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RESEARCH ARTICLE

Negative plant–soil feedback influences a dominant seeded species, Western yarrow (*Achillea millefolium*), in grassland restoration

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Plant community ecology guides restoration of degraded lands, yet seed-based restorations sometimes fail or result in unpredictable outcomes, necessitating a better understanding of community trajectory and stability. Western yarrow (*Achillea millefolium*) declined suddenly in two separate restoration projects after initial high relative abundance. To assess the potential role of soil pathogens, we surveyed plant and soil fungal communities in these restorations, and used an 8-year-old field experiment that crossed yarrow planted in varying densities with a fungicide treatment. Two greenhouse experiments then evaluated whether the suppressive effect in yarrow soil spread to native species used in restoration. Lower yarrow cover in a restoration project 5 years compared to 3 years after seeding coincided with higher relative abundance of fungal taxa that can cause disease, particularly Crown-rot fungi (*Paraphoma* spp.). *Paraphoma* increased over time in experimental plots and coincided with yarrow decline. Decline onset was density-dependent, occurring faster in plant communities where yarrow density was higher. Fungicide applications altered fungal pathogen communities and promoted yarrow cover relative to control plots. In the greenhouse, yarrow grew larger with fungicide, consistent with suppression of fungal pathogens. However, biomass of natives grown in yarrow-conditioned soil was not affected by fungicides, suggesting pathogens did not spread. The rapid establishment and competitive nature of yarrow, followed by pathogen-induced decline, make it an attractive early transitional "bridge species," so long as its pathogens are species-specific. Our results suggest negative plant-soil feedback can drive rapid decline of individual species, and considering plant–soil feedback could improve restoration predictability.

Key words: bridge species, fungal pathogens, ITS, *Paraphoma*, plant–soil feedbacks, priority effects

Implications for Practice

- Early transitional or so-called "bridge species" used in restoration at high densities may accumulate pathogens.
- Density-dependent pathogen accumulation may be predictable and could control bridge species without herbicides, mowing, or other management.
- Open niche space following suppression may allow for staggered introduction of later successional species if pathogens are host-specific and do not negatively affect other native plants.
- Monitoring soil pathogens may give practitioners a better understanding of the sources of success or failure in restoration outcomes.

Introduction

Restoration ecology emerged from plant community ecology (Temperton et al. 2004; Palmer et al. 2016) and tends to be plant-centric with community assembly and succession theories guiding selection of seed mixtures and planting order (Sheley et al. 2006; Wainwright et al. 2018). However, seed-based restorations sometimes fail or result in unexpected outcomes (Shackleford et al. 2021). Although drought (Hardegree et al. 2018), temperature extremes (James et al. 2019), non-native invasive plants (Martin & Wilsey 2014), or cryptic demographic and biotic filters (James et al. 2011) contribute, often the causes of failure are complex or simply unknown (Svejcar et al. 2017). As such, restoration ecologists continue to search for factors that can improve predictability.

Plants influence both abiotic and biotic properties of soils, which affect their growth and competitive interactions with

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other plants (Bever et al. 1997). These so-called plant–soil feedbacks can drive plant succession, influence relative abundance and diversity of plant species in communities, and either aid or prevent non-native plant invasions (Kulmatiski et al. 2012; van der Putten et al. 2013). Plant–soil feedback can affect plant performance as strongly as competition (Lekberg et al. 2018), yet it is rarely addressed or invoked in ecological restoration, even though its potential has been discussed for some time (Eviner & Hawkes 2008; Harris 2009). Most plant–soil feedback studies have been conducted under controlled greenhouse conditions (Lekberg et al. 2018), although the role of soil biota has also been documented in the field. For example, Wubs et al. (2016) showed that large-scale soil inoculations can steer plant and microbial community development toward different target communities. Similarly, inoculations by arbuscular mycorrhizal fungi (AMF), which colonize most plant roots and aid in nutrient uptake and possibly other services in exchange for plant carbon, have been used in prairie restoration (Koziol & Bever 2017).

In addition to AMF, plant pathogens are important players in plant–soil feedback. They tend to be more host-specific than AMF but can also affect plant diversity (Thakur et al. 2021). In some instances, rare species suffer more from pathogens than abundant species (Klironomos 2002; Mangan et al. 2010). In other instances, pathogens accumulate in a density-dependent fashion, suppressing growth more for abundant than less abundant species (Maron et al. 2011; van Ruijven et al. 2020). Pathogens affect early seral species more than late seral species (Kardol et al. 2006), which contributes to successional shifts in plant communities. But to what extent plant pathogens affect assembly processes and succession in communities undergoing restoration is not completely understood.

Bridge species are used during initial phases of restoration to stabilize soil, compete with weeds, or ameliorate poor site conditions, which often occur on degraded lands with low plant density or simplified communities resulting from overgrazing, mining activities, or herbicide applications (Kildisheva et al. 2016; Svejcar et al. 2017). These early, transitional species are often planted at high densities to preempt niche space from undesirable species (Fukami 2015; Delory et al. 2019). Due to their short lifespan and high density, they are probably susceptible to pathogens, but whether the abundance of bridge species is affected by pathogens is little known (Kulmatiski et al. 2017).

Western yarrow (*Achillea millefolium* L. var. *occidentalis* DC., hereafter yarrow) is a common species used in restoration of semiarid grasslands (detailed life history description in Supplement S1). Yarrow usually establishes quickly (Lambert et al. 2011; but see Jensen et al. 2022), competes well with common grassland invaders, and is available in large quantities. It also associates with AMF and has the capacity to build AMF abundance in degraded soils (Wilson & Hartnett 1998; Sonneman et al. 2012). However, yarrow can also generate negative plant–soil feedback when grown in monocultures or intraspecific conditioned soils (Maron et al. 2011; Jing et al. 2015). Yarrow reproduces from both rhizomes and seeds, so defining a lifespan for an individual is not straightforward (Bourdôt et al. 1985). Typically, yarrow is not classified as a short-lived ruderal species (Aleksoff 1999), yet we have observed a yarrow

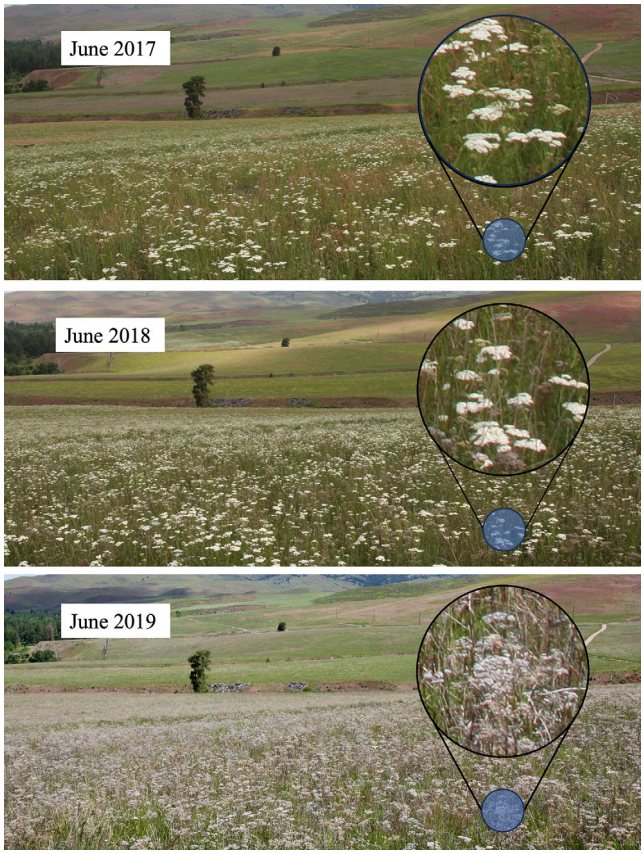


Figure 1. Time series photos from the Older restoration site (seeded 2015) on MPG Ranch. White flowering yarrow corymbs already abundant in 2017 peak in 2018. Between 2018 and 2019, many plants were in the process of dying, as indicated by the substantial reduction in flowering yarrow, with most flower stalks in 2019 being dead-standing from 2018.

boom and bust after 3–5 years across several restoration projects (Fig. 1). This rapid establishment and predictable decline without management interventions could make yarrow a promising bridge species.

Consequently, we asked: (1) is yarrow successful at establishing from seed relative to other native species commonly included in restoration seed mixes; (2) is the sudden yarrow decline due to soil pathogen accumulation; (3) is yarrow decline in-part a density-dependent process; (4) do soil pathogens spread to other native plants used in restoration? To address these questions, we surveyed plant species cover and characterized fungal and oomycete (another important group of soil pathogens, including *Phytophthora* and *Pythium*; Derevnina et al. 2016) communities in two restoration areas seeded 17 months apart and where yarrow cover was visibly less 5 years compared to 3 years after seeding. We then took advantage of an ongoing field experiment where plant cover data and soil had been collected over time in communities planted with different densities of yarrow and heterospecifics and crossed with a fungicide treatment. Finally, a series of greenhouse experiments explored the direct and indirect effects of fungicides to help interpret

results from the field experiment and assess whether soil biota cultured by yarrow suppressed the growth of four plants commonly used in restoration.

Methods

Study Area

Field surveys and experiments took place on MPG Ranch, a 6400 ha private conservation property and former cattle ranch in Western Montana, United States (46°40′48.92″N, 114°1′40.73″W, https://www.mpgranch.com; Fig. S1). Large areas are undergoing restoration from monocultures of Crested wheatgrass (*Agropyron cristatum*) and other introduced forage grass back to native cool-season bunchgrass communities typical of this area (Durham et al. 2017). Yarrow was detected at 23 out of 98 never-cultivated survey points on MPG Ranch in 2016 and comprised 1.5% average total foliar cover where it occurred (unpublished data). Annual precipitation averaged 316 mm and mean temperature 7.5°C between 1991 and 2020 based on a weather station 25 km south of MPG Ranch (www.ncei.noaa.gov). Elevation ranged from 995 m at the field experiment to 1080 m at the restoration sites. Soils are fine to gravelly, loamy mollisols in the Bigarm gravelly loam series (www.nrcs.usda.gov).

Field Survey of Ongoing Restoration

To examine the relationship between soil pathogens, yarrow's establishment, and subsequent decline during restoration (questions 1 & 2), we chose two adjacent sites (15 and 24 ha) with different restoration ages (seeded in 2015 and 2017) and yarrow cover based on visual observations (low vs. high) with otherwise similar soil conditions (Table S1), elevation, and aspect. While restoration age only differed by 17 months, hereafter we refer to these sites as Older and Younger. Time lapse photos of the Older site indicate visual declines in yarrow cover over time (Fig. 1).

Site History. Restoration activities during and after seeding are described in detail in Supplement S1. Briefly, the Older site was seeded on 3 November 2015, and the Younger site was seeded on 4 April 2017. Seed mixes are shown in Table S3a–c. We used Eagle germplasm Western yarrow (Granite Seed Company, Lehi, UT, U.S.A.), recommended for quick establishment and no known detrimental diseases (Lambert et al. 2011). Yarrow was seeded at 57 seeds/m^2 at the Older site and 97 seeds/m^2 at the Younger site, accounting for 8.6 and 13% of a seed mix (0.78 and 0.57% by weight), respectively. Yarrow seeding rates were determined as part of a mix targeting a site appropriate total number of seeds per area. Sites were drill seeded with a Truax Roughrider no-till rangeland seed drill, which cut furrows approximately 1.9 cm deep (Truax Corp., Minneapolis, MN, U.S.A.). A nearby weather station recorded similar precipitation (149–157 mm) between March and November during the initial growing season of each seeding. In November 2019, 11 ha were re-seeded with a diverse mix (Table S3c) in the Older site to increase diversity and cover after yarrow declined.

Root and Soil Sampling. In June 2020, we dug up yarrow plants from 10 randomly chosen locations at both sites and collected roots and soil adhered to yarrow roots (0–15 cm depth). Soil samples were sieved (2 mm), freeze-dried (Labconco Freezone benchtop freeze-dry system, Labconco, Kansas City, MO, U.S.A.), and stored at −20°C for future DNA extraction and amplification. Yarrow fine roots (<1.5 mm diameter) from individual plants were rinsed in tap water, placed in a 1.5 mL microcentrifuge tube, freeze-dried, and homogenized using a 1600 MiniG tissue homogenizer and cell lyser (Spex SamplePrep, Metuchen, NJ, U.S.A.) before DNA extraction and amplification. To compare fungal and oomycete communities in the rhizospheres of yarrow within the two restoration areas with naturally occurring yarrow, we also sampled fine roots from 10 randomly selected yarrow plants growing in a native community approximately 1 km northeast of the Older site (Fig. S1).

Vegetation Sampling. In June 2020, at 10 randomly selected locations at both sites, we used a 1 × 1 m frame to visually estimate plant canopy and ground cover (to the nearest 5% increment). Species cover under 5% was assigned to 1%, and we used 0.1% to indicate trace cover presence below 1%. Total cover could exceed 100% because overlapping cover was included. To determine what replaced yarrow after its decline, we surveyed the same fixed plots ($n = 10$) at each site again in June, 2022. To capture more variation and patchy species distribution, we also surveyed an additional 10 randomly located plots per site in 2022.

Field Experiment

In 2013, we established a large field experiment consisting of 448 plots (1.2 × 1.2 m with 1 m buffer) in a completely randomized design harboring different communities of seven natives and three non-native forbs treated with fungicide or not. Details of the establishment and species composition in the experiment are in Supplement S1, and initial nutrient availabilities and pH are in Table S1. To address questions 2 and 3, we focused on the 48 plots that harbored yarrow planted in community types consisting of (1) monocultures (32 yarrow seedlings), (2) dominant (20 yarrow seedlings and two seedlings each of six other native forb species), and (3) even (four seedlings each of yarrow and the six native forbs plus four randomly selected seedlings) communities. Communities were established from seedlings grown from seeds (same source as above) in the greenhouse and inoculated with soil from nearby native and non-native communities to ensure the presence of diverse soil biota. Communities were watered during the first summer to ensure good establishment. Plots were weeded to remove non-planted species.

Fungicide was applied in September 2013 and then twice a year in April and October. Fungicide-treated plots received a 1 L/m^2 mixture of Ridomil Gold EC (Syngenta Corporation, Greensboro, NC, U.S.A.; 0.117 mL/L active ingredient mefenoxam) and

Topsin 4.5FL (United Phosphorus, Inc., King of Prussia, PA, U.S.A.; 2.5 mL/L active ingredient thiophanate-methyl) applied with hand-wand from an all terrain vehicle mounted tank. Control plots received the same amount of water. Starting in 2015 and annually thereafter, we estimated plant cover for species rooted within a 1 × 1 m quadrat at peak biomass at the end of June (three community types × two fungicide treatments × eight reps).

To determine if fungal and oomycete communities change over time and respond to fungicide applications, we collected soil samples from the 16 yarrow monoculture plots in 2014, 2016, and 2019, although samples were not collected from fungicided plots in 2016. From each plot, we collected three soil cores (2.5 × 10 cm) randomly located within the plot but at least 15 cm from the edge. The three cores were mixed into one composite sample per plot, freeze-dried, and stored in the freezer prior to DNA extractions.

Greenhouse Experiments

Direct Effects of Fungicides. To determine if fungicides have direct beneficial or detrimental effects on yarrow, we grew yarrow in sterile soil that we then treated with fungicide or not. On 31 October 2019, 2-week-old seedlings germinated on MiracleGro Seed Starting Potting Mix (Marysville, OH, U.S.A.) and grown from the same seed source as above were planted into 10 cm diameter pots containing 450 mL of a 1:1:1 soil:sand:turface (v:v:v) mixture. Soil collected from the field experiment area was sterilized by three rounds of autoclaving (3 × 3 hours at 121°C). Four days after planting, 20 mL of the same fungicide mixture used in the field was added to half the pots, and the other half received water. The volume was based on the surface area of the pot to ensure that the same amount of fungicide was used in the greenhouse and the field. This application was repeated on 10 December, 44 days after planting. Due to yellowing of leaves indicating nitrogen deficiency in both treatments, we fertilized all plants with 20 mL of a 0.5 g/L of 20.2.20 (N-P-K, Peters Professional fertilizer, JR Peters, Inc., Allentown, PA, U.S.A.) solution four times during the experiment, starting 27 days after planting and then every other week. Plants were harvested on 16 January 2020, 11 weeks after planting. Shoot biomass (all biomass above the soil surface) was recorded after drying at 65°C until constant weight.

Pathogen Spread. To assess if the pathogens associated with the suppression of yarrow in the field could negatively affect other native forbs (question 4), we conducted a plant–soil feedback study. In a greenhouse, we grew Blanketflower (*Gaillardia aristata*), Lewis flax (*Linum lewisii*), Maximilian sunflower (*Helianthus maximiliani*), and Upright prairie coneflower (*Ratibida columnifera*) in soils that had previously harbored yarrow. To generate the yarrow-trained soil inoculum, we planted yarrow seedlings on 6 November 2019 in 10 cm diameter pots containing a 1:1:1 soil:sand:turface mixture. The soil fraction was harvested from eight heterospecific monoculture plots (two plots each of Hairy golden aster [*Heterotheca villosa*], Sticky cinquefoil [*Drymocallis glandulosa*], Leafy spurge [*Euphorbia esula*], and Sulfur cinquefoil [*Potentilla recta*]) from the large field experiment outlined above and in Supplement S1 to ensure a diverse mixture of soil fungi but where no prior training by yarrow had occurred (Fig. S2). Soil from each field plot was divided into two pots, one receiving water and one receiving fungicide (same amount and concentration as above), which was added on 12 November and 17 December 2019. All 16 pots were fertilized twice monthly using the same concentrations and volumes as above when assessing the direct effects of fungicides. On 6 April 2020, we cut the shoots at the soil surface, removed coarser roots to prevent re-growth, and replanted yarrow seedlings to condition soils twice to increase the likelihood of pathogen build-up. Plants were grown as before, and fungicide or water was applied on 4 May and 2 June 2020. Plants were harvested on 20 July 2020, and shoot biomass was dried as before. On 4 August 2020, we added 70 mL of either yarrow control or yarrow fungicide inoculum on top of the same sterilized soil:sand:turface (1:1:1) mix as before into 200 mL conetainers and planted seeds. Thus, in total, we had 64 pots (4 species × 2 inocula × 8 replicates). Pots were watered as needed and fertilized as before. Maximilian sunflower seeds had very low germination, so this species was omitted from the study. Shoot biomass of the remaining species was harvested on 1 October 2020.

DNA Extraction, Amplification, and Bioinformatics

We used the Dneasy PowerSoil Pro DNA isolation kit (Qiagen) for DNA extraction from soil, roots, and control samples containing no roots or soil. Samples were prepared for Illumina sequencing through a two-step Polymerase Chain Reaction amplification. We amplified the internal transcribed spacer 2 (ITS2) for general fungi and internal transcribed spacer 1 (ITS1) for oomycetes in all soil and root samples. We amplified the ITS2 region using a mix of forward fungal primers flITS7 and flITS7o and the reverse primer ITS4, following Bullington et al. (2018). For the ITS1 region, we used the forward primer OOM18S (Legeay et al. 2019) and the reverse primer ITS7ae, a degenerate form of ITS7 (Taheri et al. 2017). Samples were assigned unique barcodes and pooled based on band strength. Sequencing was performed at the University of Montana Genomics Core (umt.edu/genomics-lab/; Missoula, MT, U.S.A.). Amplicon libraries were sequenced using 2 × 300 paired end reads on the Illumina MiSeq platform (Illumnia Inc.). All sequences have been submitted to Sequence Read Archive on National Center for Biotechnology Information under accession number PRJNA983826. Oomycete communities did not amplify well from roots and soil in the field survey and were therefore not assessed.

Raw sequence data was processed using QIIME 2 (version 2018.4; Caporaso et al. 2010). For the ITS2 gene, forward and reverse reads were trimmed when median quality score fell below 30, which was 200 bp for the forward reads and 240 bp for the reverse reads. For the ITS1 gene, reverse reads were trimmed to 240 bp. Only reverse reads were used for the ITS1 amplicons, as the forward reads covered the 18S rDNA region, which lacks nucleotide polymorphisms needed to distinguish oomycete species (Legeay et al. 2019). The q2-dada2 plugin

was used to produce sequence clusters with 100% similarity. Because we were interested in taxonomic rather than genetic resolution, and because functionality of fungi is often conserved at the level of genus or family (Tedersoo et al. 2022), we clustered the sequences at 97% similarity using the q2-vsearch plugin de novo to produce operational taxonomic units (OTUs). The database UNITE (Nilsson et al. 2019) was used to assign taxonomy to representative fungal and oomycete sequences. We then used the database FUNGuild to assign potential function to all OTUs that were assigned taxonomy (Nguyen et al. 2016). We used all OTUs identified as "highly probable" and "probable" plant pathogens to represent the plant pathogen community, which should be considered putative pathogens as it is uncertain if they cause disease in our system and conditions. Analysis of fungal and oomycete communities was rarified using sequence depth curves before statistical analysis to 4000 and 1000 sequences per sample using the q2-diversity plugin. Samples with fewer sequences were removed to allow for a greater sequencing depth, leaving 20 out of 20 soil samples and 25 out of 30 yarrow root samples (Older, $n = 7$; Younger, $n = 8$; and Native, $n = 10$) in the field survey and 39 of 40 soil samples from the field experiment. Also, an average of 59% of OTUs across samples was assigned putative function in FUNGuild.

Statistical Analyses

Field Survey. To determine if mean absolute yarrow cover differed between the two restoration sites, we used a Welch *t*-test since data were normally distributed but had unequal variance.

Field Experiment. We assessed the effect of the fungicide treatment on percent cover of yarrow in the different communities over time visually and using a linear-mixed effects model. Our full model included fungicide application, community (i.e. monoculture, dominant, or even), year-since-planting, and community by year interaction as fixed effects and plot id as a random effect to account for repeated measures ($cover \sim fungicide + community + year + community \times year + 1|plot$). We built this model from a null model of effect (cover ~ year + 1|plot), adding terms sequentially. Models were fit using maximum likelihood estimation via "lmer" from the lme4 package (Bates et al. 2015). Residuals were visually examined for normality and homoscedasticity. We used "anova.merMod" to run a likelihood ratio test of all models, which confirmed that the full model explained more variance than the reduced models. We then tested each coefficient from the full model in a type III analysis of variance (ANOVA) with Satterthwaite's degrees of freedom method via "anova.lmerModLmerTest" from the lmerTest package (Kuznetsova et al. 2017). Finally, we did a pairwise comparison across communities using "emmeans" from the emmeans package (Lenth 2024). We present the data as mean and 95% CIs (*t*-distribution) of cover for each community by fungicide by year combination.

Greenhouse Experiments. We tested for a direct effect of fungicide on yarrow by comparing total plant biomass from the fungicide and control treatment using a two-sample *t*-test. We looked for potential pathogen carry-over to restoration species with a two-way ANOVA to examine the main effects and interactions of (1) fungicide: treated and untreated control and (2) restoration species: Blanketflower, coneflower, and flax on the shoot biomass of restoration plants. Data met assumptions of normality and homogeneity of variance by Levene and Shapiro–Wilk tests. All analyses were performed with R Software (version 4.2.2; R Core Team 2021).

Fungal and Oomycete Communities. Differences in community compositions were analyzed in R using the vegan package (Oksanen et al. 2019) unless noted otherwise. We used the vegdist function based on OTU sequence abundance and Bray–Curtis distances to calculate dissimilarity. We performed non-metric multidimensional scaling using the metaMDS function to visualize changes to the fungal and oomycete communities ($k = 2$). We tested whether OTU composition differed among plant pathogen communities via permutational multivariate ANOVA (perMANOVA; Anderson 2017) and adonis2 function. Dispersion of points among groups was tested using the betadispers function. We used sequence abundance as a measure of the relative abundance of putative pathogens. While this may not reflect absolute abundance due to differences in gene copy numbers among taxa and primer bias (e.g. Corradi et al. 2007; Kohout et al. 2014), sequence numbers have been shown to correlate well with abundant taxa (Skelton et al. 2022), and any potential discrepancies in our approach should apply equally to all samples.

To test for differences in relative pathogen sequence abundance, we used ANOVA. Normality and equal variance assumptions were assessed visually. Lastly, to locate potential indicator species using relative sequence abundances of plant pathogen OTUs, we used the indval function in the labdsv package (Roberts 2019).

Results

Field Survey of Restoration Sites

Despite being a minor component (<15% by seed number) of the overall seed mix, yarrow was the dominant species in both restoration sites 5 and 3 years after seeding, although yarrow cover was lower in the Older site than the Younger site ($p < 0.006$). In contrast, perennial grasses, sown at a higher abundance (>45%), exhibited much lower coverage relative to their initial seeding density (<13%; Tables S2 & S3; Fig. 2B & 2D). By 2022, relative yarrow cover dwindled to <5% in both sites (Table S2; Fig. 2B & 2D). Thus, yarrow established rapidly and outperformed other species in the seed mix but declined dramatically 5–7 years after planting.

The lower yarrow cover in the Older site coincided with a higher relative abundance of fungal pathogens ($F_{[2,22]Site} = 17.25$, $p < 0.001$; Fig. 3B). Across all sites, we identified 609 and 577 fungal OTUs in soil and roots, respectively, of which 74 and 66 OTUs were probable or highly probable plant pathogens. The composition of pathogens in yarrow roots also differed

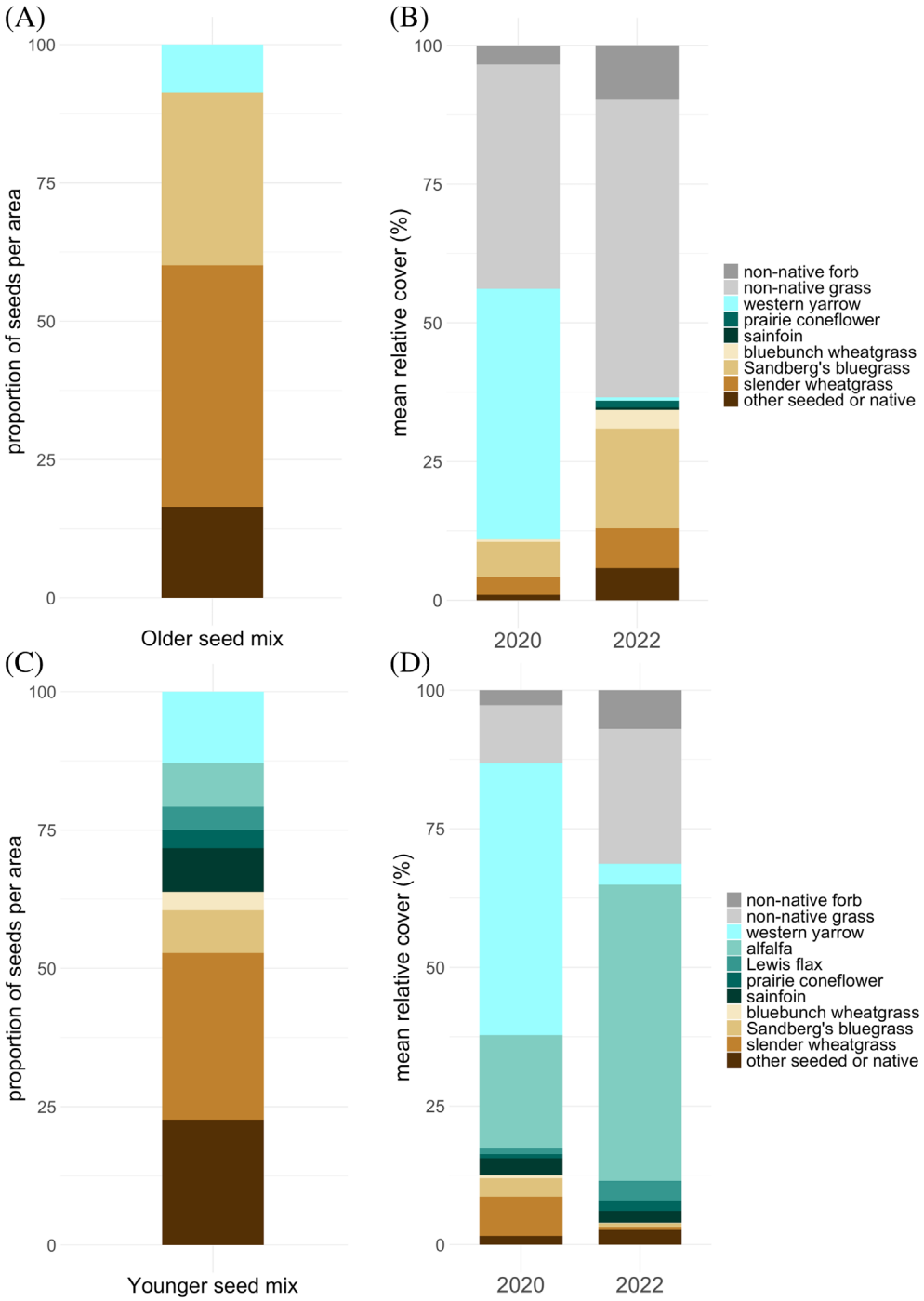


Figure 2. The proportion of species seeded (A) and their relative cover (B) in the Older site. (C) and (D) show the Younger site. In 2022, there were 35 total species, of which 2 were seeded non-native, 8 were seeded native, 10 were unseeded native, and 15 unseeded-non-native species at the 5-year post-seeding, Younger site. At the Older site, 7 years after the first seeding and 3 years following the additional seeding, there were 58 total species, of which were 12 seeded native, 15 unseeded native, and 31 unseeded-non-native species.

among sites ($F_{[2,22]\ \text{Site}} = 6.8$, $r^2 = 0.38$, $p = 0.001$; Fig. 3A), with less clear differences in the soil ($F_{[1,19]\text{Site}} = 1.8$, $r^2 = 0.09$, $p = 0.07$; Fig. S3). Overall, roots in the Older site harbored pathogens that were more dissimilar from the native site than the Younger site. This pattern was similar when repeating the analysis on presence–absence data (Jaccard: $r^2 = 0.09$, $F_{[2,22]\text{Site}} = 5.69$, $p = 0.001$), suggesting sites harbored different taxa, not just variations in relative abundance.

An indicator species analysis revealed that an OTU identified as *Paraphoma* spp. was preferentially associated with the Older

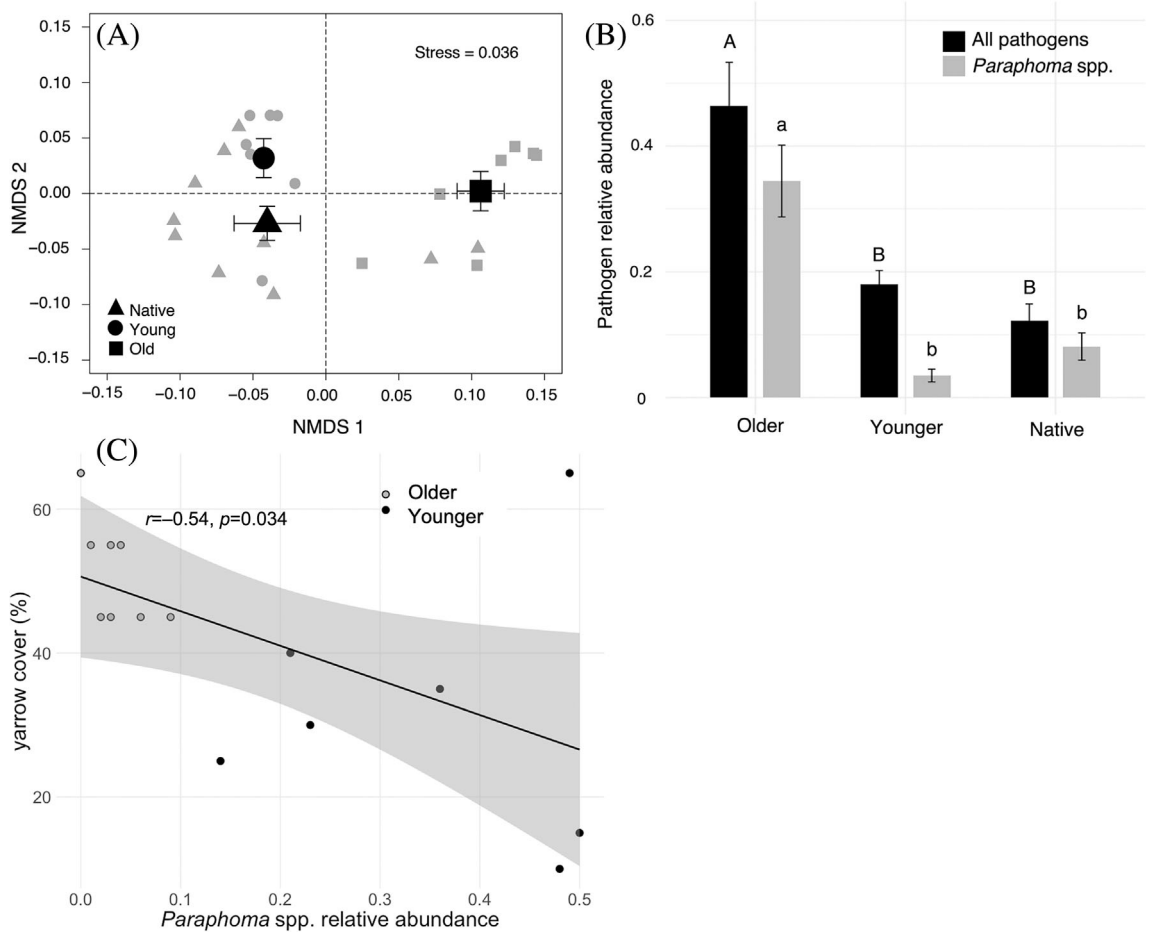


Figure 3. Communities (A), relative abundance (B), and correlation (C) of putative fungal pathogens in yarrow roots collected in 2020 from the then 3-year post-seeding Younger and 5-year post-seeding Older restoration sites as well as from native yarrow growing in an adjacent community (see Fig. S1 for location), including root rot-causing *Paraphoma* spp. Error bars show mean ± SE, Older site, $n = 7$; Younger site, $n = 8$; and Native, $n = 10$. The 95% CI for the mean is shown as a light gray band surrounding the line.

site ($F_{[1,13]\text{Site}} = 32.51$, $p < 0.001$; Fig. 3B). To ensure this result was not driven by responses of other taxa, we ran an analysis of composition of microbiomes (ANCOM), which controls for false discovery rate and potential biases associated with compositional data (Weiss et al. 2017). This additional analysis on absolute sequence abundance also showed significant difference between the Older and Younger site ($W = -3.72$, $p = 0.002$), suggesting our indicator species analysis was robust. *Paraphoma* spp. are known to cause crown-rot (Moslemi et al. 2016), and the closest match in NCBI with our OTU was with *Paraphoma chrysanthemicola* (accession number MT596045.1; www.ncbi.nlm.nih.gov). *Paraphoma* spp. relative abundance correlated negatively with yarrow cover across both sites in 2020 ($r = -0.54$, $t_{[13]} = -2.4$, $p = 0.034$; Fig. 3C).

In 2022, plant communities differed in the Older and Younger sites. At the Older site, unseeded-non-native grasses and forbs (>60% canopy cover) filled the niche space left open by yarrow's decline, with an increase in native perennial grass canopy coverage from approximately 10% in 2020 to approximately 30% in 2022 (Fig. 2B). At the Younger site, yarrow's rapid decline between 2020 and 2022 coincided with a dominance by "Don" variety alfalfa (*Medicago sativa* L. ssp. *Falcata* [L.] Arcang.), and an increase in unseeded-non-native grasses and forbs (Fig. 2D).

Field Experiment

Fungicide increased yarrow cover across years and community types ($F_{[1,48.6]} = 28.8$, $p < 0.001$, Table S4a & S4b; Fig. 4). Peak cover differed among community types, with monocultures at approximately 100% cover 2 years after planting and even communities at approximately 50% cover 4 years after planting (Fig. 4). Cover in dominant communities varied, yet peak year and mean cover at peak fell between monoculture and even communities (Fig. 4). The significant interactions of community type by year in the full model ($F_{[2,292.1]} = 29.4$, $p < 0.001$; Table S4b) indicate that the rate of yarrow decline depended on community type; declining faster in monoculture plots relative to dominant plots ($t_{53.6} = 4.2$, $p = 0.003$) and even plots ($t_{53.6} = 7.0$,

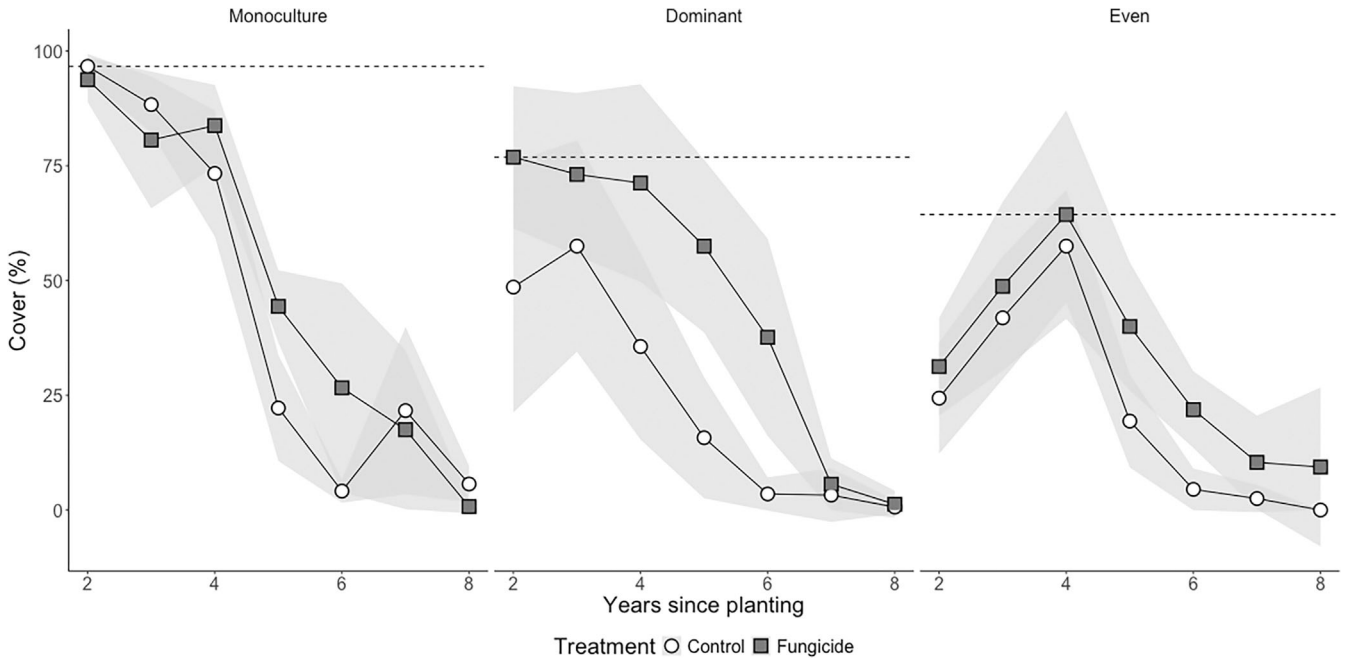


Figure 4. Percent cover of yarrow grown in field experiment, including monoculture, dominant, or even communities that were sprayed with fungicides (dark gray squares) or water (white circles) from 2 to 8 years after planting. The 95% CIs for the means are shown as a light gray band surrounding each line. Dashed lines correspond to the peak percent cover of yarrow, $n = 8$ in each community type.

$p < 0.001$; Table S4c; Fig. 4). Thus, while yarrow cover eventually declined to approximately 0% by year 8 in all plots, the onset of this crash happened earlier and proceeded more rapidly in monoculture plots where yarrow density was highest.

In all, we identified 547 fungal OTUs, of which 77 were classified as probable or highly probable pathogens. We found that communities of putative pathogens differed between control and fungicided plots ($F_{[1,34]\text{Fungicide}} = 3.8$, $r^2 = 0.09$, $p = 0.003$; Fig. 5A) and changed over time ($F_{[2,34]\text{Time}} = 1.9$, $r_2 = 0.09$, $p = 0.03$). Similar significant results were obtained when analyzing presence/absence data (not shown), suggesting species turnover. However, while fungicide affected the composition of putative pathogens, it did not affect their relative abundance ($F_{[1,28]} = 1.04$, $p = 0.317$; Fig. 5B), which ranged from 30 to 36% of total fungal sequences. Nor did the relative abundance of fungal pathogens increase with time ($F_{[1,28]} = 0.61$, $p = 0.441$). However, focusing solely on *Paraphoma* spp., the crown-rot pathogen that we detected in the field survey, we found that its relative abundance increased over time in control plots ($F_{[2,34]\text{Time}} = 7.13$, $p = 0.002$) and trended lower in fungicide plots (Fig. 5). Fifty-three oomycete OTUs were identified, but oomycete communities were not affected by fungicide applications and only differed marginally by year ($F_{[2,34]\text{Time}} = 1.8$, $r^2 = 0.10$, $p = 0.08$).

Greenhouse Experiment

Direct Effects of Fungicides. Biomass of yarrow grown in sterilized soil trended lower ($t_{[14]} = 1.7$, $p = 0.101$) in pots receiving fungicide (0.42 ± 0.04 g, mean $\pm$ SE) than pots receiving water (0.49 ± 0.02 g). Thus, when yarrow was grown without soil biota, fungicide had either no effect or effects were opposite those found in the field.

Spread to Other Native Species. In the soil conditioning phase when yarrow was grown with soil biota, fungicide affected yarrow performance similar to the field ($F_{[1,28]} = 9.16$, $p = 0.005$), and shoot biomass was higher with fungicide (1.17 ± 0.07 g, mean $\pm$ SE) than without fungicide (0.67 ± 0.08 g).

Testing for pathogen spread, we found shoot biomass differed among the three species inoculated with yarrow-trained soil ($F_{[2,37]} = 3.46$, $p = 0.04$), but not between fungicide and controls ($F_{[1,37]} = 0.02$, $p = 0.89$; Fig. 6). There was no interaction between restoration species and fungicide treatment ($F_{[2,37]} = 0.82$, $p = 0.45$). Thus, while fungicide promoted yarrow growth due to suppressive effects in control treatments, this suppressive effect was not transmitted to other species.

Discussion

Predicting restoration success is notoriously difficult (Rinella et al. 2012), indicating a need to better understand plant community development (Brudvig 2017; Zirbel et al. 2017). Surveys of restoration projects and a field experiment showed that a native forb, Western Yarrow, favored in restoration projects underwent boom and bust patterns that may be linked to a relative increase of a crown-rot pathogen. Greenhouse experiments showed that

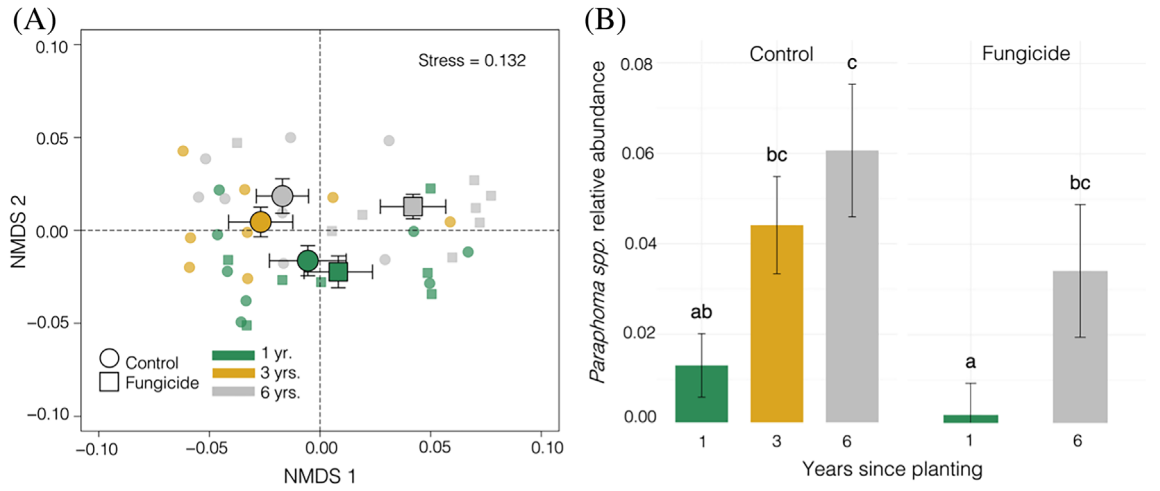


Figure 5. Nonmetric multidimensional scaling (NMDS) ordination of fungal pathogen communities in the field experiment and how they changed over time in control and fungicided yarrow monoculture plots (A) and relative sequence abundance of the indicator species *Paraphoma* sp. in the control and fungicided plots over time (B). No samples were collected from fungicided plots in 2016. Mean ± SE, $n = 8$.

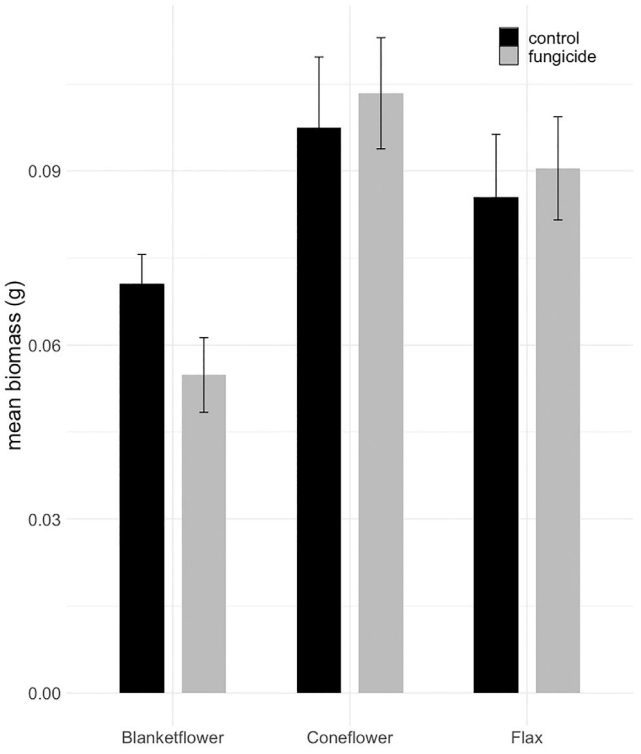


Figure 6. Shoot biomass of Blanketflower, coneflower, and flax grown in the greenhouse in yarrow-trained soil where either fungicide (black bars) or not (gray bars) was added. There were no significant differences between control and fungicide treatments for any species. Error bars show mean ± SE, $n = 8$.

the self-suppressive effect of yarrow on soil did not harm other native forbs commonly used in restoration. Plant–soil feedback has predominately been studied in the greenhouse, where responses are often more negative than in the field (Kulmatiski et al. 2008). Our findings indicate that plant–soil feedbacks can be negative and strong outside of controlled greenhouse experiments, possibly due to the high host density (Liao et al. 2015; Yang et al. 2015) and multi-year conditioning, and that these effects can have implications for restoration success.

Rapid Decline in Yarrow Linked to Shifts in Fungal Pathogens

When it comes to soil biota, restoration ecologists have centered their attention around mutualists (Asmelash et al. 2016; Koziol & Bever 2017), whereas pathogens have received less attention. This is unfortunate because pathogens can maintain diversity (Fitzsimons & Miller 2010; van Ruijven et al. 2020) and reduce the life span, fecundity, and competitive ability of early successional plants (Kardol et al. 2006), especially when grown in high densities (Kulmatiski et al. 2012; Bauer et al. 2017). One exception is the use of pathogens to control cheatgrass, although it has had little success as a management practice so far (Dooley & Beckstead 2010; Reinhart et al. 2020). To our knowledge, the possibility of managing naturally occurring pathogens to aid in restoration has not yet been explored.

Here, we present data suggesting the rapid decline of yarrow is at least partly due to fungal pathogens. First, the lower yarrow cover in the Older restoration site coincided with a higher relative abundance of putative pathogens, consistent with pathogen suppression. This was especially true for *Paraphoma* spp. a basidiomycete that causes crown-rot in several forbs (Moslemi et al. 2016; Cao et al. 2020), and that has been found in yarrow roots and rhizosphere soil previously (Schöps et al. 2018; Ampt et al. 2022). In fact, Francioli et al. (2020) found *Paraphoma chrysanthemicola*—the closest match with the *Paraphoma* in our study—in a common garden growing yarrow in the Netherlands. Second, throughout the 10-year field experiment, yarrow cover was higher in plots receiving fungicide, and yarrow also grew better in soils receiving fungicide in the greenhouse. While unintended fungicide effects can

complicate interpretations (Paul et al. 1989; Burdon et al. 2006), our greenhouse experiment showed the beneficial effect on yarrow was mediated by soil biota, because fungicide promoted growth of yarrow only when soil biota was present but not when fungicide was added to sterile soil. Fungicides can also increase fungal necromass, resulting in higher nutrient availabilities, but this is unlikely to have caused the promotion of growth and cover in fungicided plots because (1) fungicides more often change the composition of fungi rather than suppress overall biomass (Helgason et al. 2007; Henning et al. 2018), and (2) nutrient availabilities in the experimental plots were high and pots in the greenhouse were fertilized regularly. Also, the fungicide used here has previously been shown to promote plant productivity, but only in low diversity plots (Maron et al. 2011). This was most likely due to density-dependent pathogen accumulation, which has been shown before across a range of native species (Mommer et al. 2018; Liu et al. 2022). Because we only assessed fungal communities in monoculture communities, we cannot determine if the relative pathogen abundance increased with yarrow density. However, the more rapid onset of yarrow decline in monoculture plots than in plots with lower yarrow density is consistent with density-dependence.

While the increased performance of yarrow with fungicides indicates a suppressive role of pathogenic fungi, yarrow ultimately declined in fungicided plots also. This particular yarrow cultivar may not live long in our area, or fungicide applications may not suppress all fungi, which has been reported previously (Henning et al. 2018). Indeed, even though the composition of fungi changed, fatty acid analyses of fungal biomass in control and fungicided plots revealed only modest declines in fungal biomass over time (Lekberg, unpublished data), possibly because we only applied fungicide twice a year or it was incorporated insufficiently into the soil. This ineffective control by fungicides was supported by brownish-black roots of the few yarrow plants remaining in fungicided plots in the field experiment (Lekberg, personal observation). Irrespective, results from the survey and experimental plots combined suggest a potential role of soil pathogens in the substantial decline in yarrow cover.

Contrary to fungal pathogens, oomycetes amplified poorly or showed no treatment effects in our various studies. This is unsurprising given that our experiments/restoration took place in semiarid soils, which may not provide the high soil moisture required for oomycete zoospore germination and spread (Raaijmakers et al. 2009).

Considering Yarrow as a Bridge Species During Restoration

Bridge species are often early successional, competitive, and short-lived plants that facilitate the establishment of native plant species during restoration (Herron et al. 2001; Meissen et al. 2020). Native plants can benefit from a bridge species' ruderal qualities in a pattern mimicking succession where longer-lived perennials are indirectly facilitated by the competitive but short-lived transition species (Sheley et al. 2006; Perry et al. 2009; Leger et al. 2014). Without a predictable die-off, herbicide, mowing, harvesting, or some other management intervention (Milchunas et al. 2011; Espeland & Perkins 2013) are often required to remove bridge species. The high initial cover and unmanaged rapid decline in yarrow highlight its potential as a bridge species. Because we seeded yarrow in both restoration projects, we cannot quantify its effect as a bridge species, but we can speculate on its potential. In our area, yarrow behaved as a ruderal, early successional species with the highest cover coinciding with a fractional proportion of non-native weeds 3 years after seeding in the Younger site, with seeded species making up most of the relative cover 5 years after seeding.

Because the decline of yarrow might have been mediated by pathogens, another requirement to be a bridge species is that pathogens are host-specific. The greenhouse experiment showed that yarrow was suppressed when grown in its own soil without fungicides and that the suppressive effect was not transmitted to other native plants grown in yarrow-trained soil. While the build-up of yarrow-specific fungal pathogens parsimoniously explains results, we cannot exclude other factors as we did not verify higher pathogen abundance in the soil without fungicides. Also, recent work suggests yarrow promotes *Paraphoma* abundance in heterospecific neighbors in the Asteraceae family (Ampt et al. 2022). Inoculations with *Paraphoma*, which we isolated from yarrow roots (DuPre, unpublished data), onto a range of native species across sites are required to unequivocally assess the degree of host specificity and generality of our findings.

What happens after bridge species declines determines the success or failure of a restoration project. At the Younger site, non-native invasives nearly doubled, and unseeded-non-natives dominated at the Older site 7 years after seeding, tripling their relative cover between years 5 and 7. Thus, while the Older site harbored long-lived native perennials such as arrowleaf balsamroot, silky lupine, and Idaho fescue, yarrow was mostly replaced by non-natives. Future projects specifically testing yarrow as a bridge species are required to assess its ability to suppress non-native plants and influence the trajectory of native plant reestablishment.

In summary, this study indicates the value of incorporating plant–soil feedback into restoration ecology. By expanding the focus from competition theory to consider plant–soil feedbacks, we may enhance our understanding and increase predictability of ecosystem restoration.

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LITERATURE CITED

Aleksoff KC (1999) Achillea millefolium. In: Fire effects information system [online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences, Fort Collins, Colorado. https://www.fs.usda.gov/database/feis/plants/forb/achmil/all.html (accessed 24 Apr 2024)

Ampt EA, Francioli D, van Ruijven J, Gomes SI, Maciá-Vicente JG, Termorshuizen AJ, Bakker LM, Mommer L (2022) Deciphering the

interactions between plant species and their main fungal root pathogens in mixed grassland communities. Journal of Ecology 110:3039–3052. https://doi.org/10.1111/1365-2745.14012

Anderson MJ (2017) Permutational multivariate analysis of variance (PERMANOVA). Pages 1–15. In: Wiley StatsRef: statistics reference online. American Cancer Society, Atlanta, Georgia. https://doi.org/10.1002/9781118445112.stat07841

Asmelash F, Bekele T, Birhane E (2016) The potential role of arbuscular mycorrhizal fungi in the restoration of degraded lands. Frontiers in Microbiology 7:1095. https://doi.org/10.3389/fmicb.2016.01095

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48. https://doi.org/10.48550/arXiv.1406.5823

Bauer JT, Blumenthal N, Miller AJ, Ferguson JK, Reynolds HL (2017) Effects of between-site variation in soil microbial communities and plant–soil feedbacks on the productivity and composition of plant communities. Journal of Applied Ecology 54:1028–1039. https://doi.org/10.1111/1365-2664.12937

Bever JD, Westover KM, Antonovics J (1997) Incorporating the soil community into plant population dynamics: the utility of the feedback approach. Journal of Ecology 85:561–573. https://doi.org/10.2307/2960528

Bourdôt GW, Field RJ, White JGH (1985) Growth analysis of *Achillea millefolium* L. (yarrow) in the presence and absence of a competitor—*Hordeum vulgarel* (barley). New Phytologist 10:507–519. https://doi.org/10.1111/j.1469-8137.1985.tb02856.x

Brudvig LA (2017) Toward prediction in the restoration of biodiversity. Journal of Applied Ecology 54:1013–1017. https://doi.org/10.1111/1365-2664.12940

Bullington LS, Lekberg Y, Sniezko R, Larkin B (2018) The influence of genetics, defensive chemistry and the fungal microbiome on disease outcome in whitebark pine trees: genetics, terpenes, fungi and disease. Molecular Plant Pathology 19:1847–1858. https://doi.org/10.1111/mpp.12663

Burdon JJ, Thrall PH, Ericson AL (2006) The current and future dynamics of disease in plant communities. Annual Review of Phytopathology 44:19–39. https://doi.org/10.1146/annurev.phyto.43.040204.140238

Cao S, Liang QW, Nzabanita C, Li YZ (2020) Paraphoma root rot of alfalfa (*Medicago sativa*) in Inner Mongolia, China. Plant Pathology 69:231–239. https://doi.org/10.1111/ppa.13131

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. (2010) QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7:335–336. https://doi.org/10.1038/nmeth.f.303

Corradi N, Croll D, Colard A, Kuhn G, Ehinger M, Sanders IR (2007) Gene copy number polymorphisms in an arbuscular mycorrhizal fungal population. Applied and Environmental Microbiology 73:366–369. https://doi.org/10.1128/AEM.01574-06

Delory BM, Weidlich EWA, von Gillhaussen P, Temperton VM (2019) When history matters: the overlooked role of priority effects in grassland overyielding. Functional Ecology 33:2369–2380. https://doi.org/10.1111/1365-2435.13455

Derevnina L, Petre B, Kellner R, Dagdas YF, Sarowar MN, Giannakopoulou A, et al. (2016) Emerging oomycete threats to plants and animals. Philosophical Transactions of the Royal Society B: Biological Sciences 371:20150459. https://doi.org/10.1098/rstb.2015.0459

Dooley SR, Beckstead J (2010) Characterizing the interaction between a fungal seed pathogen and a deleterious rhizobacterium for biological control of cheatgrass. Biological Control 53:197–203. https://doi.org/10.1016/j.biocontrol.2009.12.007

Durham RA, Mummey DL, Shreading L, Ramsey PW (2017) Phenological patterns differ between exotic and native plants: field observations from the Sapphire Mountains, Montana. Natural Areas Journal 37:361–381. https://doi.org/10.3375/043.037.0310

Espeland EK, Perkins LB (2013) Annual cover crops do not inhibit early growth of perennial grasses on a disturbed restoration soil in the northern Great Plains, U.S.A. Ecological Restoration 31:69–78. https://doi.org/10.3368/er.31.1.69

Eviner VT, Hawkes CV (2008) Embracing variability in the application of plant–soil interactions to the restoration of communities and ecosytems. Restoration Ecology 16:713–729. https://doi.org/10.1111/j.1526-100X.2008.00482.x

Fitzsimons MS, Miller RM (2010) The importance of soil microorganisms for maintaining diverse plant communities in tallgrass prairie. American Journal of Botany 97:1937–1943. https://doi.org/10.3732/ajb.0900237

Francioli D, van Ruijven J, Bakker L, Mommer L (2020) Drivers of total and pathogenic soil-borne fungal communities in grassland plant species. Fungal Ecology 48:100987. https://doi.org/10.1016/j.funeco.2020.100987

Fukami T (2015) Historical contingency in community assembly: integrating niches, species pools, and priority effects. Annual Review of Ecology, Evolution, and Systematics 46:1–23. https://doi.org/10.1146/annurev-ecolsys-110411-160340

Hardegree SP, Abatzoglou JT, Brunson MW, Brunson MW, Germino MJ, Hegewisch KC, et al. (2018) Weather-centric rangeland revegetation planning. Rangeland Ecology & Management 71:1–11. https://doi.org/10.1016/j.rama.2017.07.003

Harris J (2009) Soil microbial communities and restoration ecology: facilitators or followers? Science 325:573–574. https://doi.org/10.1126/science.1172975

Helgason T, Merryweather JW, Young JP, Fitter AH (2007) Specificity and resilience in the arbuscular mycorrhizal fungi of a natural woodland community. Journal of Ecology 1:623–630. https://doi.org/10.1111/j.1365-2745.2007.01239.x

Henning JA, Weiher E, Lee TD, Freund D, Stefanski A, Bentivenga SP (2018) Mycorrhizal fungal spore community structure in a manipulated prairie. Restoration Ecology 26:124–133. https://doi.org/10.1111/rec.12548

Herron GJ, Sheley RL, Maxwell BD, Jacobsen JS (2001) Influence of nutrient availability on the interaction between spotted knapweed and Bluebunch wheatgrass. Restoration Ecology 9:326–331. https://doi.org/10.1046/j.1526-100x.2001.009003326.x

James JJ, Sheley RL, Leger EA, Adler PB, Hardegree SP, Gornish ES, Rinella MJ (2019) Increased soil temperature and decreased precipitation during early life stages constrain grass seedling recruitment in cold desert restoration. Journal of Applied Ecology 56:2609–2619. https://doi.org/10.1111/1365-2664.13508

James JJ, Svejcar TJ, Rinella MJ (2011) Demographic processes limiting seedling recruitment in arid grassland restoration. Journal of Applied Ecology 48:961–969. https://doi.org/10.1111/j.1365-2664.2011.02009.x

Jensen S, Christensen WF, Roundy B, Anderson VJ, Kitchen SG, Allphin L (2022) Temporal and spatial factors influence native form emergence more than sowing depth. Rangeland Ecology & Management 83:41–49. https://doi.org/10.1016/j.rama.2022.03.003

Jing J, Bezemer TM, van der Putten WH (2015) Interspecific competition of early successional plant species in ex-arable fields as influenced by plant–soil feedback. Basic and Applied Ecology 16:112–119. https://doi.org/10.1016/j.baae.2015.01.001

Kardol P, Martijn Bezemer T, van der Putten WH (2006) Temporal variation in plant–soil feedback controls succession. Ecology Letters 9:1080–1088. https://doi.org/10.1111/j.1461-0248.2006.00953.x

Kildisheva OL, Erickson TE, Merritt DJ, Dixon KW (2016) Setting the scene for dryland recovery: an overview of key findings from a workshop targeting seed-based restoration. Restoration Ecology 24:S36–S42. https://doi.org/10.1111/rec.12392

Klironomos JN (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417:67–70. https://doi.org/10.1038/417067a

Kohout P, Sudová R, Janoušková M, Čtvrtlíková M, Hejda M, Pánková H, Slavíková R, Štajerová K, Vosátka M, Sýkorová Z (2014) Comparison of commonly used primer sets for evaluating arbuscular mycorrhizal fungal communities: is there a universal solution? Soil Biology and Biochemistry 68:482–493. https://doi.org/10.1016/j.soilbio.2013.08.027

Koziol L, Bever JD (2017) The missing link in grassland restoration: arbuscular mycorrhizal fungi inoculation increases plant diversity and accelerates

succession. Journal of Applied Ecology 54:1301–1309. https://doi.org/10.1111/1365-2664.12843

Kulmatiski A, Beard KH, Heavilin J (2012) Plant–soil feedbacks provide an additional explanation for diversity–productivity relationships. Proceedings of the Royal Society B: Biological Sciences 279:3020–3026. https://doi.org/10.1098/rspb.2012.0285

Kulmatiski A, Beard KH, Norton JM, Heavilin JE, Forero LE, Grenzer J (2017) Live long and prosper: plant–soil feedback, lifespan, and landscape abundance covary. Ecology 98:3063–3073. https://doi.org/10.1002/ecy.2011

Kulmatiski A, Beard KH, Stevens JR, Cobbold SM (2008) Plant–soil feedbacks: a meta-analytical review. Ecology Letters 11:980–992. https://doi.org/10.1111/j.1461-0248.2008.01209.x

Kuznetsova A, Brockhoff PB, Christensen RHB (2017) lmerTest package: tests in linear mixed effects models. Journal of Statistical Software 8:21–26. https://doi.org/10.18637/jss.v082.i13

Lambert SM, Monsen SB, Shaw N (2011) Notice of release for eagle germplasm western yarrow (selected germplasm, natural track). US Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fort Collins, Colorado

Legeay J, Husson C, Cordier T, Vacher C, Marcais B, Buée M (2019) Comparison and validation of oomycetes metabarcoding primers for phytophthora high throughput sequencing. Journal of Plant Pathology 101:743–748. https://doi.org/10.1007/s42161-019-00276-9

Leger EA, Goergen EM, Forbis de Queiroz T (2014) Can native annual forbs reduce Bromus tectorum biomass and indirectly facilitate establishment of a native perennial grass? Journal of Arid Environments 102:9–16. https://doi.org/10.1016/j.jaridenv.2013.10.015

Lekberg Y, Bever JD, Bunn RA, Callaway RM, Hart MM, Kivlin SN, et al. (2018) Relative importance of competition and plant–soil feedback, their synergy, context dependency and implications for coexistence. Ecology Letters 21:1268–1281. https://doi.org/10.1111/ele.13093

Lenth R (2024) emmeans: estimated marginal means, aka least-squares means. R package version (1.10.1) [computer software]. https://CRAN.R-project.org/package=emmeans (accessed 2 May 2024)

Liao H, Luo W, Peng S, Callaway RM (2015) Plant diversity, soil biota, and resistance to exotic invasion. Diversity and Distributions 21:826–835. https://doi.org/10.1111/ddi.12319

Liu X, Parker IM, Gilbert GS, Lu Y, Xiao Y, Zhang L, Huang M, Cheng Y, Zhang Z, Zhou S (2022) Coexistence is stabilized by conspecific negative density dependence via fungal pathogens more than oomycete pathogens. Ecology 103:3841. https://doi.org/10.1002/ecy.3841

Mangan SA, Schnitzer SA, Herre EA, Mack KM, Valencia MC, Sanchez EI, Bever JD (2010) Negative plant–soil feedback predicts tree-species relative abundance in a tropical forest. Nature 466:752–755. https://doi.org/10.1038/nature09273

Maron JL, Marler M, Klironomos JN, Cleveland CC (2011) Soil fungal pathogens and the relationship between plant diversity and productivity. Ecology Letters 14:36–41. https://doi.org/10.1111/j.1461-0248.2010.01547.x

Martin LM, Wilsey BJ (2014) Native-species seed additions do not shift restored prairie plant communities from exotic to native states. Basic and Applied Ecology 15:297–304. https://doi.org/10.1016/j.baae.2014.05.007

Meissen JC, Glidden AJ, Sherrard ME, Elgersma KJ, Jackson LL (2020) Seed mix design and first year management influence multifunctionality and cost-effectiveness in prairie reconstruction. Restoration Ecology 28:807–810. https://doi.org/10.1111/rec.13013

Milchunas DG, Vandever MW, Ball LO, Hyberg S (2011) Allelopathic cover crop prior to seeding is more important than subsequent grazing/mowing in grassland establishment. Rangeland Ecology & Management 64:291–300. https://doi.org/10.2111/REM-D-10-00117.1

Mommer L, Cotton TA, Raaijmakers JM, Termorshuizen AJ, van Ruijven J, Hendriks M, et al. (2018) Lost in diversity: the interactions between soil-borne fungi, biodiversity and plant productivity. New Phytologist 218:542–553. https://doi.org/10.1111/nph.15036

Moslemi A, Ades PK, Groom T, Crous PW, Nicolas ME, Taylor PW (2016) Paraphoma crown rot of pyrethrum (*Tanacetum cinerariifolium*). Plant Disease 100:2363–2369. https://doi.org/10.1094/PDIS-05-16-0628-RE

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG (2016) FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology 20:241–248. https://doi.org/10.1016/j.funeco.2015.06.006

Nilsson RH, Larsson KH, Taylor AFS, Bengtsson-Palme J, Jeppesen TS, Schigel D, et al. (2019) The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Research 47:D259–D264. https://doi.org/10.1093/nar/gky1022

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. (2019) vegan: community ecology package (2.5-6) [computer software]. https://CRAN.R-project.org/package=vegan

Palmer MA, Zedler JB, Falk DA (2016)Pages 3–26. In: Palmer MA, Zedler JB, Falk DA (eds) Ecological theory and restoration ecology. Foundations of restoration ecology Island Press, Washington, D.C.

Paul ND, Ayres PG, Wyness LE (1989) On the use of fungicides for experimentation in natural vegetation. Functional Ecology 3:759–769. https://doi.org/10.2307/2389509

Perry LG, Cronin SA, Paschke MW. (2009) Native cover crops suppress exotic annuals and favor native perennials in a greenhouse competition experiment. Plant Ecology 204:247–259. https://doi.org/10.1007/s11258-009-9588-1

R Core Team (2021) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/ (accessed 20 Feb 2021)

Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, Moënne-Loccoz Y (2009) The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant and Soil 321:341–361. https://doi.org/10.1007/s11104-008-9568-6

Reinhart KO, Carlson CH, Feris KP, Germino MJ, Jandreau CJ, Lazarus BE, et al. (2020) Weed-suppressive bacteria fail to control *Bromus tectorum* under field conditions. Rangeland Ecology & Management 73:760–765. https://doi.org/10.1016/j.rama.2019.07.006

Rinella MJ, Mangold JM, Espeland EK, Sheley RL, Jacobs JS (2012) Long-term population dynamics of seeded plants in invaded grasslands. Ecological Applications 22:1320–1329. https://doi.org/10.1890/11-1103.1

Roberts DW (2019) labdsv: ordination and multivariate analysis for ecology. R package version (2.0-1) [computer software]. https://CRAN.R-project.org/package=labdsv (accessed 2 Sep 2019)

Schöps R, Goldmann K, Herz K, Lentendu G, Schöning I, Bruelheide H, Wubet T, Buscot F (2018) Land-use intensity rather than plant functional identity shapes bacterial and fungal rhizosphere communities. Frontiers in Microbiology 9:2711. https://doi.org/10.3389/fmicb.2018.02711

Shackleford N, Paterno GB, Winkler DE, Erickson TE, Leger EA, Svejcar LN, et al. (2021) Drivers of seedling establishment success in dryland restoration efforts. Nature Ecology & Evolution 5:1283–1290. https://doi.org/10.1038/s41559-021-01510-3

Sheley RL, Mangold JM, Anderson JL (2006) Potential for successional theory to guide restoration of invasive-plant-dominated rangeland. Ecological Monographs 76:363–379. https://doi.org/10.1890/0012-9615(2006)076[0365:PFSTTG]2.0.CO;2

Skelton J, Cauvin A, Hunter ME (2022) Environmental DNA metabarcoding read numbers and their variability predict species abundance, but weakly in non-dominant species. Environmental DNA 5:1092–1104. https://doi.org/10.1002/edn3.355

Sonnemann I, Baumhaker H, Wurst S (2012) Species specific responses of common grassland plants to a generalist root herbivore (*Agriotes* spp. larvae). Basic and Applied Ecology 13:579–586. https://doi.org/10.1016/j.baae.2012.09.010

Svejcar T, Boyd C, Davies K, Hamerlynck E, Svejcar L (2017) Challenges and limitations to native species restoration in the Great Basin, U.S.A. Plant Ecology 218:91–94. https://doi.org/10.1007/s11258-016-0648-z

Taheri AE, Chatterton S, Gossen BD, McLaren DL (2017) Degenerate ITS7 primer enhances oomycete community coverage and PCR sensitivity to

Aphanomyces species, economically important plant pathogens. Canadian Journal of Microbiology 63:769–779. https://doi.org/10.1139/cjm-2017-0100

Tedersoo L, Bahram M, Zinger L, Nilsson RH, Kennedy PG, Yang T, Anslan S, Mikryukov V (2022) Best practices in metabarcoding of fungi: from experimental design to results. Molecular Ecology 31:2769–2795. https://doi.org/10.1111/mec.16460

Temperton VM, Hobbs RJ, Nuttle T, Halle S (2004) Assembly rules and restoration ecology: bridging the gap between theory and practice. Vol 5. Island Press, Washington, D.C.

Thakur MP, van der Putten WH, Wilschut RA, Veen GC, Kardol P, van Ruijven J, Allan E, Roscher C, van Kleunen M, Bezemer TM (2021) Plant–soil feedbacks and temporal dynamics of plant diversity–productivity relationships. Trends in Ecology & Evolution 36:651–661. https://doi.org/10.1016/j.tree.2021.03.011

van der Putten WH, Bardgett RD, Bever JD, Bezemer TM, Casper BB, Fukami T, et al. (2013) Plant–soil feedbacks: the past, the present and future challenges. Journal of Ecology 101:265–276. https://doi.org/10.1111/1365-2745.12054

van Ruijven J, Ampt E, Francioli D, Mommer L (2020) Do soil-borne fungal pathogens mediate plant diversity–productivity relationships? Evidence and future opportunities. Journal of Ecology 108:1810–1821. https://doi.org/10.1111/1365-2745.13388

Wainwright CE, Staples TL, Charles LS, Flanagan TC, Lai HR, Loy X, et al. (2018) Links between community ecology theory and ecological restoration are on the rise. Journal of Applied Ecology 55:570–580. https://doi.org/10.1111/1365-2664.12975

Weiss S, Xu ZZ, Peddada S, Amir A, Bittinger K, Gonzalez A, et al. (2017) Normalization and microbial differential abundance strategies depend upon data characteristics. Microbiome 5:1–18. https://doi.org/10.1186/s40168-017-0237-y

Wilson GW, Hartnett DC (1998) Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. American Journal of Botany 85:1732–1738. https://doi.org/10.2307/2446507

Wubs ERJ, van der Putten WH, Bosch M, Bezemer TM (2016) Soil inoculation steers restoration of terrestrial ecosystems. Nature Plants 2:1–5. https://doi.org/10.1038/nplants.2016.107

Yang L, Maron JL, Callaway RM (2015) Inhibitory effects of soil biota are ameliorated by high plant diversity. Oecologia 179:519–525. https://doi.org/10.1007/s00442-015-3351-1

Zirbel CR, Bassett T, Grman E, Brudvig LA (2017) Plant functional traits and environmental conditions shape community assembly and ecosystem functioning during restoration. Journal of Applied Ecology 54:1070–1079. https://doi.org/10.1111/1365-2664.12885

Supporting Information

The following information may be found in the online version of this article:

Supplement S1. Detailed restoration history for the two restoration sites.
Figure S1. Map of MPG Ranch in western Montana inset with large-scale map showing locations of the field experiment.
Figure S2. Communities of putative fungal pathogens in yarrow soil collected from the young (NIR) and old (RP) restoration, mean ± SE, $n = 10$.
Table S1. (a) Older (RP), and (b) Younger (NIR).
Table S2. Seed mixes for Older (RP), 2 November 2015 (a), Younger (NIR) 4 April 2017 (b), and Older (RP) supplemental planting 12 November 2019 (c).

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