn X Seed	0.69 $_{1,61.217}$ 0.409	2.35 $_{1,63.127}$ 0.130
Litter X Seed	0.54 $_{1,61.217}$ 0.466	0.91 $_{1,63.127}$ 0.343
Burn X Litter X Seed	0.12 $_{1,61.217}$ 0.726	0.63 $_{1,63.127}$ 0.431
Soil Surface Temperature		
Burn Completion	25.93 $_{1,62.787}$ <0.0001	21.27 $_{1,63.305}$ <0.0001
Litter Treatment	27.18 $_{1,62.787}$ <0.0001	19.34 $_{1,63.305}$ <0.0001
Seed Treatment	0.09 $_{1,62.787}$ 0.771	0.28 $_{1,63.305}$ 0.599
Burn X Litter	1.40 $_{1,62.787}$ 0.241	0.34 $_{1,63.305}$ 0.562
Burn X Seed	0.53 $_{1,62.787}$ 0.469	0.47 $_{1,63.305}$ 0.497
Litter X Seed	2.63 $_{1,62.787}$ 0.110	0.36 $_{1,63.305}$ 0.552
Burn X Litter X Seed	1.46 $_{1,62.787}$ 0.232	1.39 $_{1,63.305}$ 0.243
Percent Available PAR		
Burn Completion	0.30 $_{1,59.361}$ 0.588	3.25 $_{1,62.278}$ 0.076
Litter Treatment	88.74 $_{1,59.361}$ <0.0001	19.18 $_{1,62.278}$ <0.0001
Seed Treatment	1.27 $_{1,59.361}$ 0.264	0.53 $_{1,62.278}$ 0.468
Burn X Litter	0.25 $_{1,59.361}$ 0.621	3.81 $_{1,62.278}$ 0.055
Burn X Seed	0.05 $_{1,59.361}$ 0.825	0.87 $_{1,62.278}$ 0.355
Litter X Seed	0.18 $_{1,59.361}$ 0.677	0.11 $_{1,62.278}$ 0.739
Burn X Litter X Seed	0.02 $_{1,59.361}$ 0.901	0.05 $_{1,62.278}$ 0.828

Table 2) Summary statistics of *Oncosiphon* responses. The first number is the F statistic, the following numbers in subscript are Sattherwaite approximations of the degrees of freedom with numerator and denominator separated by a comma, and the final number is the p value. Field response of total *Oncosiphon* cover in 2020 and 2021, and viable seed counts were all ln(x+1) transformed. *Viable seed was estimated from a seedbank study of soil collected once in 2021.

	***Oncosiphon* cover 2020**	***Oncosiphon* cover 2021**	**Viable Seed 2021***
	F stat, $_{DF,}$ p value	F stat, $_{DF,}$ p value	F stat, $_{DF,}$ p value
Burn Completion	63.01 $_{1,63}$ <0.0001	22.14 $_{1,63}$ <0.0001	50.51 $_{1,55.093}$ <0.0001
Litter Treatment	12.97 $_{1,63}$ 0.001	0.09 $_{1,63}$ 0.768	21.10 $_{1,55.093}$ <0.0001
Seed Treatment	0.02 $_{1,63}$ 0.898	0.19 $_{1,63}$ 0.668	0.01 $_{1,55.093}$ 0.920
Burn X Litter	4.66 $_{1,63}$ 0.035	0.61 $_{1,63}$ 0.437	6.30 $_{1,55.093}$ 0.015
Burn X Seed	0.61 $_{1,63}$ 0.438	0.01 $_{1,63}$ 0.930	0.18 $_{1,55.093}$ 0.672
Litter X Seed	0.09 $_{1,63}$ 0.763	2.23 $_{1,63}$ 0.140	0.11 $_{1,55.093}$ 0.745
Burn X Litter X Seed	0.02 $_{1,63}$ 0.885	0.30 $_{1,63}$ 0.585	2.12 $_{1,55.093}$ 0.151

Table 3) Summary statistics of California native species responses. The first number is the F statistic, the following numbers in subscript are Sattherwaite approximations of the degrees of freedom with numerator and denominator separated by a comma, and the final number is the p value. Results from the first year are on the left, and results from the second year are on the right. Total native cover, seeded species cover, and seeded species richness were all ln(x+1) transformed.

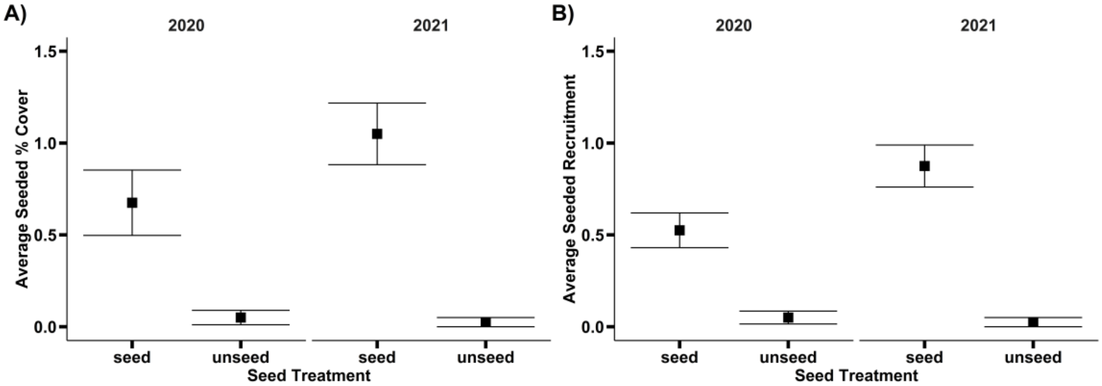
	2020	**2021**
Total Native Cover	F stat, $_{\text{DF}}$, P value	F stat, $_{\text{DF}}$, P value
Burn Completion	28.86 $_{1,63.239}$ <0.0001	1.31 $_{1,72}$ 0.256
Litter Treatment	1.57 $_{1,63.239}$ 0.215	3.89 $_{1,72}$ 0.052
Seed Treatment	2.08 $_{1,63.29}$ 0.155	0.72 $_{1,72}$ 0.400
Burn X Litter	3.39 $_{1,63.29}$ 0.070	1.47 $_{1,72}$ 0.229
Burn X Seed	0.07 $_{1,63.239}$ 0.798	0.17 $_{1,72}$ 0.681
Litter X Seed	3.53 $_{1,63.239}$ 0.065	1.28 $_{1,72}$ 0.262
Burn X Litter X Seed	0.06 $_{1,63.29}$ 0.810	0.04 $_{1,72}$ 0.848
Seeded Species Cover		
Burn Completion	2.78 $_{1,63}$ 0.100	0.34 $_{1,72}$ 0.564
Litter Treatment	1.36 $_{1,63}$ 0.249	0.43 $_{1,72}$ 0.515
Seed Treatment	17.57 $_{1,63}$ <0.0001	53.94 $_{1,72}$ <0.0001
Burn X Litter	0.03 $_{1,63}$ 0.874	2.56 $_{1,72}$ 0.114
Burn X Seed	3.95 $_{1,63}$ 0.051	0.02 $_{1,72}$ 0.886
Litter X Seed	0.71 $_{1,63}$ 0.401	0.05 $_{1,72}$ 0.828
Burn X Litter X Seed	0.44 $_{1,63}$ 0.508	1.36 $_{1,72}$ 0.248
Seeded Species Richness		
Burn Completion	1.56 $_{1,63}$ 0.216	0.00 $_{1,72}$ 1.000
Litter Treatment	0.06 $_{1,63}$ 0.803	0.17 $_{1,72}$ 0.677
Seed Treatment	22.58 $_{1,63}$ <0.0001	50.50 $_{1,72}$ <0.0001
Burn X Litter	0.06 $_{1,63}$ 0.803	1.57 $_{1,72}$ 0.214
Burn X Seed	1.56 $_{1,63}$ 0.216	0.17 $_{1,72}$ 0.677
Litter X Seed	0.06 $_{1,63}$ 0.803	0.00 $_{1,72}$ 1.000
Burn X Litter X Seed	0.56 $_{1,63}$ 0.456	0.70 $_{1,72}$ 0.406

Table 4) Summary statistics of viable seed responses for all native species and the seed addition species. The first number is the F statistic, the following numbers in subscript are Sattherwaite approximations of the degrees of freedom with numerator and denominator separated by a comma, and the final number is the p value. Total native viable seeds and seeded species viable seeds were both ln(x+1) transformed for residual normality.

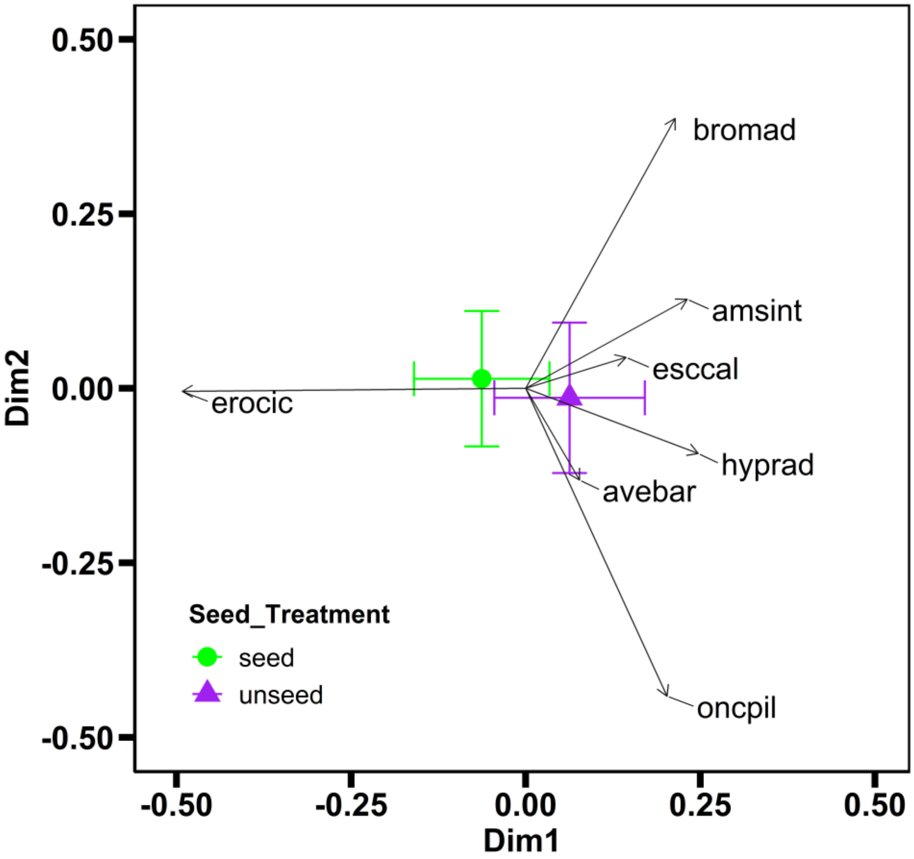
	Total Native Viable Seeds	**Seeded species Viable Seeds**
	F stat, $_{\mathrm{DF}}$, P value	F stat, $_{\mathrm{DF},}$ P value
Burn Completion	$8.18_{1,63}$ 0.006	$0.88_{1,63.504}$ 0.352
Litter Treatment	$0.33_{1,63}$ 0.570	$6.35_{1,63.504}$ 0.014
Seed Treatment	$2.70_{1,63}$ 0.105	$44.83_{1,63.504}$ <0.0001
Burn X Litter	$0.12_{1,63}$ 0.732	$1.47_{1,63.504}$ 0.231
Burn X Seed	$1.65_{1,63}$ 0.204	$0.84_{1,63.504}$ 0.363
Litter X Seed	$0.15_{1,63}$ 0.704	$7.37_{1,63.504}$ 0.009
Burn X Litter X Seed	$1.43_{1,63}$ 0.236	$1.41_{1,63.504}$ 0.239

Table 5) Summary statistics of community level responses. For diversity and richness, the first number is the F statistic, the following numbers in subscript are Sattherwaite approximations of the degrees of freedom with numerator and denominator separated by a comma, and the final number is the p value. For the PERMANOVA, the first number is the F statistic and the following number in subscript is the R^2, and the final number is the p value.

	2020	**2021**
Diversity (H)	F stat, $_{DF}$, P value	F stat, $_{DF}$, P value
Burn Completion	0.26 $_{1,72}$ 0.611	10.11 $_{1,63}$ 0.002
Litter Treatment	1.61 $_{1,72}$ 0.208	1.15 $_{1,63}$ 0.287
Seed Treatment	0.14 $_{1,72}$ 0.710	0.44 $_{1,63}$ 0.508
Burn X Litter	2.49 $_{1,72}$ 0.119	0.72 $_{1,63}$ 0.398
Burn X Seed	0.18 $_{1,72}$ 0.669	0.00 $_{1,63}$ 0.976
Litter X Seed	0.08 $_{1,72}$ 0.777	1.01 $_{1,63}$ 0.320
Burn X Litter X Seed	0.12 $_{1,72}$ 0.728	0.00 $_{1,63}$ 0.995
Richness		
Burn Completion	0.31 $_{1,72}$ 0.581	7.84 $_{1,63}$ 0.007
Litter Treatment	0.69 $_{1,72}$ 0.408	0.09 $_{1,63}$ 0.769
Seed Treatment	5.56 $_{1,72}$ 0.021	6.27 $_{1,63}$ 0.015
Burn X Litter	0.69 $_{1,72}$ 0.408	7.03 $_{1,63}$ 0.010
Burn X Seed	0.94 $_{1,72}$ 0.335	0.54 $_{1,63}$ 0.464
Litter X Seed	1.56 $_{1,72}$ 0.216	0.00 $_{1,63}$ 1.000
Burn X Litter X Seed	0.48 $_{1,72}$ 0.490	0.00 $_{1,63}$ 1.000
PERMANOVA results	F stat, $_{R2}$, P value	F stat, $_{R2}$, P value
Burn Completion	33.59 $_{0.302}$ 0.001	15.36 $_{0.161}$ 0.001
Litter Treatment	2.51 $_{0.023}$ 0.050	2.21 $_{0.023}$ 0.048
Seed Treatment	0.23 $_{0.002}$ 0.826	2.59 $_{0.027}$ 0.032
Burn X Litter	2.08 $_{0.019}$ 0.084	1.26 $_{0.013}$ 0.208
Burn X Seed	0.43 $_{0.004}$ 0.572	-0.06 $_{-0.001}$ 0.993
Litter X Seed	0.09 $_{0.001}$ 0.943	1.06 $_{0.011}$ 0.259
Burn X Litter X Seed	0.24 $_{0.002}$ 0.783	0.96 $_{0.010}$ 0.303



Supplemental Figure 1. Seeded species responses in the field. A) Average seeded percent cover with standard error B) Average seeded species recruitment listed as proportion of species recruited with standard error.



Supplemental Figure 2. Principal Coordinates Analysis visualization of community level differences indicated by permutational analysis of variance (PERMANOVA). Points are average scores of every plot, and bars are 95% confidence intervals. Species codes, full names, years present, and common names are listed in table S1.

Table S1. Species present in field sampling, with species six letter code of genus and species, full name, years present, and origin. Asterix indicates seeded species.

Six Letter Code	Full Name	Year(s) Present	Origin
amsint	*Amsinckia intermedia*	2020, 2021	native
avebar	*Avena barbata*	2020, 2021	non-native
avefat	*Avena fatua*	2020, 2021	non-native
brator	*Brassica tournefortii*	2021	non-native
brodia	*Bromus diandrus*	2020, 2021	non-native
bromad	*Bromus madritensis*	2020, 2021	non-native
calmen	*Calandrinia menziesii*	2020, 2021	native
cambis	*Camissoniopsis bistorta**	2020, 2021	native
cracon	*Crassula connata*	2020, 2021	native
croset	*Croton setiger*	2020	native
cryspp	*Cryptantha spp*	2020, 2021	native
erocic	*Erodium cicutarium*	2020, 2021	non-native
esccal	*Eschscholzia californica**	2020, 2021	native
eupspp	*Euphorbia spp*	2020	non-native
hirinc	*Hirschfeldia incana*	2020	non-native
hormur	*Hordeum murinum*	2021	non-native
hyprad	*Hypochaeris radicata*	2020, 2021	non-native
lacser	*Lactuca seriola*	2020, 2021	non-native

lascal	*Lasthenia californica*	2020, 2021	native
laypla	*Layia platyglossa**	2020, 2021	native
lupbic	*Lupinus bicolor*	2020, 2021	native
oncpil	*Oncosiphon piluliferum*	2020, 2021	non-native
plaspp	*Plagiobothrys spp*	2020	native
salcol	*Salvia columbariae**	2021	native
schbar	*Schismus barbatus*	2020, 2021	non-native
trilan	*Trichostema lanatum*	2020	native
urolin	*Uropappus lindleyii**	2020, 2021	native

Table S2. Species present in seedbank study, with species six letter code of genus and species, full name, and origin. Asterix indicates species with seed added.

Six Letter Code	Full name	Origin
acmstr	*Acmispon strigosus*	native
amaalb	*Amaranthus albus*	non-native
amsint	*Amsinckia intermedia*	native
avefat	*Avena barbata*	non-native
branig	*Brassica nigra*	non-native
brator	*Brassica tournefortii*	non-native
brodia	*Bromus diandrus*	non-native
bromad	*Bromus madritensis*	non-native
calmen	*Calandrinia menziesii*	native
cambis	*Camissoniopsis bistorta**	native
cheber	*Chenopodium berlandieri*	native
cracon	*Crassula connata*	native
croset	*Croton setiger*	native
crycot	*Cryptantha cotata*	native
cryint	*Cryptantha intermedia*	native
erobot	*Erodium botrys*	non-native
erocic	*Erodium cicutarium*	non-native

esccal	*Eschscholzia californica**	native
hirinc	*Hirschfeldia incana*	non-native
hyprad	*Hypochaeris radicata*	non-native
lacser	*Lactuca seriola*	non-native
lascal	*Lasthenia californica*	native
laypla	*Layia platyglossa**	native
lupbic	*Lupinus bicolor*	native
oncpil	*Oncosiphon piluliferum*	non-native
pecspp	*Pectocaria spp*	native
phadis	*Phacelia distans*	native
salcol	*Salvia columbariae**	native
schbar	*Schismus barbatus*	non-native
sisiri	*Sisymbrium irio*	non-native
trilan	*Trichostema lanatum*	native
urolin	*Uropappus lindleyii**	native

Table S3. Average vegetative cover and standard error of seeded species recruitment in seeded and unseeded treatments. One seeded species, *Stipa pulchra*, did not recruit in seeded or unseeded treatments and is not included in this table.

Seed Treatment	**Species**	**Average Cover 2020**	**Average Cover 2021**
seed	*Camisoniopsis bistorta*	0±0.000	0.175±0.061
seed	*Escscholzia californica*	0.0125±0.013	0.075±0.055
seed	*Layia platyglossa*	0.5125±0.164	0.4±0.100
seed	*Salvia columbariae*	0±0.000	0.25±0.078
seed	*Uropappus lindleyii*	0.15±0.074	0.15±0.092
unseed	*Camisoniopsis bistorta*	0.0375±0.038	0±0.000
unseed	*Escscholzia californica*	0±0.000	0.025±0.025
unseed	*Layia platyglossa*	0.0125±0.013	0±0.000
unseed	*Salvia columbariae*	0±0.000	0±0.000
unseed	*Uropappus lindleyii*	0±0.000	0±0.000

Chapter 2: Invasion creates a dynamic plant soil feedback reducing invader and native plant performance.

Abstract:

Invasive plants disrupt recipient communities through a variety of mechanisms; however, the interactions between invasive plants and soil communities are especially problematic as they develop plant soil feedback (PSF) mechanisms which mediate plant abundance. Plant invaders can drive PSF by altering the amount of arbuscular mycorrhizal fungi (AMF) or through increasing the presence of pathogens in the soil, either of which can impact resident or invader performance. The strength and direction of invader driven PSF is dependent upon the degree of soil conditioning by the invader and the traits of the resident species. Certain resource acquisition traits such as specific root length (SRL) and specific leaf area (SLA) can be indicative of fundamental plant strategies and tradeoffs including the costs and benefits of symbiosis and the growth-defense tradeoff. We investigated the possibility of an invasive forb, *Oncosiphon pilulifer,* to alter native plant growth via PSF over a soil invasion gradient. Six native forbs and *Oncosiphon* were grown in pots inoculated with whole soil from four invasion histories within three reserves. After eight weeks, we measured growth via shoot and root biomass, changes in AMF via root colonization rates, and resource acquisition traits of SLA and SRL. We found evidence for an invader driven PSF reducing native growth, where the average of all six native plant shoot response ratios decreased in lightly invaded soil, heavily invaded soil, and longest invasion history compared to uninvaded soil, with corresponding reductions in AMF colonization. *Oncosiphon* also decreased in

performance with increased soil conditioning, but we generally did not find AMF in Oncosiphon roots implying belowground enemies as drivers of its reduced growth. We found that SLA was negatively predictive of native plant response to invader PSF independent of soil conditioning, suggesting a growth defense tradeoff, which might be strengthened with the loss of AMF mutualists. Our findings suggest that *Oncosiphon* is reducing native plant performance via reductions in AMF partners, and that species with fast growth strategy are the most vulnerable to its soil conditioning. Functional traits may elucidate responses to invader PSF, but the presence of multiple invader mediated dynamics require considering broader plant strategies and tradeoffs to produce a more generalizable framework for predicting native response to invader PSF.

Introduction:

Plant-soil interactions are important drivers of plant community dynamics and influence plant abundance (Klironomos 2002; Mangan et al. 2010; Eppinga et al. 2018). These interactions form plant soil feedback (PSF), which can elicit positive to negative plant responses with different belowground constituents (e.g., symbionts, pathogens) and may shift over time within a community (Hawkes et al. 2013; Ke, Zee & Fukami 2021). Invasions have provided unique opportunities to explore how PSF may impact community dynamics (Teste et al. 2019), as novel invasive plants can contribute to their invasion success through changes in soil communities that enhance their performance (Callaway & Reinhart 2006). In some cases, the PSF can have different effects among species, enhancing the growth of some groups while reducing the growth of others (Ke Le & Fukami 2021). However, predicting the impacts of invader mediated PSF on the recipient community has been challenging due to the variety of mechanisms by which invaders may promote PSF and that studies often miss the temporal development of these dynamics (Grove et al 2017). Plant functional traits and functional strategies present a promising opportunity to improve our understanding of plant-soil interactions (Xi et al 2021), as they may indicate growth trade-offs that are predictive of the different PSF mechanisms. Leveraging traits' predictive capacity to understand PSF responses is contingent on simultaneously investigating the temporal development of invader-mediated PSF.

Plant invaders alter PSF in ways that impact resident species and themselves over time, through alterations to the belowground soil biota. PSF are assumed to be density dependent (Bever et al. 1997; Chung 2023), with the expectation that invader PSF should develop over time as the density of the invader increases. However, this development may or may not be directly related to invader abundance depending on the nature of mechanism, resulting in delayed impacts on resident species. For instance, when an invader disrupts mutualisms (i.e., reduces symbiont presence) by outcompeting and excluding hosts, invader impacts on soil symbiont abundance and belowground communities are indirect, and will develop slowly, effecting native performance at later stages of invasion (Grove et al. 2017). Conversely, when invaders disrupt mutualisms via allelopathy, the impact on symbionts is direct and the impact on native plants is indirect and may more reflect a legacy of plant activities and not instantaneous invader abundance (Lankau and Lankau 2014). Additionally, allelochemical production varies with plant age and abundance (Chung 2023), which would promote step-like reductions in fungal richness and abundance and delayed responses from resident species. Capturing when invader PSF has direct or delayed impacts on the recipient community is key to ensure that invader impacts can be mitigated before any long-term impacts are developed, yet few studies explore this temporal dimension. Importantly, any changes made to the soil community can also impact a plant invader over time. More established invaders might have less need for mycorrhizae over the course of invasion if their dominance over time allows them to depend less on fungi (Seifert, Bever & Maron 2009). Alternatively, some invaders may develop stronger mycorrhizal relationships over time, either because of the increased probability of the introduction of their symbionts or because they are able to foster a relationship with an existing fungal partner (Reinhart & Callaway 2006). These dynamics may impact the time frame at which an invader benefits or begins to be negatively impacted by changes in the soil community.

Invaders can also interact with belowground enemies, like pathogens and root herbivores to develop PSF, which can limit their performance (Diez et al. 2010) or spillover to impact resident species (Kelly et al. 2009). The accumulation of pathogens often occurs over longer time scales (Flory and Clay 2013) as time is needed to meet conditions that facilitate pathogen growth and transmission, such as a minimum host population size (Garrett & Mundt 1999; Flory & Clay 2013) or through changes in microclimate facilitating pathogen populations (Alexander 2010). However, after pathogens have accumulated in the soil, native species may be more sensitive to increases in pathogens than invaders (Kelly et al. 2009; Kendig et al. 2021). The temporal development of these different impacts on recipient species vs the plant invader is often overlooked as many studies focus on soils from a single time point (Kardol et al. 2013; Chung 2023). Improving our understanding of invader mediated PSF is contingent on determining if changes are occurring to pathogen and mutualist soil biota, and how native plant growth strategies are differentially impacted.

Plant functional traits can play an important role for predicting the response of species in recipient communities and to elucidate mechanisms of native resistance to invader PSF**.** While efforts are emerging for a general trait-PSF framework for species in uninvaded communities (Cortois et al. 2016; Xi et al. 2021), functional traits have yet to be linked to invader mediated PSF. Emerging trait-PSF frameworks highlight the importance of considering fundamental trade-offs in plant strategies. In one key trade-off, plants may exhibit a “do it yourself” soil exploration strategy vs an “outsourcing” strategy that relies on mycorrhizal partnerships (Bergmann et al. 2020). This tradeoff reflects specific root length (SRL), where collaborative “outsourcing” species tend to have lower SRL (McCormack & Iversen 2019). In the case of disrupted mutualism PSF, the reduction in symbiont availability would have stronger impacts on plants with high mycorrhizal dependency, as they rely on soil biota to maintain growth, resulting in an inverse relationship between SRL and response to invader mediated PSF. In another key plant strategy trade-off, plants may invest more in growth or defense (Weigelt et al. 2021). In uninvaded systems, high SRL and high SLA species tend to be most vulnerable to soil biota exhibiting negative PSF (Cortois et al 2016; Xi et al. 2021). If an invader increases the amount of belowground enemies, species with higher growth rates, and associated higher SRL and higher SLA, would be more susceptible to the impacts of pathogens than species with more investment in defenses (Newsham et al. 1995; Cappelli et al. 2020; Xi et al. 2021). Predictive frameworks linking traits and native PSF response must identify the PSF that negatively impact native species (i.e., mutualism disruption, pathogen spillover) and incorporate fundamental tradeoffs (i.e., costs & benefits symbiosis, growth-defense) that mediate responses to invader PSF. As invader mediated impacts on mutualists and pathogens take time to develop and impacts on native species are not always directly related to current invader abundance, novel invasions provide a unique opportunity to elucidate the temporal development of invader PSF and associated impacts on soil biota.

Oncosiphon pilulifer is a novel annual invasive forb in the Southwest United States, thought to have been originally introduced to Southern California with a soil shipment from South Africa in the 1980's. *Oncosiphon* has substantially altered the landscapes within reserves across the Inland Empire area and is expanding in density and cover regionally across Southern California and Arizona (Hedrick & McDonald 2020; Schwab, Jenerette & Larios 2023). As a recently expanding invader, less is known about the community dynamics and potential for *Oncosiphon* driven PSF to restructure community dynamics. *Oncosiphon's* original introduction point is known (Lake Perris State Park, CA) and there are surrounding reserves that have experienced differing degrees of invasion from single point introductions typically near visitor parking lots. In addition to the reserve level differences, there are areas within each reserve that have substantially different levels of invasion, providing an excellent opportunity to explore the temporal development of invader driven PSF within and between sites.

To better identify how a new species in a community can influence PSF we tested a series of hypotheses on the temporal development of PSF following the introduction of *Oncosiphon pilulifer*. Using this recent invasion as a model for PSF dynamics we asked how novel PSF develop over time and influence both the invader and native species? We predicted that a positive PSF would increase *Oncosiphon* growth rates through associating with a subset of symbionts (i.e., *enhanced mutualisms;* increased plant growth, increased AMF colonization) and that these growth effects would increase with the time since invasion, with limited impacts on native species. Alternatively, we tested the prediction that a negative PSF response in *Oncosiphon* would cause a decrease in growth via increased belowground enemies (i.e., *pathogen driven invasive decline;* reduced plant growth, no change in AMF), with strongest impacts in the later stages of invasion. For native species, we predicted a negative PSF from *Oncosiphon* that will reduce native growth more with increasing invasion, and that there will either be fewer symbionts with greater invasion levels (i.e., *disrupted mutualisms:* reduced plant growth, reduced AMF colonization) or increased belowground enemies. (i.e., *pathogen spillover:* reduced plant growth, no change in AMF). We further asked how plant functional traits predict native species responses to invader driven PSF? For disrupted mutualisms, we predicted that the trait relationships associated with costs and benefits of maintaining symbiosis (i.e., low SRL) may be a better predictor of native PSF responses indicating which species are more dependent on and therefore more sensitive to losses in AMF. Alternatively, if invader driven PSF were result of increased belowground enemies, we predicted trait relationships associated with higher growth and decreased defense (i.e., high SRL, high SLA) would be the most sensitive to invader PSF. Enhancing our understanding of how invasive species PSF develop over time to influence both the invader and native performance, and how functional traits may predict native species responses to invader driven PSF can elucidate mechanisms of invasion success and native resistance to invasion.

Methods:

Soil Inocula Collection:

To assess the temporal development of *Oncosiphon* soil legacies, we collected soil from three reserves and four invasion levels within each reserve. We collected soil from Lake Perris State Park (33.868530, -117.176620), Motte Rimrock Reserve (33.800570, -117.255322) and Lake Matthew's Estelle Preserve (33.808138, -117.426358). To assess intensity of soil conditioning, we used a space for time substitution, where within each reserve, invasion levels were determined as "uninvaded" having no *Oncosiphon* present for at least 5m in every direction, "Light" if *Oncosiphon* patches were less than 0.25mX0.25m and had interspaces greater than 2m, "heavy" if *Oncosiphon* patches were greater than 10m^2 without interspaces, and "origin" soils were determined as the original sightings of *Oncosiphon* within the reserve. Three 10cm diameter X 15cm deep cores were taken from each invasion level within each reserve and kept separate as analytical replicates. Soil cores were sieved with a sterilized (via 90% Ethanol spray) 2mm soil sieve to remove rocks and debris. We then measured 350ml of sieved soil for each live soil replicate and pooled the remaining soil from each reserve and invasion level to have a paired sterilized inoculant. The sieved cores were stored in a refrigerator for no more than 72 hours before being used to inoculate pots in the greenhouse. We used a whole soil inoculation, where inoculum was added to pots at a ratio of 1:30 by volume of live soil to sterilized bulk soil from each reserve. To create the bulk soil and to account for nutrient-based differences between reserves, we first collected background field soils from each reserve near the origin point. The background field soil was sieved through a 2mm sieve, and mixed 1:1 with UC sand mix #3 (contents of soil mix in Table S1), then steam sterilized to 80˚C for 1 hour, rested for one hour, then sterilized again for 1 hour. This sterile soil was used as the background bulk soil to fill the pots for the greenhouse experiment.

Greenhouse experiment:

To assess PSF within the *Oncosiphon* invasion gradient, we grew six native plants and *Oncosiphon* in a greenhouse experiment. The native plants were *Amsinckia intermedia, Nemophila menziesii, Eschscholzia californica, Lasthenia californica,* and *Layia platyglossa.* These species represent a breadth of common and rare native plants with different resource acquisition strategies. All species occur at the three different study sites but at differing frequency and abundance, and seeds were purchased from S&S Seeds (Carpinteria, CA). Each species was grown in a 300ml pot with soil inoculated from one of the 12 soil types (3 sites X 4 soil histories). We collected three soil cores for each soil type to maintain the statistical independence of samples (Reinhart & Rinella 2016) for a total of 36 experimental cores. We pooled and sterilized the remaining soil from each experimental group to act as a control, making a total of 48 inoculants (36 experimental cores + 12 sterilized controls for each soil type = 48). To account for greenhouse level variation, we grew three replicates per inoculant and averaged their values before analysis, resulting in a total greenhouse size of 1008 individuals (48 inoculants X 7 species X 3 greenhouse replicates). Utilizing a blocked approach would have resulted in three large blocks where a single block would have covered a significant area of a greenhouse, resulting in variable greenhouse conditions for a single block. Therefore, to minimize potential greenhouse effects on growth, we implemented a completely random design and further randomized tray locations within the greenhouse weekly during the experiment. Plants were grown over 10 weeks in a University of California Riverside greenhouse and watered with spray emitters for ten minutes three times a week for the first seven weeks, then additional watering *ad libitum* for the remaining three weeks ranging from an additional 5 minutes at 2pm on non-watering days, to 5 minutes at 2pm every day depending on plant wilting due to increasing temperatures.

After 10 weeks we harvested plants to assess overall plant growth, plant functional traits, and percent root mycorrhizal colonization. To estimate plant growth, we measured shoot and root biomass for each individual plant. We calculated response ratios for only shoot biomass as $\frac{dry\ mass\ live - dry\ mass\ sterile}{dry\ mass\ sterile}$ and use shoot response ratios as the main response to *Oncosiphon* PSF as a way to standardize across species level differences in growth. Shoot masses were tightly correlated with full plant mass (Pearson's correlation p>0.0001). Plants were first cut at the base of the shoot and had one leaf removed and scanned then later assessed for area using imageJ. Shoot dry mass and leaf dry mass were taken after shoot and leaf samples were placed in a 60˚C drying oven for 48 hours. Plant roots were kept in a refrigerator between 0 and 48 hours after shoots were removed. Plants roots were de-potted and roots were washed and cleaned free of all soil, then placed in a wet paper towel and scanned for root length with ‘WinRhizo™’ scanner within 24 hours. After root scans were measured, root samples were air dried, and the full air-dry mass was measured. We then subsampled a portion of the air-dried roots to stain for mycorrhizal analysis, and then re-weighed the air-dried roots to acquire the percentage of root mass remaining. The remaining roots were placed in a 60˚C drying oven for 48 hours. Total root mass was calculated as percentage air dry * oven dry weight. Specific leaf area (SLA) was calculated as leaf area (cm^2) from imageJ divided by leaf dry mass (g), and SRL was calculated as total root length (m)/root dry mass (g). The root to shoot ratio was calculated as root dry mass (g)/shoot dry mass (g). Therefore, we estimated 2 functional traits (SRL, SLA). We selected these traits as they are indicative of resource acquisition strategy (Poorter & Bongers 2006), as well as fundamental tradeoffs in plant ecology (Wright et al. 2004, Mommer & Weemstra 2012). Root mycorrhizal colonization was estimated after roots were cleared with 2.5% KOH and stained with 0.5% Trypan Blue. We utilized the point intersect method (Brundrett et al. 1996) using ten 1cm roots per plant with 10 fields of view per root at 400X magnification.

Analysis:

To evaluate changes in belowground communities, we focused on the root mycorrhizal colonization rate as a proxy to infer changes in AMF communities and a disruption in mutualisms. As our inoculations made up a very small component of the total soil (1:30 ratio) nutrient levels are consistent across *Oncosiphon* soil conditioning gradient, and therefore decreases in plant performance without changes in root mycorrhizal colonization imply an impact of belowground enemies. Our response ratio calculations are also designed to infer strength and direction of PSF from biological drivers (Brinkman 2010), where belowground enemies including pathogens and root herbivores are the only possible biological agents that can form a negative PSF. To evaluate *Oncosiphon's* response to its own PSF, we ran linear mixed effects models with either shoot response ratio or percent root mycorrhizal colonization as the response variables, with only soil history as a fixed effect and reserve as a random effect. We performed two sets of post-hoc tests for the response ratios, and one for the root mycorrhizal colonization. The first set of post-hoc tests was a Tukey Honestly Significant Difference Test (Tukey HSD) to infer differences between treatment groups, which was performed on both AMF root colonization models and shoot response ratio models. The second set of post-hoc tests was a one-way t-test to infer that treatment groups are statistically different from 0, indicating biologically different from the sterile conditions, which was only performed on the shoot response ratio models. To quantify native response to *Oncosiphon* PSF, we performed linear mixed effects models on either shoot response ratios or root mycorrhizal colonization with the fixed effects of soil history, species, and their interaction, with reserve as the random effect. We performed the same two sets of Tukey HSD and t-test post-hoc tests as the conspecific PSF model. Due to the distribution of root mycorrhizal colonization data for native forbs being heavily skewed from low colonization in certain species, the colonization rates were natural log(x+1) transformed to normalize residual distribution.

To address how functional traits predict native responses to invader PSF, we compared native plant trait values in uninvaded soils to shoot response ratios from all invasion levels to focus on the response to invader driven PSF without associated changes in traits due to PSF. The trait-based models focused on shoot response ratios as the response, with the fixed effects of SLA or SRL from uninvaded soils (two models), and soil history (light, heavy, origin) as well as all interactions, with reserve as a random effect. Post-hoc tests only include Tukey HSD. To eliminate outliers, we filtered the data to exclude the lowest 2.5% and greatest 2.5% of response variables before averaging greenhouse replicates. We only removed the individual datapoint and retained other response parameters for a given outlier. All analyses were run utilizing the 'lme4' package (Bates et al. 2015) in R (v.4.1.1) Post hoc tests were performed using the 'emmeans' package (Russel 2022) for Tukey HSD and the t.test function in base R for the secondary post-hoc tests.

Results:

Summary statistical tables for *Oncosiphon* models are in supplementary table S2, while native PSF responses are in supplementary table S3, and trait-based predictions of native responses to invader PSF are in supplementary table S4.

Oncosiphon PSF response:

Overall, *Oncosiphon* shoot response ratios decreased with more intense invasion levels (soil conditioning p=0.0005). *Oncosiphon* shoot response ratios were similarly positive in uninvaded and light invaded soils (Tukey HSD p=1.000). *Oncosiphon* shoot responses in heavily invaded soils and origin point soils were lower than uninvaded (Tukey HSD: heavy p=0.009, origin p=0.005) and light invaded soils (Tukey HSD: heavy p=0.013, origin p=0.009; Figure 1A). While we observed that shoot responses differed among soil histories, *Oncosiphon* did not grow different from sterile conditions except for the lightly conditioned soil (t-test p=0.008), indicating *Oncosiphon* grew better in live lightly conditioned soil than sterile soil. The shoot response was not different from sterile, positively or negatively, in any other soil history (t-test uninvaded p=0.052, heavy p=0.218, origin p=0.077; Figure 1A), indicating that *Oncosiphon* did not grow significantly better or worse than sterile soils than in live soil for uninvaded, heavily invaded, and origin soils.

Oncosiphon root mycorrhizal colonization differed significantly across soil conditioning intensities (p=0.033). The uninvaded and light soils had similar colonization rates (Tukey HSD p=0.573). Root mycorrhizal colonization was higher in lightly invaded soils compared to heavy conditioned soil (Tukey HSD p=0.045) but not origin soils (Tukey HSD p=0.066). There were no differences between uninvaded root mycorrhizal colonization rates and heavy and origin conditioned soils (Tukey HSD heavy p=0.466, Origin p=0.573) (Figure 1B). The range of root mycorrhizal colonization in *Oncosiphon* was very low overall with the highest overall percentage infected being 10% in one greenhouse replicate of lightly invaded soils at Lake Matthews.

Native forb response to Oncosiphon PSF:

Native shoot response ratios were significantly lower with greater *Oncosiphon* soil conditioning (soil conditioning p=0.005) and differed among species (species p=0.0004). On average for all six native forbs, plants had the highest shoot response ratios in uninvaded soils which were also the only soil significantly different from zero (t-test, p=0.0004), indicating plants grew significantly better in the live uninvaded soil than sterile soils. Compared to the uninvaded soil, the average native forb response was 25% lower in lightly invaded soils but was statistically similar to uninvaded soils (Tukey HSD p=0.168) and not statistically different from sterile soil (t-test p=0.381) (Figure 2A), indicating native forbs did not grow significantly larger in live lightly invaded soil than sterile soil. The heavily invaded and origin point soils had 102% and 110% respective reductions in shoot response ratios and were statistically lower than shoot responses in uninvaded soils (Tukey HSD heavy p=0.011, origin p=0.011) but were not different from zero (t-test p=0.923, p=0.661), indicating any benefits from live soil was eliminated for native plants growing in heavily invaded and origin point soils.

The shoot responses were not consistent across species. *Layia platyglossa* is the only species with an average negative response ratio that was significantly different from zero (t-test p=0.004) indicating *Layia* grew worse in live soil than sterile soil independent of soil history (Figure 2B). Both *Lupinus bicolor* and *Nemophila menziesii* had overall positive responses significantly different from zero (t-test p=0.005, p=0.047 respectively), indicating these species grew better in living soil than sterile soil independent of soil conditioning (Figure 2B). On average across all soil histories, *Amsinckia intermedia, Eschscholzia californica,* and *Lasthenia californica* did not have significantly different responses from sterile soils (t-test p=0.630, p=0.370, p=0.151 respectively) indicating there was not a significant difference between living and sterile soils independent of soil history, or from other species (Figure 2B). On average across all soil conditioning, *Layia* had a 219% lower response than *Lupinus* and a 172% lower shoot response than *Nemophila* which were statistically significant (Tukey HSD p=0.0001, p=0.005 respectively). The interaction between species and soil history was not significant for shoot response ratios (soil history X species p=0.251).

Root mycorrhizal colonization was significantly different between soil histories (p<0.0001) and species (p<0.0001). The average root mycorrhizal colonization was statistically similar in lightly invaded soils compared to uninvaded soils (Tukey HSD p=0.996; Figure 2C). Root mycorrhizal colonization was 70.3% lower in heavily invaded and 71.5% lower for origin point conditioned soils when compared to uninvaded soil (Tukey HSD p<0.0001, p<0.0001). The root mycorrhizal colonization was not statistically different between heavy and origin soils (Tukey HSD p=0.905; Figure 2C). The species level differences in root mycorrhizal colonization can roughly be grouped into high levels with *Amsinckia* and *Nemophila*, intermediate levels in *Lupinus*, *Layia* and *Lasthenia*, and *Eschscholzia* consistently had the lowest root mycorrhizal colonization rates. Both *Amsinckia* and *Nemophila* had the highest root mycorrhizal colonization rates at 20.5±2.8% and 15.5±2.4% respectively and were similar to each other (Tukey HSD p=0.534) independent of soil conditioning. *Lupinus* and *Layia* had similar intermediate levels of root mycorrhizal colonization at 10.4±1.9% and 10.1±1.6% (Tukey HSD p=1.00), which were similar to *Nemophila* (Tukey HSD p=0.170, p=0.197 respectively) but significantly lower than *Amsinckia* (Tukey HSD p=0.001, p=0.001 respectively). *Lasthenia* had lower root mycorrhizal colonization rates compared to most other species, at 7.4±1.5%, which was statistically similar to intermediate levels in *Lupinus* (Tukey HSD p=0.755) and *Layia* (Tukey HSD p=0.713) but were lower than *Nemophila* (Tukey HSD 0.003) and *Amsinckia* (Tukey HSD p<0.0001). *Eschscholzia* consistently had the lowest root mycorrhizal colonization at 2.3±0.3% and was lower than every other species (Tukey HSD: *Amsinckia* p<0.0001, *Nemophila* p<0.0001, *Lupinus* p<0.0001, *Layia* p<0.0001, *Lasthenia* p=0.007; Figure 2D). The interaction between species and soil history was not significant for root mycorrhizal colonization (p=0.730).

Trait predictions of native response to Oncosiphon PSF:

We found that SLA was negatively predictive of native PSF response to *Oncosiphon* soil conditioning (SLA p=0.007; Figure 3A), but SRL was not predictive of native PSF responses (SRL p=0.448; Figure 3B). Plants with higher SLA values in uninvaded conditioned soil had lower shoot response ratios, independent of invasion stage (soil history p=0.778; interaction SLA X soil history p=0.861). Soil history or the interaction between SRL and soil conditioning were not predictive of native response to Oncosiphon soil conditioning (soil conditioning p=0.700, soil conditioning X SRL p=0.930).

Discussion:

We investigated how invader driven PSF dynamics over an invasion gradient can alter the growth of native plants, the invader, and how native traits predict native responses to invader driven PSF. Our study demonstrates that *Oncosiphon* develops a PSF that drives a dynamic negative response in native species and *Oncosiphon* alike, as well as the importance of functional traits in predicting PSF responses. We found evidence for three key PSF processes occurring within the *Oncosiphon* invasion. 1) *Oncosiphon* itself has reduced growth with greater soil conditioning, but mostly at later stages of invasion. Reductions in *Oncosiphon* growth are not associated with a decline in AMF, therefore implying pathogens or belowground enemies as the mechanism. 2) On average, native species growth benefits from live soil declined rapidly and were lost even in early stages of invasion (i.e., "light"); however, AMF colonization rates were resistant to decline until late-stage invasion (i.e., heavy) suggesting a threshold dynamic of fungal resistance. Importantly, native forb responses varied widely and independent of soil conditioning, where certain species (*Layia*) had a growth cost associated with live soil, while others (*Nemophila, Lupinus*) had a growth benefit from live soil. 3) Our results support a growth-defense tradeoff mediating PSF responses, where high growth functional strategies (i.e., high SLA) were more susceptible to the impacts of invader driven PSF. Together, our results highlight how the development of invader PSF can reduce native forb performance and invader performance dynamically through soil conditioning gradients.

Invader responses to and impacts on soil biota strongly mediate their invasion success. Symbiotic mutualisms may provide a barrier to invasion success if a species requires obligate mutualisms; however non-mycorrhizal or facultative mutualist invaders may not be strongly impacted by the availability of symbionts (Pringle et al 2009). Here the low AMF colonization rates within *Oncosiphon* roots suggest that AMF likely do not directly contribute to *Oncosiphon* performance. This response has been found in other invasive plants, including *Plantago virginica* (Luo et al. 2021), *Salsola tragus* (Hovland et al. 2019), and many species in the *Brassica* family (see Grove et al. 2017 for additional studies). The decreased growth *Oncosiphon* experienced over the course of invasion soil conditioning, given the minimal changes in mycorrhizal association, indicates that the decreased growth was mediated by increased pathogen accumulation. This is consistent with previous work on invasions developing negative PSF (Diez et al. 2010). Our design utilizes microscopy to identify if AMF are associating with plants and isolates biological drivers to infer pathogens in negative shoot responses; however, utilizing a sequencing approach along an invasion gradient to identify specific subsets of AMF species as well as directly quantifying changes in pathogen abundance would improve our understanding of these dynamic associations between invaders and soil biota. Importantly, despite the reductions in *Oncosiphon* performance, the pathogen driven decreases are likely not sufficient to negatively impact the spread and dominance of this invader. *Oncosiphon* produces a large number of seeds per individual that can saturate the soil seedbank (Hedrick & McDonald 2020), and it invades areas where the native seedbank has already been severely depleted (Cox & Allen 2008, Schwab et al. 2023). Moreover, waiting for pathogen accumulation to decrease invader performance or control an invader may allow for other invader mediated legacies to develop that constrain the recovery of a system (Policelli et al. 2018); therefore, management actions should be taken to constrain invader growth as early during an invasion as possible.

The degree of mycorrhizal dependency and sensitivity to pathogens both mediate how strongly native plants are impacted by invader driven changes to soil biota. In our study, the uninvaded soils provided a growth benefit to native plants, but native plants grew progressively worse in soils that were more invaded. By measuring AMF colonization rates, we were able to detect that AMF colonization was initially resistant to soil conditioning, and it was not until heavy and origin invaded soil conditioning that colonization rates dropped, indicating a threshold dynamic in fungal tolerance to invader mediated impacts. These results support recent findings that invaders may first disrupt native performance by suppressing root nutrient acquisition vs disrupting mycorrhizal associations (Chen et al. 2022). Importantly, not all native species were impacted to the same degree, likely due to differences in the native plant species relationships with AMF (i.e., obligate species more sensitive) (Bunn et al. 2015). In addition to driving reductions in AMF colonization, invaders can differentially reduce native plant performance via increasing the amount of soil pathogens present (Lucas 2020), resulting in reduced growth despite maintaining some degree of AMF relationships as we saw here. Pathogen accumulation can drive increases in pathogen presence well beyond pre-invasion population levels resulting in differentially reduced native plant growth compared to invaders (Kendig et al. 2021). The pathogen loading can contribute to long term impacts preventing native establishment (Polliceli et al. 2018) especially if symbionts like AMF are depleted (Middleton & Bever 2012). The temporal development of invader driven PSF might have differential timing on symbionts and pathogens driving a substantial range in native plant responses, where functional traits may be helpful in producing more predictive frameworks of native responses to invader PSF.

The functional traits of native plants in uninvaded soils suggest that there is a growth-defense tradeoff mediating pathogen impact. In our study, SLA for plants grown in uninvaded soil was negatively related to PSF response independent of invasion stage, indicating plants with fast growth strategies were more susceptible to invader mediated changes in soil biota. This finding is consistent with previous research investigating the relationship between SLA and sensitivity to pathogens in uninvaded communities (Xi et al. 2021). Contrary to our expectations SRL was not predictive of PSF response. This could have arisen due to our species selection. While our project investigated native plant species that co-occur with the invader, due to other degradation factors we may have been working with a restricted species pool, where species with more extreme trait values (i.e., very low SRL) and greater dependence on AMF may have already been extirpated locally due to other degrading factors like nitrogen deposition and other previous allelopathic invaders reducing AMF presence (Vogelsang and Bever 2009). Alternatively, we may have not found a relationship between SRL and plant responses to invader mediated impacts on soil biota, as the overall response was neutralized due to the combination of invader PSF both increasing pathogens and reducing symbionts. Species with high SRL may experience reduced performance from pathogens, while low SRL species are negatively impacted by symbiont loss, resulting in no linear relationship between SRL and native PSF response. The impacts of invasive plants on soil biota are multifaceted, and trait-based approaches to predict native responses to invader mediated impacts on soil biota might require both integration of economic spectrums and more comprehensive species palettes with broader trait values to capture trait-PSF relationships.

The legacies of invasion are complex and multifaceted, where invader driven losses in AMF can have impacts years after invader removal (e.g., Lankau and Lankau 2014) and pathogens may continue to impede native recovery (Flory & Clay 2013; Polliceli et al. 2018). The integration of temporal dynamics, and historical factors impacting soils need to be included to isolate the effects of invaders and elucidate when invaders are drivers or passengers of change in communities. Here, we found evidence of an invader driven PSF impacting native performance due to altered AMF and likely pathogen accumulation. The temporal development of these PSF mechanisms is nuanced as plants and AMF may be able to buffer some impacts early during an invasion. We found evidence of high-growth strategies having increased susceptibility to pathogen spillover even in early stages of invasion, evidenced by the negative relationship between SLA and PSF response to *Oncosiphon* soil conditioning. However, singular traits may not always be readily predictive of PSF responses (i.e., SRL) due to multiple invader mechanisms differentially impacting species on opposite ends of a trait gradient, (i.e., where disrupted mutualisms reduce low SRL species growth and pathogens reduce high SRL growth). Overall, the impact of invasive species on soil and plant communities is complex and multifaceted, with both short-term and long-term effects that depend on a variety of factors such as temporal dynamics, plant functional traits, and resistance in the soil community.

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Figures & Tables

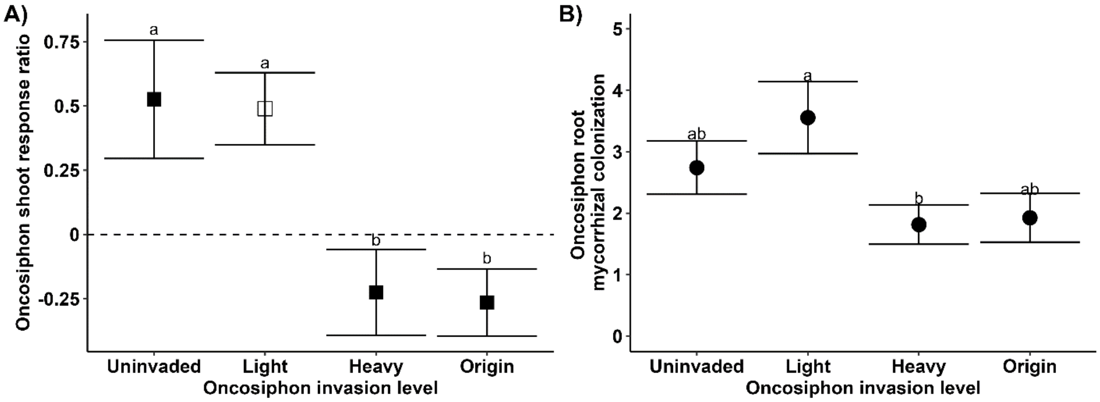

Figure 1) *Oncosiphon* response to *Oncosiphon* driven PSF. A) Average *Oncosiphon* shoot response ratio for each invasion level, with standard error bars. Fill indicates results of t-test, and letters display results of Tukey-HSD. B) Average *Oncosiphon* percent root mycorrhizal colonization for each invasion level with standard error bars. Letters display results of Tukey-HSD.

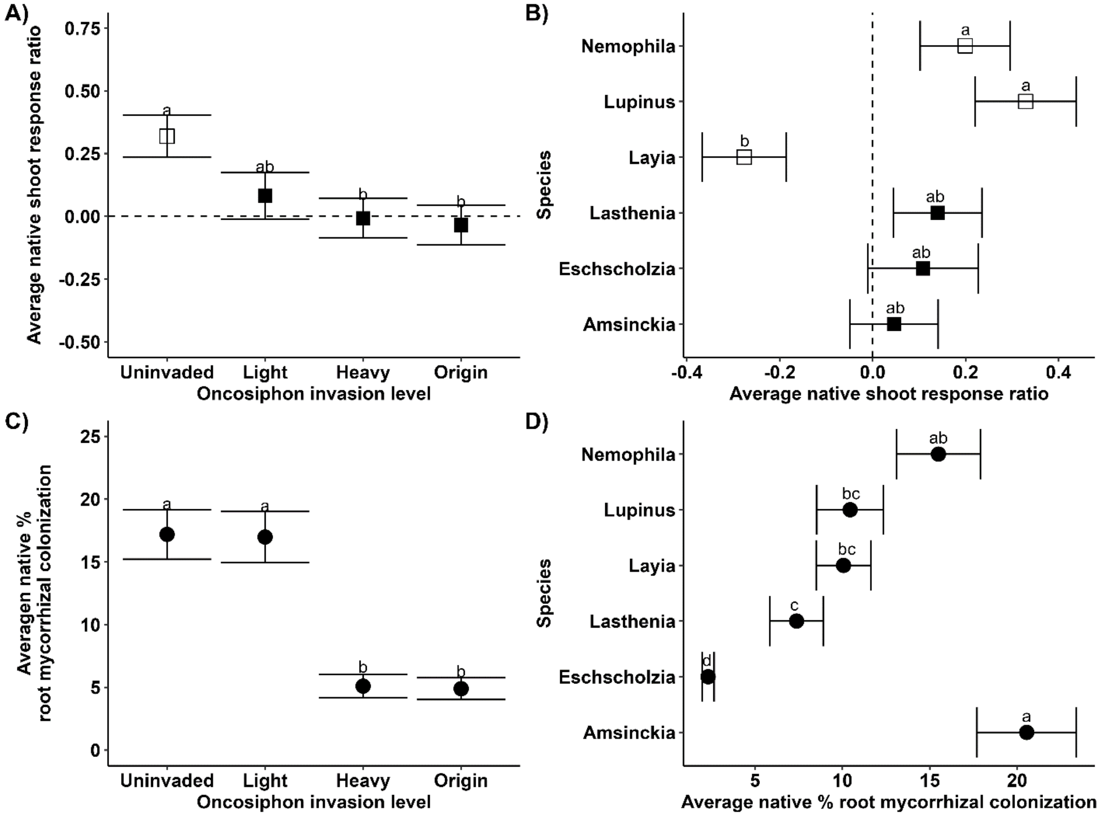

Figure 2) Native responses to *Oncosiphon* driven PSF. A) Average shoot response ratios for all native plants for each invasion level with standard error bars. Fill indicates results of t-test where unfilled indicates significantly different from sterile. Letters displayed are the results of Tukey HSD. B) Average shoot response ratio of every soil history for each species with standard error bars. Fill indicates t-test results, where unfilled indicates significantly different from sterile. Letters indicate results of Tukey HSD. C) Average percent mycorrhizal colonization of all native species for each invasion level, with standard error bars. Letters indicate results of Tukey HSD. D) Average native percent mycorrhizal colonization for all soil histories by species. Letters indicate results of Tukey HSD.

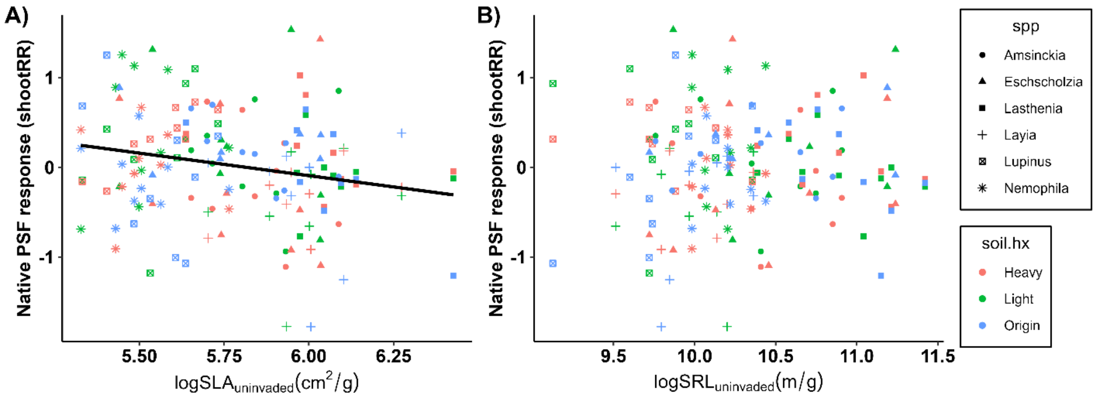

Figure 3) Trait based relationship with native responses to *Oncosiphon* PSF using uninvaded traits. Color indicates the invasion history, and shape represents species in both graphs. A) Relationship between Specific Leaf Area (SLA) and native response to *Oncosiphon* PSF. Black line indicates significant relationship of fixed effect specific leaf area. B) Specific root length relationship of native response to *Oncosiphon* PSF.

Supplemental Figures & Tables

Supplementary Table 1) Contents of UC Soil mix #3. Plaster sand and Peat moss are in percentage, and nutrients are in grams per cubic meter.

Soil Content	**Quantity**
Plaster Sand	0.57%
Peat Moss	0.43%
KNO3	86.70 g/m^3
Limestone Flour	520.19 g/m^3
Phosphate	433.50 g/m^3
Dolomite	1300.49g/m^3
Magnesium	24.28 g/m^3
Iron	45.08 g/m^3
Manganese	10.40 g/m^3
Zinc	17.34 g/m^3
Copper	38.15 g/m^3

Supplementary Table 2). Summary statistics of *Oncosiphon* responses to *Oncosiphon* PSF. The first number is the F statistic, the following numbers in subscript are Sattherwaite approximations of the degrees of freedom with numerator and denominator separated by a comma, and the final number is the p value.

	Shoot Response Ratio	**AMF colonization**
Soil conditioning	$8.059_{3,29.025}<0.0001$	$3.323_{3,30}0.033$

Supplementary Table 3). Summary statistics of native species responses to *Oncosiphon* PSF. The first number is the F statistic, the following numbers in subscript are Sattherwaite approximations of the degrees of freedom with numerator and denominator separated by a comma, and the final number is the p value.

	Shoot Response Ratio	**AMF colonization**
Soil conditioning	$4.378_{3,184}0.005$	$26.697_{3,192}<0.0001$
Species	$4.735_{5,184.01}<0.0001$	$19.082_{5,192}<0.0001$
Soil conditioning X Species	$1.231_{15,184.01}0.252$	$0.751_{15,192}0.730$

Supplementary Table 4) Summary statistics of relationships between Specific Leaf Area (SLA) and Specific Root Length (SRL) in uninvaded soil, and native plant shoot response ratios across invasion gradient (light, heavy, origin soils).

	Specific Leaf Area	**Specific Root Length**
Trait	$7.286_{1,146.54}0.008$	$0.580_{1,144.59}0.448$
Soil conditioning	$0.147_{2,146.02}0.864$	$0.357_{2,143}0.700$
Trait X Soil Conditioning	$0.131_{2,146.02}0.877$	$0.072_{2,143}0.930$

Chapter 3: Managing in the face of multiple constraints: the impacts of seed availability, litter accumulation, and symbiont depletion.

Abstract:

The impacts of plant invaders can drive ecological resilience preventing effective restoration which further entrenches their negative impacts on recovery and desired management outcomes. Managers often want increased native cover, and reduced invasive cover; however, balancing these two goals remains a challenge and may require different strategies. The addition of native seed is necessary to establish native plants in areas with depleted native seedbanks, but seeding efforts alone often fail to produce desired outcomes even with repeated long-term applications. The muted impact of seeding alone might be due to constraints of invasive species. Plant invaders can reinforce their dominance through positive feedback with litter and interrupting symbioses, like the arbuscular mycorrhizal fungi (AMF)-plant symbiosis that native species are dependent upon. While there are multifaceted impacts of plant invaders, enacting many management actions at the same time can result in costly and unnecessary treatments that hinder long term management success. We aimed to address how singular vs integrated strategies addressing the constraints of seed availability, litter accumulation, and symbiont depletion differentially achieve desired outcomes. To address this question, we performed a factorial field experiment at the Sierra Foothills Research Extension Center, and used a seed mix of 15 species, litter removal via clipping, and a commercial AMF inoculation. We collected community composition data over two years (2021, 2022), and focused on the management responses of total native cover, total seeded

species cover, total *Elymus caput-medusae* cover, diversity and richness as well as changes in rank and abundance to address overall community change. We found that of the singular constraints (seed, light, symbionts), seed availability and litter accumulation were the strongest singular constraints, and that inoculation benefits were contingent upon other treatments like adding seed. There was no singular management treatment for all goals, but the combination of seeding, clipping, and inoculating together had positive outcomes for all management goals and the greatest change in the rank and abundance of the community. Determining how ecological drivers constraining recovery interact with each other is critical to both enhancing our understanding of community dynamics and increasing the efficacy of management efforts.

Introduction:

Managing native plant diversity is an integral component of restoration and conservation, but it is increasingly complicated by the varied impacts of invasive plants (Suding et al. 2004). Plant invaders may limit native establishment by altering native seed availability (Gioria et al. 2019) or creating conditions that limit plant establishment such as increasing litter layers (Molinari & D’Antonio 2020) or depleting beneficial soil symbionts (Grove et al. 2017). These multiple mechanisms can individually or interactively result in constraints to native establishment resulting in variable outcomes in restoration (Brudvig et al. 2017). Addressing multiple constraints at once can increase management success (e.g., Mishra et al. 2021) but can easily result in costly unnecessary expenditures (Kimball et al 2015). Moreover, reducing invader cover, while also increasing native cover can create conflicts in achieving management goals (Skurski et al.

2013), if the same strategies that are successful at reducing invader cover have negative impacts on native performance (e.g., Sherrill et al. 2022). Achieving native plant diversity goals is contingent on not only identifying the main mechanisms that are either limiting native establishment or allowing invaders to persist (Kettenring & Adams 2011), but also understanding when constraints can be addressed individually or simultaneously without resulting in conflicting management outcomes (Suding et al 2004).

Long-term invasions can result in depleted native seedbanks that restrict the ability of native species to recover post-invader removal (Cox & Allen, 2008; Gioria et al., 2019). Native seed addition is an intuitive starting point for enhancing native recruitment (Nolan et al. 2021). Yet, seeding alone often has muted responses (James et al. 2013; Shackelford et al. 2021) or variable responses (e.g., Torok et al. 2021), even with repeated long term seed addition efforts (Copeland et al. 2019). Native species may fail to recruit for a variety of reasons, including stress from abiotic conditions (Shackelford et al. 2021), as well due to shifts in species interactions (Fenner & Thompson 2005, Mangla et al. 2011). These constraints may also be exacerbated by the plant invader, as invaders can reduce native establishment by increasing litter accumulation (Molinari & D’Antonio 2020; Mariotte et al. 2017) or can reduce native performance by negatively impacting plant-arbuscular mycorrhizal fungi (AMF) interactions (Grove et al. 2017). As a result, removing litter via grazing or clipping (Ruprecht et al. 2010) or adding beneficial symbionts like AMF via soil inoculations (Koziol et al. 2022) are commonly proposed management strategies to enhance native recruitment. However, implementing just one of these treatments may differentially favor other resident non-native species (Charles, Maron, Larios 2022) or fail to benefit focal native species (Perkins and Hatfield 2015). Therefore, to successfully restore native plants, it is crucial to identify when integrated management plans that account for the potential of invasive plant impacts and native dispersal limitations are needed.

Impacts of noxious plant invaders are often focused on community metrics such as species richness and diversity (Crystal & Lockwood 2020), but their dominance may result in larger changes in overall community structure. Plant community structure is classically described by a log-normal distribution with a few dominant species and many rare species (i.e., Rank Abundance Curves; MacArthur 1957; Whittaker 1965) but species invasions may change the overall shape of these curves, increasing its steepness as a single species becomes dominant or reduce its tail as species are lost in a system (Powell, Knight, Chase 2013). Therefore, when assessing management strategies, it is helpful to understand how treatments are also changing the relative abundance of species within a community. Seed addition treatments can increase richness (Foster & Tilman 2003) and thus the tail of a curve, but seed addition may not change the overall shape of the curve if all seeded species occur at low abundances. This could be used as an early indicator that management actions may result in more transitory dynamics as the overall structure of the community still mimics that altered invaded system. Similarly, as litter removal interrupts the positive feedback reinforcing dominance of invasive grasses (Mariotte et al. 2017), litter removal strategies may result in curves that are less steep, indicating that abundances within the community are becoming more evenly dispersed across species rather than being dominated by one species. However, litter removal alone may result in recruitment of other nonnative species (Charles et al. 2022), and if dominant grasses can re-invade, then the impacts of litter removal are likely to be short term from re-invasion (Pearson et al. 2016). The addition of soil symbionts in inoculations can enhance native survivorship and accelerate succession (Koziol & Bever 2019), which should be reflected in increased numbers of unique species present, as well as in alterations to the ranking of subdominant species if they receive growth benefits. The benefits of inoculation are most likely to be seen in how species ranks change with treatments and may enhance the number of species that can be supported (i.e., lengthening the curve). If inoculations produce robust populations of symbionts, then these impacts should be long lasting. Determining the community dynamics, and mechanisms of invader dominance are key to elucidating the most cost effective and most ecologically effective treatment plans. Integrated management strategies can have impacts beyond single univariate metrics of diversity which may influence the trajectory of a restoration site (Catano et al. 2022).

Rangeland systems make up nearly one third of the total land area of the United States and are managed for multiple ecosystem services including native biodiversity (Havstad et al. 2015). These critical ecosystems coincidentally are typically heavily invaded, with plant invaders that negatively impact ecosystem services (Tomaso, Masters & Peterson 2010), making them an excellent model system to investigate ecological dynamics of multiple constraints in response to management treatments. Certain non-native species were intentionally introduced as they are desirable for livestock (e.g., *Avena*, certain *Bromes*) (Larson-Praplan 2014); however, other introduced non-native species have strong negative impacts reducing ecosystem services and biodiversity, making those species of high priority to manage (Heady 2019). Notably, medusahead (*Elymus caput-medusae)* is one such invader, reducing forage quality, increasing fuel loads for fires, and reducing biodiversity (Nafus & Davies 2014), making its removal a priority for management efforts (Kyser et al. 2014). California rangelands are emblematic of these dynamics as they host a suite of invaders including *E. caput-medusae* but are focal areas of conservation due to their high biodiversity (Plieninger et al. 2012). Californian rangelands are constrained by litter accumulation dynamics preventing native establishment (Mariotte et al. 2017), as well as depleted native seedbanks (Cox & Allen 2008; Clark et al. 2020) and altered symbiont availability (Vogelsang & Bever 2009). Rangeland management could be greatly improved by providing more insights into when these constraints need to be tackled individually or in an integrated manner.

To better identify how seed limitation, litter accumulation, and the depletion of soil symbionts constrain restoration outcomes, we tested a series of hypotheses on the impacts of these treatments and potential interactions in a rangeland system. Using rangelands as a model system, we asked how do single treatment vs integrated management strategies addressing constraints of native seed availability, litter accumulation, and depletion of soil symbionts differentially achieve desired management outcomes and alter community dynamics? Seeding native species, and removing litter should independently increase total native cover, diversity, and richness, but clipping and seeding together should produce the highest total native cover and greatest increases in diversity and richness. Total native cover is not as likely to increase with commercial inoculations, but there may be increases in diversity and richness from other non-native species and because commercial inoculations can reduce dominant invader performance. If *E. caput-medusae* dominance is maintained through litter feedbacks and symbiont availability is also exacerbating existing competitive differences, then inoculations and litter removal should have the greatest impact on reducing *E. caput-medusae* cover especially when done simultaneously. Seeding native species will not have a detectable impact on *E. caput-medusae* cover individually, but seeding will enhance the impact of clipping and inoculating on *E. caput-medusae* cover. In terms of community dynamics in rank and abundance, seeding alone should have greater impacts on richness differences than clipping or inoculating, but seeding alone will not alter evenness rank or rank abundance curve differences relative to control, as seeded species will occur at low abundances. Evenness should be enhanced most by inoculations and clipping as they should disrupt the feedbacks of dominance, and subsequently rank differences relative to control should be greatest in inoculation and clipping treatments. For interactive treatments, we expect that if seeded or other resident species are constrained by more than seed availability, the interaction between seeding X clipping, and seeding X inoculating will have greater increases in richness, evenness, rank differences, and greater changes in rank abundance curves than seeding alone. The interaction between clipping and inoculating should also increase the richness and evenness in resident species and seeded species, as well as curve differences if many species are limited by light and symbiont availability.

Methods:

Study Area and Design:

Our study took place in annual grasslands at the Sierra Foothills Research Extension Center (SFREC; 39°15′ N, 121°17′ W) in Browns Valley California in 2021 and 2022. SFREC experiences a Mediterranean-type climate with hot dry summers and cool wet winters. Temperatures were consistent between years, with average annual high temperatures of 23.0℃ in 2021 and 23.1℃ in 2022 and the same average low temperature of 11.1℃ in 2021 and 2022. The annual rainfall varied between years of this study, with a total of 52.7 cm in 2021 and 23.0cm in 2022 (Browns Valley Weather Station). Several dominant annual invasive grasses (e.g., *Avena fatua, Elymus caput-medusae, Festuca perennis*) and invasive forbs (e.g., *Carduus pycnocephalus, Erodium botrys, Centaurea solstitialis*) are found at SFREC along with annual native forb genera (e.g., *Plagiobothrys, Navarretia, Madia*). This site is a working rangeland with cattle; however, our experiment was performed entirely in cattle exclosures.

We set up a factorial experiment to evaluate the effectiveness of single management treatments (seed addition, litter removal, commercial inoculation) vs integrated management strategies. This project was performed within existing experimental infrastructure, such that cattle exclosures had been set up since 2014 and litter removal was started over 2014 and 2015 (Charles et al. 2022). We selected 5 out of the eight experimental blocks within this infrastructure to carry out our experiment. Within each block, we set up 8 0.5 m x 0.5 m plots, which were assigned to one of 8 treatments that represented the factorial combination of seed addition (seed added or unseed), litter presence (litter intact, litter removed), and fungal inoculation (commercial inoculation or uninoculated), for a total of 40 plots.

Seeds for our seed addition were sourced from Hedgerow Farms (Winters, CA) and include 15 native species (Grasses: *Bromus carinatus, Poa secunda, Elymus glaucus, Stipa pulchra;* Forbs*: Phacelia cicutaria, Plantago erecta, Clarkia gracilis, Achillea millefolium, Eschscholzia californica, and Eriophylum lanatum, Ascelpia fascicularis, Madia elegans;* Nitrogen fixing forbs*: Lepidium nitidum, Lupinus succulentus, Trifolium wildenovii*). Species were seeded at a rate of 2 g/m^2 of pure live seed, making 30 g/m^2 of seed added per plot. Pure live seed was calculated by multiplying the reported purity and germination rates from Hedgerow Farms. Litter removal treatments were implemented by SFREC staff for six years prior to this experiment, beginning in 2014. Briefly, previous year's growth was clipped just above ground level and removed from the litter removal plots in October before the growing season began. Fungal inoculations were performed in December 2020 using MycoApply (trademark) and following manufacturer's directions. We mixed 8 g of inoculant per liter and added one liter of inoculant solution to inoculation plots, and one liter of water to controls at dawn. Litter removal was repeatedly conducted during the study in Fall 2020, 2021, 2022 to ensure that light limitation did not subsequently impact establishment, while seed additions and inoculations were done once as these were applied at a rate that would carry over from one year to the next.

Field measurements: To measure plant community responses, we made visual estimates of species composition where we recorded percent cover for each vascular plant species within a plot during peak biomass (April-May) and late season (May) for two years (2021, 2022) following the Jepson Flora species names and classifications (Jepson 2022). For each species, we used the maximum value observed across these two sampling points to calculate one matrix of species composition per year for a plot. All layers of canopy were included in visual estimates of percent cover, thus total plot values could exceed 100%. A cover value of 0.125% (0.5% per ¼ plot, * 4=0.125) was assigned to small individual species that only occurred once per plot

Auxiliary measurements: To quantify if the commercial inoculation changed overall mycorrhizal root colonization (i.e., positive control), we conducted a greenhouse bioassay experiment at the University of California Greenhouse between May-July 2021. We collected soil cores from every field plot in 2021 to use as whole soil inoculant (n=40) with a sterilized (via 90% ethanol spray) 10 cm diameter X 15 cm deep core. Three cores were taken per plot, (1 for nitrogen extractions, 1 for inoculations, 1 for sequencing) where soil cores were sterilized between plots but not for each individual core taken. Field cores were placed in a new whirlpak® bag, with a 90% ethanol sterilized glove used as needed to transfer the cores. Cores were then placed in an ice chest with ice and driven back to University of California Riverside laboratory refrigerator within 12 hours. They were then sieved through a sterilized (via 90% ethanol) 2mm sieve to remove rocks and large debris the next day and used as inoculant within 7 days. A subset of the soil was immediately stored after sieving in a -80℃ freezer for storage. We do not report on the sequencing data here, as too many samples did not successfully sequence making it such an incomplete dataset we could not compare between groups. We additionally collected bulk soil from SFREC and sieved through a 2mm soil sieve, and mixed sieved soil with plaster sand in a 1:1 ratio to use as our background soil that plants were grown in. This 1:1 bulk soil was steam sterilized for one hour, rested for one hour, then steam sterilized for another hour. Inoculations were performed as whole soil inocula, with a ratio of 1-part inoculant to 30-parts bulk soil by volume, and sterilized controls were steamed as described for bulk soil. We split up the soil inocula for a given field plot to inoculate 5 individual 300 ml pots, in which we grew *Stipa pulchra* as a native bioassay. We chose *Stipa* as our bioassay as it is an obligate generalist (Hausmann & Hawkes 2009), and was included in our seed mix for the seed addition treatment in the field. Each plot inoculant was paired with a sterilized control for a total of 400 replicates (40 plots X 5 replicates X 2 live or sterile = 400). We used a complete random design as this design would only have five very large blocks with different greenhouse conditions within each block. We randomized the location of each tray every week to minimize greenhouse effects. After 10 weeks, we de-potted plants and separated shoots, and subsampled the roots by removing approximately 50 ml (by volume) lateral roots. These root subsamples were then washed again with deionized water and left to air dry for one week before staining. Mycorrhizal analysis was performed on the root subsamples, where roots were cleared with 2.5% KOH and stained with 0.05% trypan blue. We then quantified mycorrhizal presence with ten fields of view on ten 1cm roots per sample at 400x magnification following published protocols

(Brundrett et al. 1996). Shoot dry mass was measured after shoots were dried in a 60℃ oven for 72 hours.

To quantify any differences in soil nitrogen availability, we conducted soil Nitrogen extractions on soils collected with a sterilized (via 90% ethanol between plot replicates) 10cm diameter X 15cm deep core. These nitrogen extraction cores were taken from every plot in 2021 on the last day of sampling of the late season composition, and at the same time as the greenhouse bioassay cores. We collected an additional round of cores at the end of the season in 2022. Soil nitrogen extractions were performed using the KCl method and following published protocols (Robertson 1999). The NH4 and NO3 analyses were performed at the University of California Riverside Environmental Science Research Lab with an AQ2 discrete analyzer.

Analysis:

Field collected data: To assess how the impacts of singular vs integrated treatments impact desired outcomes, we calculated effect size as the difference between treatment and control, resulting in seven treatment groups (Seeding, Clipping, Inoculating, Seeding X Clipping, Seeding X Inoculating, Clipping X Inoculating, Seeding X Clipping X Inoculating) for multiple response variables. For all calculations the control used was the unseeded, uninoculated plot, with litter intact for a given block. To assess the impact on native establishment and diversity, we calculated effect sizes for total cover of native species and total cover of seeded species. To estimate the impact on the dominant invader, we calculated an effect size for *Elymus caput-medusae (E. caput-medusae*) cover, and to assess impact on community diversity, we calculated an effect size for Diversity (Shannon Weiner), and Species Richness. For each of these response variables, we assessed differences in effect sizes among treatment groups and between years by performing linear mixed effects models with the fixed effects of treatment (7 groups), year, and their interaction, with block as the random term. To assess if treatments had different impacts relative to each other, we performed a Tukey Honestly Significant Difference post-hoc tests (Tukey HSD). To determine if treatments were significantly different from control, we performed a one-way t-test to infer that treatment groups are statistically different from 0, indicating ecologically different from controls. Alpha values were divided by the total number of comparisons for a Bonnferroni correction to account for inflated type I error (Dunn 1961). Diversity was ln(x+10) transformed to normalize residual distribution.

To quantify how these management treatments were impacting overall community structuring, we assessed shifts in species rank and abundances. To assess differences in rank and abundance, we used the RAC_difference() and curve_difference() functions in the ‘codyn’ package (Hallett et al. 2016; Avolio et al. 2019) to calculate differences in richness, evenness, species’ ranks, shared species, and differences in rank abundance curves (RAC) between our manipulated treatment plots and untreated controls. This approach allows us to compare how species abundance and identity change with treatments, while accounting for unique species between plots. To determine differences in species richness between treatment and control plots, species richness differences was calculated as the difference in the number of species in the treatment and control divided by the total number of unique species ($(S_{trt} – S_{ctrl})/S_{total})$), resulting in a value ranging from 1 to -1, where higher values indicate higher changes in species richness. To determine how evenly species abundance are distributed within a plot, we calculated species evenness and subtracted evenness values from our manipulated plot from species evenness of our control plot, resulting in a value between 1 and -1, where larger negative values indicate greater declines in species evenness. To determine differences in species rank (i.e., reordering within the community), we compared the rank of species between control and treated plots, resulting in a value ranging from 0 to 0.5, where 0.5 would indicate the highest rank change allowed in the community. We also calculated species differences between control and treatment communities by measuring species differences between plots while accounting for both shared and unique species present within the treatment and control plots being compared. Lastly, we calculated differences in the shape of RAC between control and manipulated treatments, allowing us to compare the degree of change in community composition between treatments (all calculations based on Avolio et al. 2019).

We then performed a linear mixed effects model with the RAC responses (richness difference, evenness difference, rank difference, species difference, curve difference) with the fixed effects of treatment and year with interaction between the two and block as a random term, for a total of 5 models.

Ancillary collected data: To assess *Stipa pulchra* growth differences, we performed a linear mixed effects model with the fixed effects of seeding treatment, litter treatment, and inoculation treatment, and if the cores were sterilized or not, and all interactions with field block as the random term. Both sterile and live replicates were included for shoot growth assessments, as we intended to infer if *Stipa* receives a growth benefit from live soil and if inoculations increase that benefit. To assess if the field commercial inoculations altered symbiont availability, we performed a linear mixed effects model on the subset of live replicates with the fixed effects of seed treatment, litter treatment, and inoculation treatment with all interactions and field block as the random term. Sterile treatments were dropped from the percent mycorrhizal colonization because they did not have, and were intended to not have, any root AMF colonization.

To assess if treatments changed nitrogen availability, we performed linear mixed effects models on total inorganic Nitrogen, NH4, and NO3 with the fixed effects of seed treatment, litter treatment, inoculation treatment, year and all interactions with block as the random term.

Results:

We found a total of 39 native species and 34 nonnative species over both years of this project (Table S1). Observed treatment means for the five focal variables of total native cover, seeded cover, *Elymus caput medusae* cover, diversity and richness across the field treatments are in supplemental figure 1.

I) Achieving management goals

Increasing Native Cover:

Total native cover effect sizes ranged from -48.00 (in the inoculation alone treatment) to +75.25 (in the seeding X inoculating treatment), with an average effect size of +15.11. There were no detectable differences between treatments (Treatment p=0.0673) or year (Year p=0.0510, Year X Treatment p=0.983; Table S2).

Despite the similarities between treatments in the linear model, not all treatments were effective at increasing total native cover (Figure 1A). Seeding alone (t-test p=0.3612), clipping alone (t-test p=0.282), inoculating alone (t-test p=0.5232), and the combination of seeding and inoculating (t-test p=0.1122) effect sizes were not significantly different from 0, indicating these treatments did not change the total native cover compared to control. The combination of seeding and clipping increased total native cover 31.8+/6.1% (t-test p=0.0006), while clipping and inoculating increased native cover 19.1+/-6.9% (t-test p=0.0226), with effect sizes significantly different from 0, indicating these treatments had greater native cover compared to control. The combination of seeding, clipping, and inoculating increased native cover 24.4+/-9.5% (t-test p=0.031).

The sum of seeded species cover effect sizes ranged from -36.75 (in the inoculation only treatment) to 98.25 (in the seed X inoculating treatment) with a mean effect size of +10.53. Not all treatments were equally effective in increasing the seeded species cover (Treatment p=0.0015; Table S2), with substantial differences in effect sizes between years (Year p=0.004; Table S2). Seeding and clipping was more effective than clipping alone (Tukey HSD p=0.029), inoculating alone (Tukey HSD p=0.041) and clipping X inoculating (Tukey HSD p=0.033) (Figure 1B). There were more positive effect sizes for seeded species cover in 2022 than 2021 (Tukey HSD p=0.004). The interaction between treatment and year was not significant (Treatment X year p=0.471).

Seeding alone increased seeded species cover by 12.1+/-5.4% (t-test p=0.049), while seeding with clipping increased seeded species cover by 24.5+/-5.8% (t-test p=0.002). The combination of seeding and inoculating increased seeded cover by 23.4+/-10.4% (t-test p=0.0509), while seeding, clipping, and inoculating together increased seeded cover by 17.8+/-6.6% (p=0.025). These treatment effect sizes were all significantly different from 0, indicating these treatments significantly increased seeded species cover (Figure 1B). Clipping alone (t-test p=0.642), inoculating alone (t-test p=0.954) and clipping with inoculating (t-test p=0.727) did not result in effect sizes that were different from 0, indicating there was not a benefit to seeded species cover from these treatments. (Figure 1B).

Reducing E. caput-medusae % cover:

The effect sizes for *E. caput-medusae %* cover ranged from +52.5 (inoculation treatment) to -71.75 (seeding X clipping treatment), with a mean effect size of -5.85. The mean effect size for all treatments were negative and did not differ from one another (Treatment p=0.067; Table S2). Reductions in *E. caput-medusae* cover were different between years (year p=0.005; Table S2), with more negative effect size values for *E. caput-medusae* cover in 2022 than 2021 (Tukey HSD p=0.005). The interaction between year and treatment was insignificant (Treatment X year p=0.467; Table S2).

Despite the similarities between treatments in the linear model, not all treatments were effective at reducing *E. caput-medusae* (Figure 1C). Seeding alone (t-test p=0.336), clipping alone (t-test p=0.155), and inoculating alone (t-test p=0.865) treatments were not statistically different from 0, indicating they did not reduce *E. caput-medusae* cover more than control (Figure 1C). The combinations of seeding and clipping (t-test p=0.513) and clipping and inoculating (t-test p=0.392) treatments also did not statistically reduce *E.*

caput-medusae cover more than control. The combination of seeding, clipping, and inoculating caused an 11.1+/-5.2% reduction and trended toward reducing *E. caput-medusae* cover (t-test p=0.062) (Figure 1C). *E. caput-medusae* cover was reduced 14.0+/-5.8% in the seeding with inoculating treatment (t-test p=0.0376), indicating this treatment reduced *E. caput-medusae* cover more than control (Figure 1C).

Increasing Diversity and Richness:

Diversity effect sizes ranged from -1.20 (seeding alone treatment) to +1.23 (seeding X clipping) with an average effect size of +0.38. Our treatments did not have detectable differences in their impacts on diversity (Treatment p=0.086; Table S2), and diversity was consistent between years (Year p=0.072, Treatment X year p=0.553; Table S2).

Despite the similarities between treatments in our linear model, not all treatments had similar impacts on diversity (Figure 1D). Seeding alone (t-test p=0.243) and inoculating alone (t-test p=0.1374) did not have effect sizes significantly different from 0, indicating these treatments did not change diversity more than in controls, while seeding and inoculating increased diversity 0.447+/-0.134 (t-test p=0.0009). All treatments that included clipping significantly increased diversity relative to the control with clipping alone increasing diversity by 0.346+/-0.126 (t-test p=0.022), clipping and inoculating by 0.301+/-0.120 (t-test p=0.033), seeding and clipping by 0.574 +/- 0.119 (t-test p=0.001) and seeding, clipping, and inoculating increased diversity by 0.603+/-0.124 (t-test p=0.0009) (Figure 1D).

Richness responses ranged from decreasing species by 7 (clipping only) to increasing the number of species by 20 (seed X clip X inoculation treatment). Not all treatments had similar impacts on richness (Treatment p=0.001; Table S2). Seeding and clipping was more effective at increasing richness than clipping alone (Tukey HSD p=0.0419), or inoculating alone (Tukey HSD p=0.002; Figure 1E). The combination of seeding, clipping, and inoculating increased species richness more than inoculating alone (t-test p=0.0041). Richness effect sizes were different between years (Year p=0.006), with more positive effect sizes in 2021 (Tukey HSD p=0.006). The interaction between year and treatment was not significant (Treatment X Year p=0.663; Table S2).

All treatments that included seeding significantly increased richness compared to the controls (i.e., effects sizes greater than 0) with seeding increasing richness by 5.8 +/- 1.8 (t-test p=0.03127), seeding and clipping by 9.7 +/- 1.6 (t-test p=0.0002), seeding and inoculating by 7.1+/-1.6 (t-test p=0.0014) and seeding, clipping and inoculating increased richness by 9.1+/-2.0 (t-test p=0.0012). The clipping alone treatment (t-test p=0.1492) and inoculating alone (t-test p=0.7286) did not have effect sizes significantly greater than 0, indicating these treatments did not increase diversity compared to control, but combined they increased richness by 5.5 +/- 1.5 (t-test p=0.005).

II) Assessing Management Impacts on Whole Community

When evaluating overall impacts on community structure, we found that differences in richness compared to control varied significantly by treatments (p=0.0185, Figure S2A; Table S3); specifically, treatment combinations of seeding, clipping, and inoculating, as well as seeding and clipping, had more positive species richness difference values compared to the control than the inoculation alone treatment (Tukey HSD p=0.041, p=0.021; Figure S2A). This indicates that there were significantly more unique species present when seeding, clipping and inoculating a plot (0.348+/- 0.080) and when seeding and clipping (0.371+/- 0.067) than with inoculations alone (0.053+/- 0.069) compared to control (unseed, litter intact, uninoculated). Differences in evenness, rank, and species did not differ among treatments (evenness diff p=0.159, rank diff p=0.107, species diff p=0.634; Figure S2B-D; Table S3). Additionally, there were significant differences between years for the richness (Year p=0.0002), rank (p=0.041) and species differences (Year p=0.002; Table S3), with greater richness change, more rank change, and more new unique species in 2022 compared to 2021 (Tukey HSD Year: richness diff p=0.0002; rank diff p=0.0413; species diff p=0.0024). The interaction between treatment and year was not significant for richness (p=0.847), evenness (p=0.937), rank (p=0.166) and species differences (p=0.967; Table S3).

We detected that some treatments had stronger changes in community composition, evidenced by the degree of change in rank abundance curves (RAC) (treatment p=0.032; Table S3). Plots with seeding, clipping, and inoculating had greater changes in RAC (56.47339+/- 6.910609) compared to the control plots than those with seeding alone (27.08157+/- 6.908560; Tukey HSD p=0.029; Figure 2; Figure S2E; Table S3). All other treatments had similar impacts on RAC differences (Figure 2). Lastly, RAC differences did not differ between years (year p=0.716, year X treatment p=0.204; Table S3).

III) Bioassay and Soil Nitrogen Availability

For the greenhouse bioassay, *Stipa* pulchra shoot mass was significantly influenced by soil sterilization (sterilization treatment p<0.0001), but not by seed treatment (p=0.41), litter treatment (p=0.07), or field inoculation treatment (p=0.273) or any interactions (Table S4). *Stipa* grew more in live soil than sterile (Tukey HSD p<0.0001).

The seed treatment and litter treatments did not have a detectable impact on *Stipa* AMF colonization (seed tx p=0.700, litter tx p=0.080), but the inoculation treatment did have a detectable impact on AMF colonization (Inoc tx p<0.0001). There was significantly greater mycorrhizal colonization in the cores from commercially inoculated plots, with an average of 26.6+/-4.14 % infection rates compared to 14.3+/-2.37% infection in the uninoculated plots (Tukey HSD p=0.0001). There was also a significant interaction between inoculant, litter, and seed treatments (p=0.038); however, no other interactions were significant (seed X litter p=0.477, seed X inoc p=0.821, litter X inoc p=0.926) Seeding, clipping, and inoculating had significantly greater AMF root colonization than uninoculated, clipped and seeded plots (Tukey HSD p=0.022), uninoculated, litter intact and seeded plots (Tukey HSD p=0.016) and uninoculated, litter intact unseeded plots (Tukey HSD p=0.0021). The unseeded, litter intact but inoculated plots had greater AMF colonization than uninoculated, litter intact, unseeded plots (Tukey HSD p=0.047; Figure S3b).

The amount of soil nitrogen varied between years and was significantly lower in 2021 (total inorganic N 0.306 +/- 0.18; NH4 2.08+/-0.14; NO3 0.025+/-0.003) than 2022 (total inorganic N 4.04 +/- 0.23; NH4 2.73 +/- 0.18; NO3 0.054 +/- 0.006) (total inorganic N year p=0.0003; NH4 year p=0.0007; NO3 year p<0.0001). Nitrogen metrics were not significantly different between seed treatments (total inorganic N p=0.876; NH4 p=0.795, NO3 p=0.803), litter treatments (total inorganic N p=0.880; NH4 p=0.293, NO3 p=0.940), or inoculation treatments (total inorganic N p=0.850; NH4ppm p=0., NO3ppm p=0.190). The interaction between seeding and litter was significant for NH4 (p=0.0303); however, in pairwise comparisons (Tukey HSD), there were not any detectable differences. No interaction terms were significant for any of the nitrogen models.

Discussion:

Meeting native biodiversity management goals is complicated by the various impacts that invasive species have on ecological communities. We investigated how treatments addressing the constraints of seed limitation, light limitation, and symbiont depletion interact to differentially achieve management goals and alter community dynamics. Our study found dynamic responses to management efforts, with mixed results from both singular and combined treatments on management goals and community impacts. Our seeding treatments aimed to overcome seed limitation, clipping treatments aimed to address the impacts of litter accumulation, and inoculation treatments aimed to address symbiont limitation. Importantly, we found that while some treatment combinations such as seeding and clipping had synergistic benefits, others such as clipping and inoculating had sub-additive benefits, indicating that different factors (i.e., seed, light, and symbiont availability) have varying impacts on management outcomes and community dynamics. Our analyses addressing how single vs combined treatments of seed addition, litter removal, and symbiont inoculations found that of the singular factors, treatments addressing light limitation and seed limitation had stronger impacts than inoculations, and are less dependent on mediating factors (i.e., inoculating was only beneficial with seeding). Secondly, we found that the combined strategies are more effective than singular strategies for most management goals by accounting for multiple constraints, but there was no single treatment to best achieve all goals. Our study exemplifies the complex nature of management making decisions in the face of multiple constraints and highlights the importance of mediating establishment conditions with seed addition efforts. Below we go into detail into the dynamics between our imposed treatments and ecological outcomes.

Identifying key recruitment limitations and whether they should be tackled individually or simultaneously is integral to the development of multipronged management plans for enhancing native diversity. Within our study system, native plant recruitment appears to be largely constrained by seed availability and light availability but how these constraints are addressed is important for achieving management goals. Our singular treatments (i.e., seeding alone, clipping alone, inoculating alone) were not as effective at increasing total native cover, seeded species cover, diversity and richness as seeding and clipping combined, highlighting the importance of treatments addressing both seed limitation and the impacts of litter. Litter accumulation has been shown to significantly reduce biodiversity to only those species that can tolerate light limitation (Dybzinski & Tilman 2007; Molinari & D'Antonio 2020). Seed limitation has also been regularly seen in other grassland systems (Seabloom et al 2003; Clark et al 2007), where adding seeds results in higher abundances. Importantly, if seeds are added without litter removal, the low light conditions created by a dense litter layer tend to differentially favor resident invasive grasses (Mariotte et al. 2017; Charles et al 2022) and reduce the survivorship and growth of seeded native species (Molinari & D'Antonio 2020), suggesting the need to tackle both constraints. Despite the history of invader mediated feedbacks on native plant species in California grassland (Vogelsang & Bever 2009, Grove et al. 2017), in our study, the impact of commercial inoculations generally did not increase native cover, seeded species cover, diversity or richness unless combined with seeding with generally sub additive effects. Whole soil inoculations can reduce re-invasion in prairie systems (Koziol et al. 2022) and may work better than commercial options for enhancing native growth (Emam 2015), suggesting the commercial inoculation might not have addressed symbiont limitation for native plants in our study. Future work that explores the use of locally sourced soil inoculants could help identify to what extent inoculations limit native species establishment. Our findings indicate that tackling limitations simultaneously is critical to enhance most management goals, but management of the invasive grass may require more nuanced approaches, where additional factors like litter accumulation should be considered.

Dominant invasive grasses may mask more nuanced responses from the rest of the community (Elmendorf & Harrison 2009; Fernandez-Going et al. 2012). We found that the combination of seeding, clipping, and inoculating together yielded the most unique species (i.e., richness differences) and caused the greatest overall difference in the rank and abundance (i.e., curve change) of recipient communities. Having greater changes in the rank and abundance, and more unique species entering the community post-treatment may fill vacant niche space, making systems less prone to invasion (Funk et al. 2008). The significance of more unique species, in combination with the greatest changes in curves, suggests that combined strategies are the most likely to have the greatest community level changes in heavily dominated systems; however, invasive species and degraded ecosystems still exhibit resilience towards an undesirable state of few dominant species, preventing restoration success. The seed addition alone did not cause significant changes in unique species or greater overall curve change, suggesting that the post-dispersal limitations of light availability and symbiont depletion interact with seed limitation. In our study, seed addition, clipping, and commercial inoculations did not differentially influence differences in evenness, rank, or species gains and losses. Alternatively, there may be resilience of the dominant invasive type (nonnative annual grasses) that are preventing the impacts of management actions from realizing their intended goals on communities.

Rangeland and grassland management often consist of multifaceted goals of reducing invader abundances while concomitantly increasing native diversity (Sherrill et al. 2021). Achieving these goals requires addressing factors that regulate invader performance, which may not align with factors to promote native species (Sherril et al. 2021). In our system we found evidence of this dynamic that was mediated by the belowground soil biota community versus clipping or adding seed alone. Like other studies (Gornish & James 2016), we found that clipping alone did not help with reducing

E. caput-medusae cover, despite its strong impacts on native diversity. However, we did observe interactive effects of seeding and inoculating together reducing *E. caput-medusae* cover. These findings are consistent with previous research showing that AMF inoculations may reduce the performance of invasive species without enhancing the growth of native species (Perkins & Hatfield 2015; Waller et al. 2016, Luo et al 2021). While plant invaders can benefit from soil biota, in many instances invasive plants do not receive growth benefits from soil (Pringle et al. 2009) and may even have reduced performance when AMF are present (e.g., *Salsola* [Hovland et al. 2019], and *Plantago* [Luo et al. 2021]). If trying to manage for both *E. caput-medusae* cover and native diversity then the conducting all three treatments may maximize outcomes as we observed increases in native cover and diversity with clipping and seeding which was maintained in the seeding, clipping, and inoculating treatment, and we also saw the greatest decrease in *E. caput-medusae* cover in seeding and inoculating treatment. Importantly, our study utilized a commercial inoculant due to practical constraints of developing a locally sourced inoculant during the COVID-19 pandemic; exploring this mechanism with locally sourced inoculum would indicate whether *E. caput-medusae* is similarly impacted by native AMF or whether there is something about the AMF species within the commercial inoculant that impacts *E. caput-medusae* growth. While commercial inoculants are an appealing option to manage large areas, these products may not assist native plant growth (Perkins & Hatfield 2015, Maltz & Treseder 2015) and have unknown lingering impacts on the soil community in natural areas. Despite the differential risks and benefits of inoculation types, community responses are constrained by several factors beyond the availability of symbionts, and inoculations may only work as a mediating factor in combination with other treatments.

In heavily invaded systems, how to effectively restore in the face of multiple constraints remains an ongoing challenge. The limited success of seeding alone highlights the importance of tackling multiple constraints to recovery (Copeland et al. 2019; Kettenring & Adams 2011). Moreover, increasing native cover while reducing invader dominance may require different treatment combinations to overcome factors limiting native recovery (Sherrill et al. 2021). In our study, native plants were primarily constrained by seed availability and the impacts of litter, and the highest response was achieved when addressing both simultaneously. However, our focal invader reductions responded more strongly to the combined treatments of seeding and inoculating. Similarly, the full community responded the most strongly to multiple treatments. Taken together, our multi-factor study on the impacts of seeding, litter removal, and inoculations suggest that integrated treatments have the strongest potential to achieve management goals, but this is contingent on targeting the appropriate constraints for both the native and invading species.

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Figures & Tables

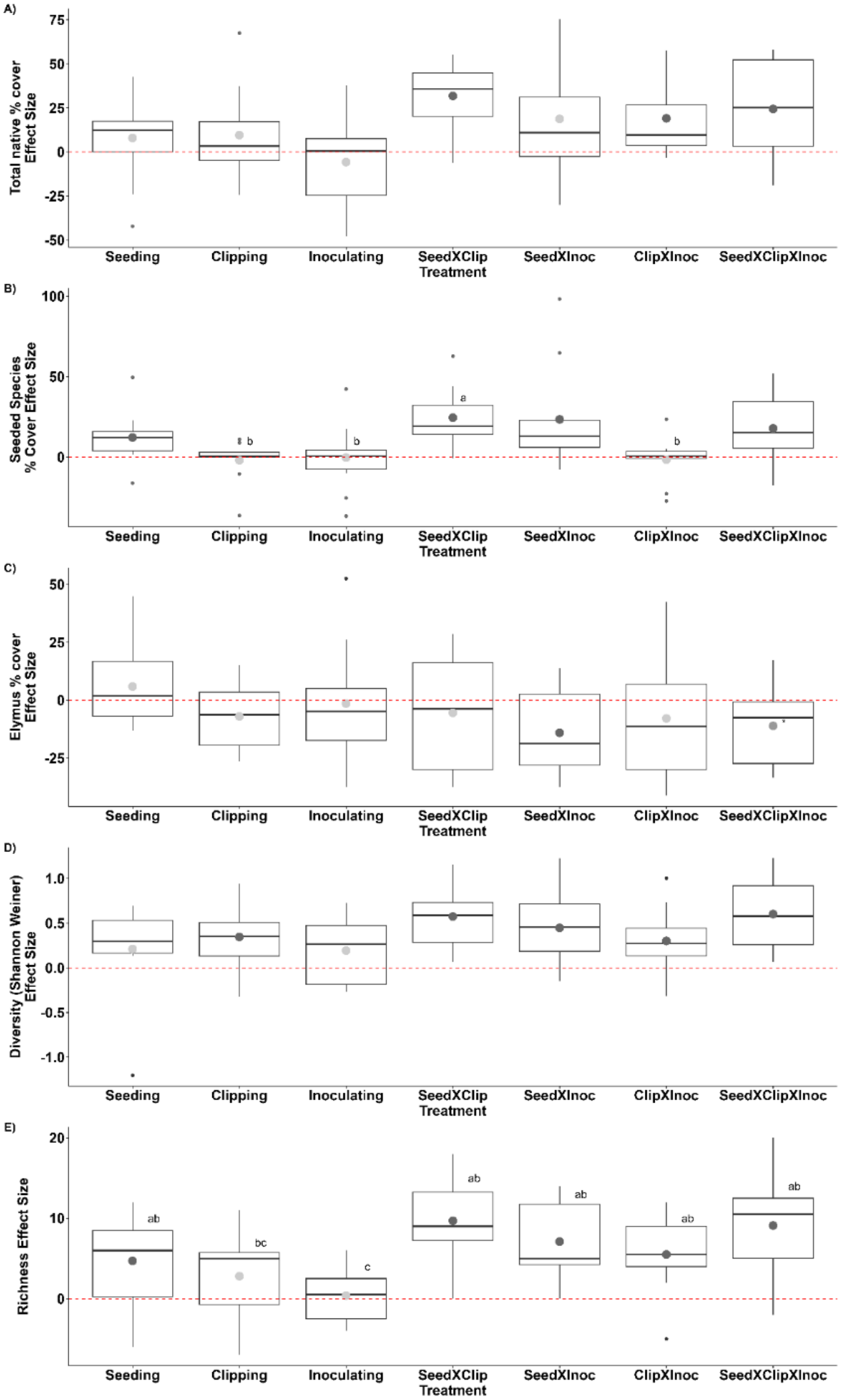


Figure 1) Treatment effect sizes on focal response variables. Plots show percentiles and median, with a colored circle representing both the average for a treatment and the results of t-test, where dark grey is significantly different from 0 and light grey is not significantly different from 0. A) Reductions in *E. caput-medusae* cover; B) Increases in total native cover, letters represent significantly different groups based on Tukey HSD; C) Increases in seeded species cover; D) Increases in Diversity; E) Increases in richness, letters indicate differences based on Tukey HSD.

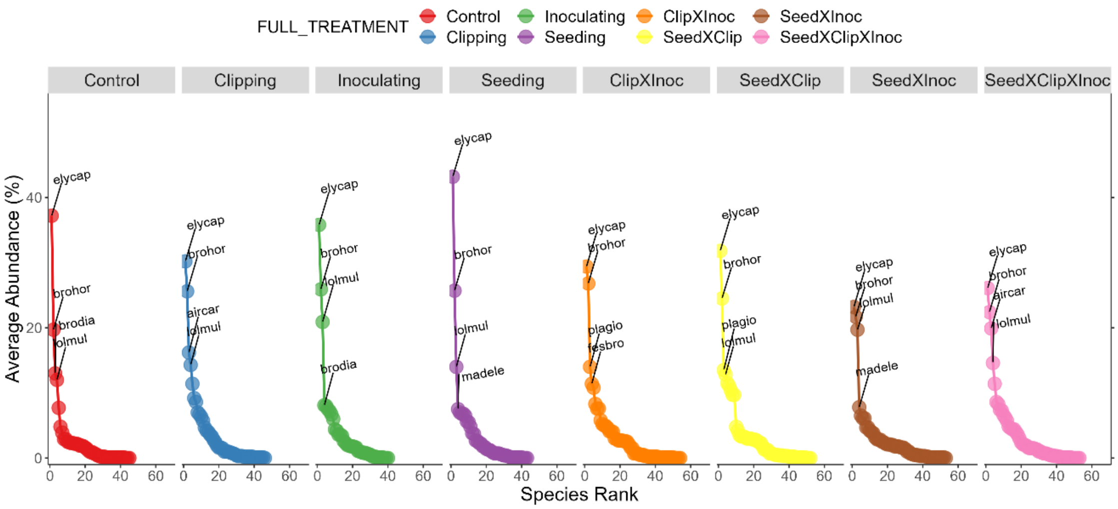
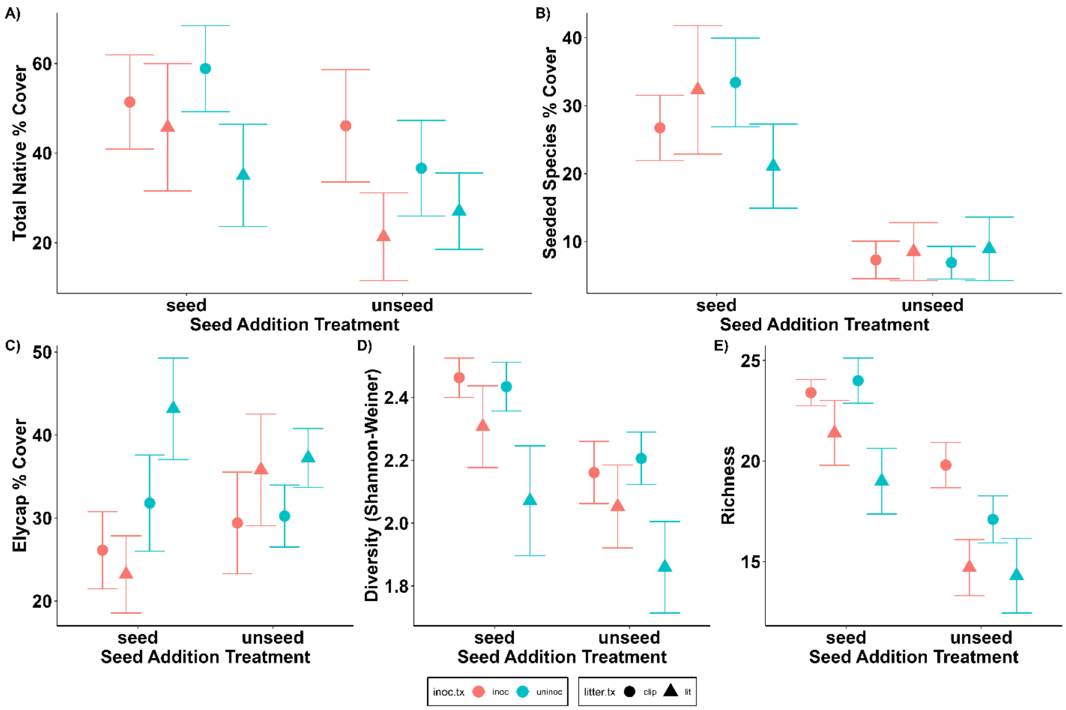
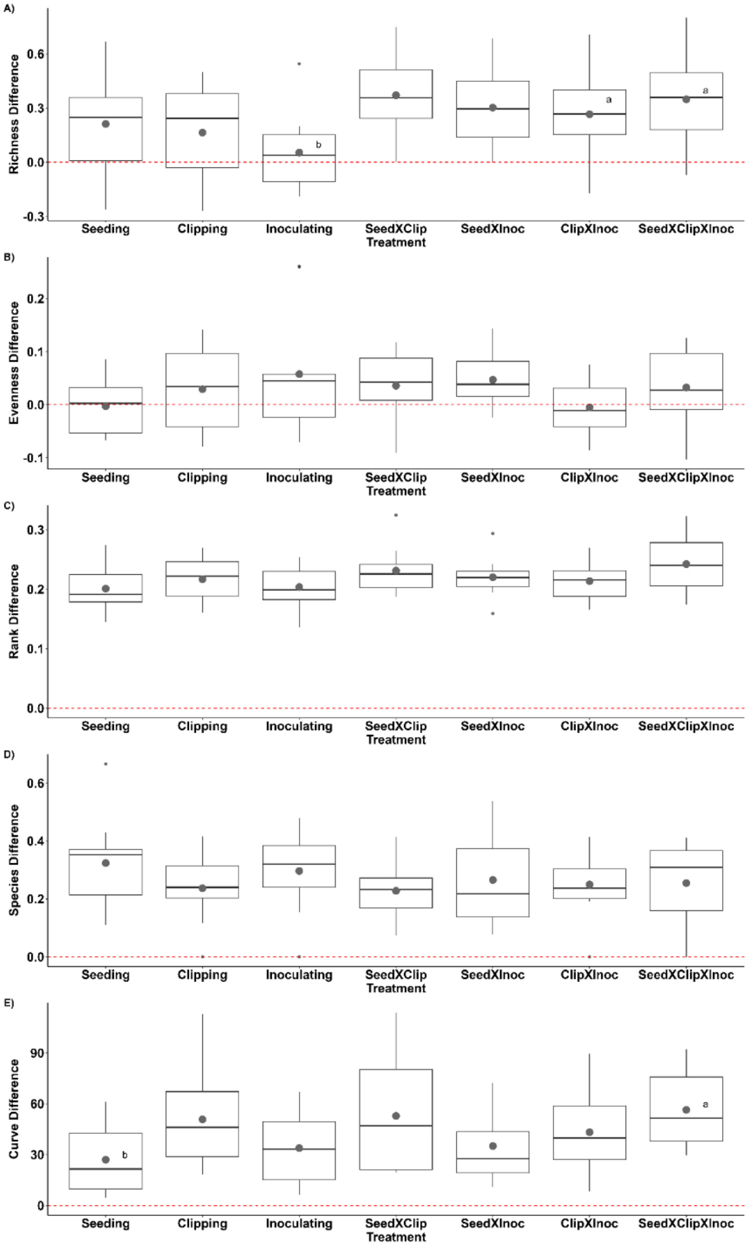


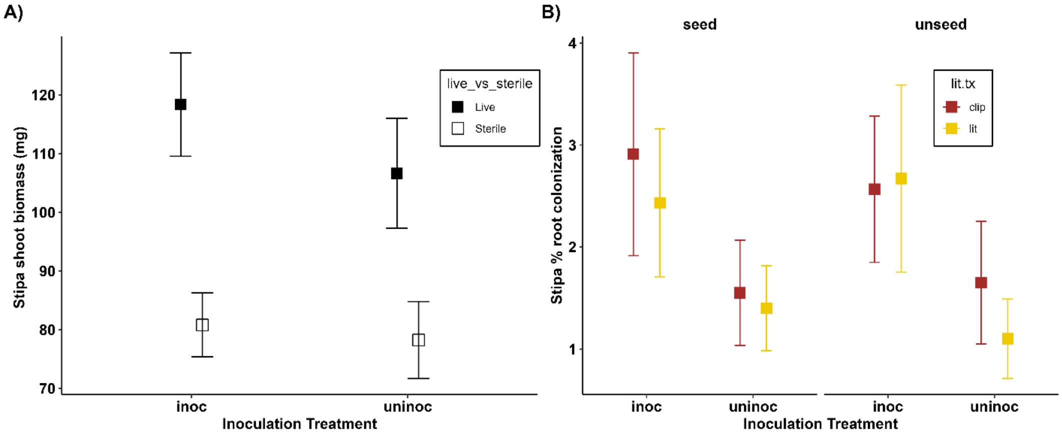
Figure 2) Average rank and abundance curve for each treatment including control. Six letter codes for the four most abundant species are labeled for each curve. Six letter codes can be found in Supplementary Table 1.



Supplemental Figure 1) Comparisons of observed data between full factorial treatment (seeding, clipping, and inoculating) compared to untreated. Points indicate treatment averages, with standard error. Shape indicates litter treatment, where triangles are litter intact and circles are clipped, and color indicates inoculation treatment, where red is inoculated and blue is uninoculated.



Supplemental Figure 2) Community responses in rank and abundance, with impacts on focal metric split by treatment A) Richness differences – letters indicate Tukey HSD results B) Evenness differences C) Rank differences D) Species differences E) Curve differences, letters represent Tukey HSD results.



Supplemental Figure 3) Greenhouse bioassay results for *Stipa pulchra*. A) Shoot Biomass response in live vs sterile soil for inoculated vs uninoculated soils. B) Percent AMF colonization across the factorial combination of treatments of inoculation (inoculated, uninoculated), litter (clipped, intact), and seed addition (seed, unseed).

Supplemental Table 1) Table of all species recorded in plots during this study, and which years they were present for. Six letter codes taken in the field are the leftmost column, with full species names following Jepson in the middle, provenance as native or non-native and years present in the right most column. Asterisk in the provenance column indicates which species were in the seed addition mix.

Six Letter Code	**Full Name**	**Provenance**	**Year(s) present**
achmil	*Achillea millefolium*	native *	2021,2022
achmol	*Achyrachaena mollis*	native	2022
acmpur	*Acmispon purpureus*	native	2021,2022
agohet	*Agoseris heterophylla*	non-native	2021,2022
aircar	*Aira caryophyllea*	non-native	2021,2022
antari	*Anthoxanthum aristatum*	native	2021,2022
ascfas	*Asclepia fascicularis*	native *	2021,2022
avebar	*Avena barbata*	non-native	2021,2022
avefat	*Avena fatua*	*non-native*	2021,2022
bradis	*Brachypodium distachyon*	*non-native*	2021,2022
brimax	*Briza maxima*	*non-native*	2021,2022
brimin	*Briza minor*	*non-native*	2021,2022
brodia	*Bromus diandrus*	*non-native*	2021,2022
brohor	*Bromus hordeaceus*	*non-native*	2021,2022
bromad	*Bromus madritensis*	*non-native*	2022
broste	*Bromus sterilis*	*non-native*	2022
carpyc	*Carduus pycnocephalus*	*non-native*	2021,2022
caryx	*Caryx species*	native	2021
casatt	*Castilleja attenuata*	native	2021,2022
casten	*Castilleja tenuis*	native	2021
censol	*Centaurea solstitialis*	*non-native*	2021
cerglo	*Cerastium glomeratum*	*non-native*	2021
cicqua	*Cicendia quadrangularis*	native	2021,2022
clagra	*Clarkia gracilis*	native *	2021
clapur	*Clarkia purpurea*	native	2022
cynech	*Cynosurus echinatus*	*non-native*	2021,2022
daupus	*Daucus pusillus*	native	2022
dicmul	*Dichelostemma multiflorum*	native	2021

dipcap	*Dichelostemma capitatum*	native	2021,2022
elycap	*Elymus caput-medusae*	*non-native*	2021,2022
erilan	*Eriophylum lanatum*	native *	2021,2022
erobot	*Erodium botrys*	*non-native*	2021,2022
erocic	*Erodium cicutarium*	*non-native*	2021,2022
escal	*Eschscholzia californica*	native *	2021,2022
fesbro	*Festuca bromoides*	*non-native*	2021,2022
fesmic	*Festuca microstachys*	native	2022
fesmyu	*Festuca myuros*	*non-native*	2022
filgal	*Filago gallica*	*non-native*	2021,2022
galpar	*Galium parisiense*	native	2021,2022
germol	*Geranium molle*	*non-native*	2022
hormur	*Hordeum murinum*	*non-native*	2022
hypgla	*Hypochaeris glabra*	*non-native*	20212022
hypper	*Hypericum perforatum*	*non-native*	2021,2022
junbuf	*Juncus bufonius*	native	2022
lepnit	*Lepidium nitidum*	native *	2022
linbic	*Linanthus bicolor*	native	2021
linbin	*Linum bienne*	native	2021
lolmul	*Lolium multiflorum*	*non-native*	2021,2022
lupbic	*Lupinus bicolor*	native	2021,2022
lupsuc	*Lupinus succulentus*	native *	2021,2022
madele	*Madia elegans*	native	2021,2022
madgra	*Madia gracilis*	native *	2021,2022
miccal	*Microcarpus californicus*	native	2021,2022
navpub	*Navarretia pubescens*	native	2021,2022
petdub	*Petrorhagia dubia*	*non-native*	2021,2022
plaere	*Plantago erecta*	native *	2021,2022
plagio	*Plagiobothrys spp*	native	2021,2022
riglep	*Rigiopappus leptocladus*	native	2021,2022
sanbip	*Sanicula bipinnata*	native	2021,2022
shearv	*Sherardia arvensis*	*non-native*	2022
silgal	*Silene gallica*	*non-native*	2022
torarv	*Torilis arvensis*	*non-native*	2022
tridep	*Trifolium depauperatum*	native	2022
tridub	*Trifolium dubium*	*non-native*	2021,2022
trihir	*Trifolium hirtum*	*non-native*	2021,2022
trihya	*Triteleia hyacinthina*	native	2021
trilan	*Trichostema lanceolatum*	native	2021
trimic	*Trifolium microcephalum*	native	2021,2022

triset			2021
trisub	*Trifolium subterraneum*	*non-native*	2022
triwil	*Trifolium willdenovii*	native *	2021,2022
vicsat	*Vicia sativa*	*non-native*	2021,2022
zigfre	*Zigadenus fremontii*	native	2021

Supplemental Table 2) Summary statistics for linear mixed effect model assessments of treatments on effect size of focal management goals. The first number is the F statistic, the following numbers in subscript are Sattherwaite approximations of the degrees of freedom with numerator and denominator separated by a comma, with p values afterwards.

	Treatment	Year	Treatment X Year
Total Native Cover	$2.114_{6,52}$0.067	$3.991_{1,52}$0.051	$0.171_{6,52}$0.983
Seeded spp cover	$4.227_{6,52}$0.002	$8.907_{1,52}$0.004	$0.945_{6,52}$0.471
E. caput-medusae cover	$2.115_{6,52}$0.067	$8.596_{1,52}$0.005	$0.952_{6,52}$0.467
Diversity	$1.976_{6,52}$0.086	$3.353_{1,52}$0.073	$0.829_{6,52}$0.553
Richness	$4.614_{6,52}$0.001	$8.358_{1,52}$0.006	$0.685_{6,52}$0.663

Supplemental Table S3) Summary statistics for linear mixed effect models assessing changes to rank and abundance metrics across treatments and years. The first number is the F statistic, the following numbers in subscript are Sattherwaite approximations of the degrees of freedom with numerator and denominator separated by a comma, with p values afterwards.

	Treatment	Year	Treatment X Year
Richness Difference	$2.828_{6,52}$0.019	$15.525_{1,52}$<0.0001	$0.442_{6,52}$0.847
Evenness Difference	$1.623_{6,52}$0.160	$1.801_{1,52}$0.185	$0.293_{6,52}$0.938
Rank Difference	$1.850_{6,52}$0.108	$4.377_{1,52}$0.041	$1.600_{6,52}$0.166
Species Difference	$0.723_{6,52}$0.633	$10.161_{1,52}$0.002	$0.223_{6,52}$0.968
Curve Difference	$2.505_{6,56}$0.032	$0.134_{1,56}$0.716	$1.474_{6,56}$0.204